


Title:	PHASE III NON-INFERIORITY CLINICAL STUDY TO COMPARE EFFICACY AND SAFETY OF PRO-169 VERSUS INTRAVITREAL RANIBIZUMAB FOR THE TREATMENT OF DIABETIC MACULAR EDEMA
Short / Alternate Title:	PRO-169 FOR DIABETIC MACULAR EDEMA
Version / Date	Version 5.0 / March 17th, 2023
Sponsor:	Laboratorios Sophia S.A. de C. V. 
Protocol number:	SOPH169-0718/III
Investigational Product:	PRO-169 (Bevacizumab) IgG1 Humanized Monoclonal Antibody Directed Against VEGF-A
Development phase:	Phase III
Study design:	Phase III, multicenter, randomized, double-blind clinical study with parallel active control to demonstrate non-inferiority.
Indication under study:	Diabetic Macular Edema
Objective of the study:	To assess the efficacy, expressed as improvement in best-corrected visual acuity one year after initiation of treatment, of PRO-169 compared to Lucentis® (ranibizumab) during the treatment of diabetic macular edema.
Compliance statement	This protocol has been carried out in accordance with the principles of the Declaration of Helsinki and will be carried out in accordance with the ICH guidelines and current local legislation.

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Organizations responsible for study conduct

Key Function	Responsible organization	Address
Sponsor	Laboratorios Sophia S.A. de C.V.	[REDACTED]
Contact for safety-related issues:	Pharmacovigilance and Technovigilance Management. Laboratorios Sophia, S.A. de C.V.	[REDACTED]
Contact for questions related to the research protocol:	Regional Management of Medical Affairs. Laboratorios Sophia, S.A. de C.V.	[REDACTED]

Signature page

From the sponsor:

Name:	
<div></div>	Signature
Title:	
Medical Head of the Study	Date

Name:	
<div></div>	Signature
Title:	
Study Director	Date

Name:	
<div></div>	Signature
Title:	
Operational Manager	Date

Name:	
<div></div>	Signature
Title:	
Protocol Author	Date

Investigator agreement

I declare that I have read and understand this protocol, the investigator's manual, and all information provided about the product by the sponsor. I agree to conduct this study in accordance with the requirements of this protocol and to protect the rights, safety, privacy, and well-being of the patients in the study in accordance with:

- The ethical principles emanating from the Declaration of Helsinki.
- The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use's guide to Good Clinical Practice E6.
- All applicable laws and regulations, including, but not limited to, laws and regulations regarding the handling and confidentiality of information. The requirements for the reporting of adverse events in accordance with applicable laws and regulations.
- The terms described in the Clinical Trial contract.

Signature

Date (dd/mm/yyyy)

Investigator's Name (printed or typed)

Title of the Investigator (printed or typed)

Name of the center (printed or typed)

Geographic location (city/state/country)

Abbreviations

Abbreviation	Abbreviated term
AUC	Area under the curve
ADA	American Diabetes Association
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ULN	Upper limit of normal
C _{max}	Maximum concentration
CMH	Cochran-Mantel-Haenszel
CMT	Central Macular Thickness
AE	Adverse Event
DME	Diabetic macular edema
RPE	Retinal pigment epithelial
ETDRS	Early Treatment Diabetic Retinopathy Study
FDA	Food and Drug Administration
eCRF	electronic Case Report Form
eGFR	estimated Glomerular Filtration Rate
HbA1c	Glycosylated hemoglobin
CI	Confidence interval
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
KDIGO	Kidney Disease Improving Global Outcomes
LogMAR	Logarithm of the Minimum Angle of Resolution
LOCS III	Lens Opacities Classification System III
MedDRA	Medical Dictionary for Regulatory Activities
OCT	Optical Coherence Tomography
WHO	World Health Organization
T _{max}	Time to Maximum Concentration
VEGF	Vascular Endothelial Growth Factor
q.s.	The amount which is enough (per its abbreviation in Latin, <i>quantum satis</i>)

Synopsis

Title: PHASE III NON-INFERIORITY CLINICAL STUDY TO COMPARE EFFICACY AND SAFETY OF PRO-169 VERSUS RANIBIZUMAB INTRAVITREAL FOR THE TREATMENT OF DIABETIC MACULAR EDEMA

Protocol number: SOPH169-0718/III

Investigational product: PRO-169 (bevacizumab) humanized IgG1 monoclonal antibody directed against VEGF-A

Development phase: Phase III

Objectives of the study:

Primary objective:

To evaluate the efficacy, expressed as improvement in best-corrected visual acuity, and non-inferiority of PRO-169 compared to Lucentis® (ranibizumab) one year after initiation of treatment, in patients with diabetic macular edema (DME).

Secondary efficacy objectives:

- To assess the efficacy, expressed as the area under the curve of improvement in best-corrected visual acuity from initiation of treatment to completion of one year of treatment, of PRO-169 compared with Lucentis® for the treatment of diabetic macular edema
- To assess the efficacy, expressed as improvement in best-corrected visual acuity at 4 months of initiation of treatment, adjusted to the baseline value, of PRO-169 compared with Lucentis® for the treatment of diabetic macular edema.
- To establish the change in central macular thickness during the treatment of diabetic macular edema with PRO-169 compared to Lucentis® at one year of treatment.
- To assess the change in central retinal volume during the treatment of diabetic macular oedema with PRO-169 compared with Lucentis® at one year of treatment.
- To determine the proportion of patients with positive responses to treatment, with permanence in one of the following areas being considered a positive response: absolute improvement, improvement and stability; described in the treatment response classification set out in section 3.1 Design Overview.

- To quantify the average number of injections administered per patient during the protocol.
- To identify the frequency of laser rescue therapy during the treatment of diabetic macular edema with PRO-169 compared to Lucentis®.

Secondary security objective:

- To determine the incidence and severity of adverse events during the treatment of diabetic macular edema with PRO-169 compared to Lucentis® with an emphasis on the following adverse events of interest:
 - Injection-related: endophthalmitis, traction retinal detachment, rhegmatogenous retinal detachment, retinal tear, cataract, vitreous hemorrhage, increased intraocular pressure.
 - Related to medication at the ophthalmic level: inflammation, new or progressive traction retinal detachment, progression of extramacular to macular retinal detachment.
 - Drug-related at a systemic level.

Study hypothesis:

The efficacy and safety of PRO-169 are not inferior to Lucentis® in one year of treatment of diabetic macular edema.

Justification for the use of the product in clinical investigation:

It has been shown that treatments aimed at neutralizing VEGF inside the eye contribute to reducing DME, improving visual acuity [1, 2]. This treatment is also effective in other proliferative retinal conditions (age-related macular degeneration, macular edema secondary to occlusion of the central retinal vein or venous branch, diabetic retinopathy, choroidal neovascularization secondary to myopia). [3]

Four previous studies [1, 4, 5, 6] comparing treatment between ranibizumab and bevacizumab have shown that the efficacy of treatment is equivalent in terms of improvement in visual acuity, but with a lower response in measurements of central macular thickness as assessed by optical coherence tomography (OCT). With the exception of one study [1], these were conducted with small sample sizes or reduced treatment times. This latest study showed that after one year of treatment with bevacizumab and ranibizumab they were equivalent in their efficacy for the

treatment of DME [1]. Despite these results, there is no ophthalmic presentation of bevacizumab on the market to date. [1]

Due to the need for low-cost therapeutic options and considering the increase in the overall incidence of diabetes mellitus, the present study aims to evaluate the non-inferiority of PRO-169, an ophthalmic presentation of bevacizumab, compared to ranibizumab (Lucentis®), with an intravitreal administration in a monthly regimen, for DME during one year of treatment.

Study design:

Phase III, multicenter, randomized, double-blind clinical study with parallel active control to demonstrate non-inferiority.

Treatment scheme:

According to randomization:

- a. **Test treatment:** Intravitreal injection of 1.25 mg of PRO-169 in a volume of 0.05mL each month according to the protocol (see Annex 1).
- b. **Reference treatment:** Intravitreal injection of 0.5 mg of Lucentis® in a volume of 0.05 mL each month according to the protocol (see Annex 1).

Sample size:

N = 442 patients (398 evaluable), randomized with a 1:1 allocation to two treatment groups of 221 each (one eye per patient).

The sample size has been calculated to determine non-inferiority in the main outcome variable (improvement in the best-corrected visual acuity with respect to its baseline value) between the treatments according to the following premises:

A previous study evaluating the improvement in visual acuity through the use of aflibercept, bevacizumab, or ranibizumab [1] showed that the efficiency with treatment using ranibizumab consisted of an average improvement of 11.2 ± 9.4 letters. This study derived its sample size (n=220 eyes per group) from a previous study with a total of 173 participants in which the standard deviation in the improvement in number of letters was 11.2 (95% CI 10.0, 12.5) and the one adjusted according to baseline visual acuity was 10.2 (95% CI 9.2, 11.4).

Using the CI values as a guide, a simulation was carried out to establish an average difference of -4 letters as non-significant:

POWER Procedure (SAS Program): t-test of two independent samples for the mean difference:

Table 1 Assumptions for sample estimation

Distribution	Normal
Method	Exact
Number of sides	1, Upper limit
Null Difference	-4
Alpha	0.05
Nominal Power	0.8
Weighting for Group 1	1
Weighting for Group 2	1

Table 2 Estimated Sample Size

Number	Difference of Means	Standard Deviation	Estimated Power	Estimated sample size
1	-3.0	9	0.800	2006
2	-3.0	10	0.800	2476
3	-3.0	11	0.800	2994
4	-2.5	9	0.800	892
5	-2.5	10	0.800	1102
6	-2.5	11	0.800	1332
7	-2.0	9	0.801	504
8	-2.0	10	0.800	620
9	-2.0	11	0.800	750
10	-1.5	9	0.800	322
11	-1.5	10	0.801	398
12	-1.5	11	0.801	482
13	-1.0	9	0.800	224
14	-1.0	10	0.802	278
15	-1.0	11	0.800	334
16	-0.5	9	0.802	166
17	-0.5	10	0.801	204
18	-0.5	11	0.801	246
19	0.0	9	0.804	128
20	0.0	10	0.800	156

Considering that the average difference between ranibizumab and bevacizumab in the study used as the basis for sample calculation [1] was 1.4 (95% CI -0.4 to +3.2 $p = 0.12$) letters, the sample size considered for this study is 398 evaluable patients (a difference of -1.5, an expected standard deviation of 10, with a non-inferiority limit of -4 (number of letters) and a nominal power of 0.8). If a 10% dropout rate is considered, the total number of eyes in the study should be 442.

Endpoints:

Primary endpoint:

- Visual acuity will be assessed with a standardized ETDRS best-corrected visual acuity test. To determine the efficacy of the treatments, the mean change in the best-corrected visual acuity between the baseline value and the value at one year of treatment will be used.

Secondary efficacy endpoints:

- The 4-month best-corrected visual acuity assessment will use the average values of the best-corrected visual acuity change between baseline and the value at visit 4 (after 4 months of treatment).
- The evaluation of the area under the curve of the change in the best corrected visual acuity will be carried out taking into account all the values obtained by each patient during all the visits.
- The evaluation of central macular thickness will be performed by optical coherence tomography. Efficacy will be determined as the mean change between the baseline value and the value at one year of treatment.
- The evaluation of the central volume of the retina will be performed by optical coherence tomography. Efficacy will be determined as the mean change in volume value between the baseline measurement and the measurement at one year of treatment.
- The assessment of the proportion of patients who responded to treatment will be carried out by determining the proportion of patients with improvement of more than 15 lines, more than 10 lines, or loss of more than 10 lines after one year of treatment.
- The assessment of the number of treatments will be quantified as the average number of injections received during the study.

- The assessment of the number of patients who required photocoagulation treatment will be quantified as a proportion of patients per treatment group.

Demographic and exploratory endpoints:

- The effect of the baseline Hb1Ac (glycosylated hemoglobin) level on treatment efficacy will be evaluated by patient stratification. This stratification will be made as follows with respect to the basal value:
 - Controlled: HbA1c levels $\leq 7.0\%$
 - Uncontrolled: HbA1c levels $> 7.0\%$
- The effect of the baseline level of best-corrected visual acuity on treatment efficacy will be further evaluated by patient stratification. This stratification will be done as follows:
 - Patients at recruitment with a best-corrected visual acuity >69 ETDRS letters.
 - Patients at recruitment with a best-corrected visual acuity ≤ 69 ETDRS letters.

Safety Rating:

The safety assessment will be carried out by analyzing the frequency and severity of adverse events.

Statistical analysis plan:

The statistical analysis will be presented to give an overview of the patients under study and an overview of the efficacy and safety of the results. The data provided by the sites will be summarized for this purpose. For qualitative variables, $p \times q$, absolute and relative frequency tables will be constructed. All percentages will be presented with a decimal. The quantitative variables will be summarized with number of patients participating (n), mean, standard deviation (SD), median, maximum and minimum. Changes in best-corrected visual acuity will be expressed as continuous variables. In the event that normal distributions are not presented, appropriate analyses will be used for this purpose.

For the primary variable, the Student's t-test statistic will be estimated for the mean difference once the values have been adjusted with respect to their baseline value within each individual. In the event that assumptions regarding normality and homogeneity of variances are not met, the non-parametric Mann-Whitney U test will be presented.

That same difference within groups will be made for each of the values observed over time and estimated least squares (LSM) averages will be compared between groups over time through an Analysis of Variance (ANOVA) of repeated measurements adjusted for the baseline value of each patient. In case of heterogeneity of variances, the Welch test will be presented.

A second analysis will be performed and the confounding variables will be included in an Analysis of Covariance (ANCOVA), to adjust for any differences that would have occurred in the random assignment procedure as long as they have been significant individually. The confounding variables determined in the protocol are baseline HbA1c, waist circumference, body mass index (BMI), concomitant medication, time of evolution of diabetes and associated comorbidities. Confidence intervals for the difference between treatments of the difference within treatments will be estimated to measure the magnitude of the effect.

According to the limits defined during the sample calculation, -4 will be considered as the non-inferiority limit. If the lower limit of the confidence interval of the difference between the control treatment and the experimental treatment is less than -4, the experimental treatment shall be considered as non-inferior.

For the analysis of secondary variables, the same primary analysis will be carried out as long as the variables are continuous and have the necessary measurements to do so. In the case of discrete variables, the analysis will compare between the groups the proportions of patients who present improvement or loss of visual acuity best corrected by means of Cochran-Mantel-Haenszel (CMH) tests. 95% confidence intervals for the difference will be presented in the analysis to observe the variation in the magnitude for the difference.

The primary efficacy analysis will be performed for the baseline control groups and for the variable of improvement or loss of the best-corrected visual acuity stratified into different types of response presented in the endpoints.

The assessment of adverse events will be monitored throughout the study, by direct observation at each visit.

All adverse events (according to the protocol) will be recorded and not only those in which the investigator suspects a causal relationship with the treatments.

Incidence rates for adverse events will be summarized by number of patients with at least one event per System Organ Classification (SOC)/Preferred Term in MedDRA version 24.0 or higher. Similar tables will also be presented involving severity (seriousness) and intensity. According to their causality or attributability to any of the treatments under investigation:

- Serious adverse events
- Non-serious adverse events
- Treatment-related adverse events
- Serious and treatment-related adverse events
- Discontinuations due to an adverse event

The statistical analysis will be carried out by means of specialized statistical software (available version of the SPSS package or R software). The data obtained from the electronic Case Report Form (eCRF) will be captured in an Excel sheet (Microsoft® Office). The analysis of data will be performed blinded.

Figure 1 General outline of the protocol

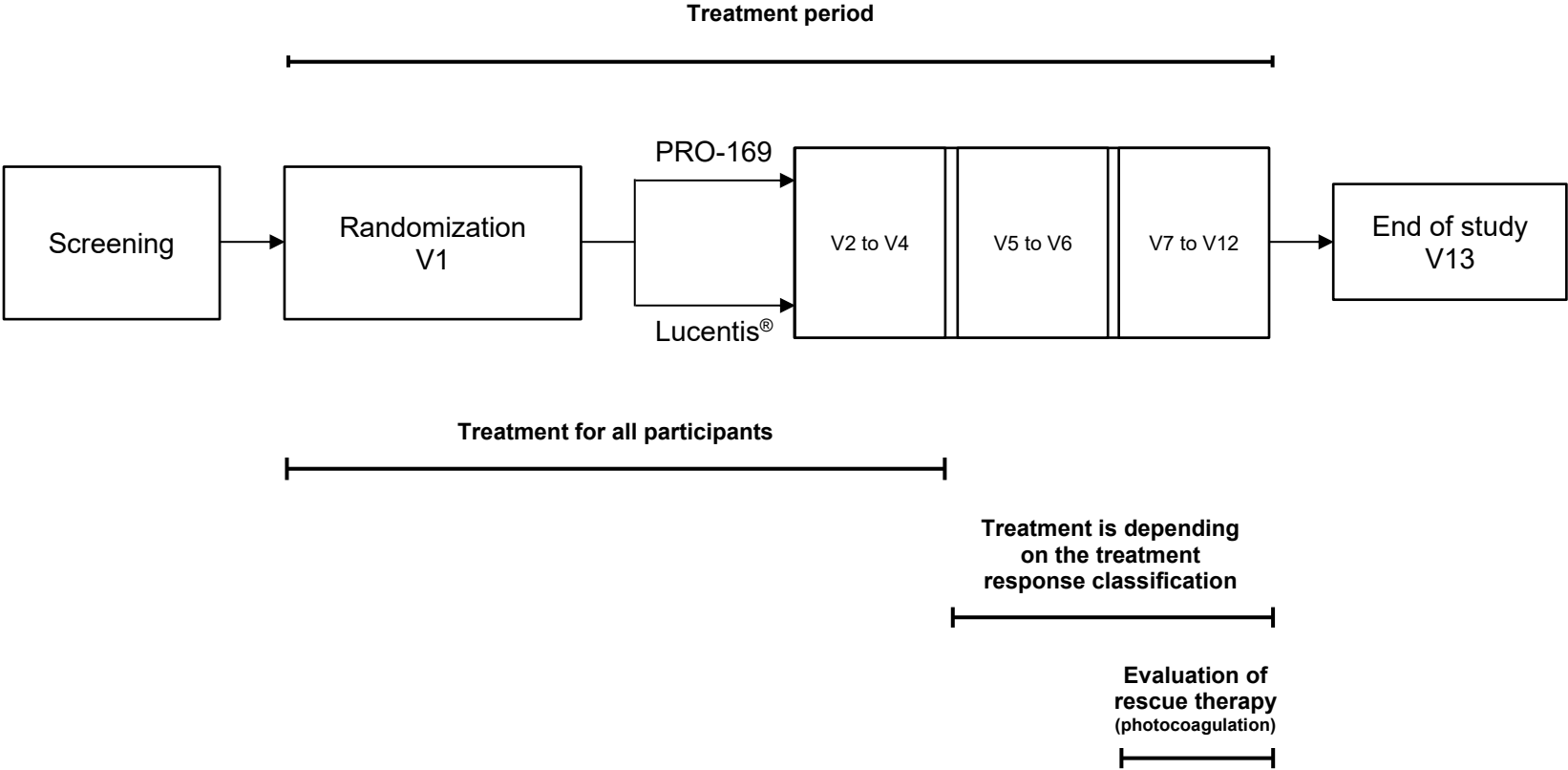


Table 3 Study activities

	Screening visit	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13
Day	-8 to -1	Randomization 1	30±3	60±3	90±3	120±3	150±3	180±3	210±3	240±3	270±3	300±3	330±3	Final visit/ Early withdrawal 360±3
Informed consent	✓													
Medical history	✓													**
Vital Signs	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
% HbA1c	✓													✓
Glomerular filtration rate (eGFR)	✓													
Concomitant medication	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Adverse event registration	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pregnancy test	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Best-corrected visual acuity	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Biomicroscopy	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Indirect ophthalmoscopy	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ocular tonometry	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Macular optical coherence tomography	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Angiography with fluorescein staining	✓							✓						✓
Randomization		✓												
Intravitreal injection		✓	✓	✓	✓	✓*	✓*	✓*	✓*	✓*	✓*	✓*	✓*	
Photocoagulation								✓*	✓*	✓*	✓*	✓*	✓*	

* According to injection assessment algorithms and rescue therapy assessment algorithms (See Annex 1)

** Waist circumference, weight and height measurements will be taken, and a BMI calculation will be done.

1) Introduction and background

1.1 Problem definition

Diabetic macular edema (DME) is the leading cause of vision loss in diabetic patients [7]. DME consists of the accumulation of both intracellular and extracellular fluid at the level of the retina. This accumulation interferes with the functions of the retina, causing visual deficiencies. [7, 8]

Although DME can occur in both types of diabetes (type 1 and type 2), its character as a public health problem is a consequence of the fact that type 2 diabetes is a serious public health problem. Two studies published in consecutive years in 2017 [9] and 2018 [10] define the diabetes prevalence estimates for 2015 and 2017 at 415 million (uncertainty range: 340–536 million) equivalent to 8.8% (7.2–11.4%) of the global population between 20–99 years and 451 million (uncertainty range: 367.5–585.5 million) equivalent to 8.4% (7.0–11.2) of the global population aged 18–99 years, respectively. Predictions for 2040 and 2045 are 642 million and 693 million adults affected by the disease, respectively.

The ophthalmological complications that occur in diabetic patients are multiple and include keratopathy, cataract, neovascular glaucoma, neuropathy, ischemic optic neuropathy, diabetic retinopathy and secondary macular edema [11, 12]. Diabetic retinopathy is the set of alterations in the retina due to the damage caused by uncontrolled hyperglycemia and affects about 4 million people worldwide [11]. Diabetic retinopathy can be subdivided into non-proliferative diabetic retinopathy and proliferative diabetic retinopathy, depending on the presence or absence of secondary ocular neovascularization. While retinal neovascularization leads to serious complications resulting in severe vision loss, the leading cause of loss of visual acuity in diabetic patients is DME. Historically, the first descriptions of diabetic retinopathy are presented at the end of the nineteenth century with descriptions by Jaeger [13], Mackenzie [14] and Noyes [15], among others. Mackenzie, on the other hand, is the first author to describe in detail the changes in the retina at a histopathological level: "The retina was thickened in all its layers... the thickening of the retina appears to be due to the result of chronic edema with connective tissue hypertrophy... microscopic cysts are observed... the thickness is two or three times greater than normal tissue".

Later, Nettleship [16] would publish histopathological observations that clearly demonstrated cystoid degeneration of the retina. Even so, the role of DME in the visual acuity of diabetic patients did not begin to be considered determinant of vision loss until the studies by Klein *et al.* [17] published in 1984, which demonstrated a clear relationship between the presence of vascular edema and the impairment of visual acuity in patients with diabetic retinopathy, mainly in adults diagnosed with diabetes after 30 years of age.

Current understanding of DME allows us to understand why it can occur at any stage of both non-proliferative and proliferative diabetic retinopathy. The presence of DME is directly dependent on hyperglycemic states [8]. Hyperglycemia conditions the abnormal glycosylation of proteins at the level of the retina, which leads to damage to pericytes and endothelial cells. Damage to vascular structures alters permeability, resulting in fluid extravasation and consequently macular edema. Once the edema is established, a vicious cycle of hypoxia and inflammation begins [8]. These two elements promote the synthesis of vascular endothelial growth factor (VEGF). VEGF values during DME are usually increased by up to 10 times their normal levels. This presence of high levels of VEGF has two effects within the pathogenesis of diabetic retinopathy and the formation of diabetic macular edema: first, it promotes the synthesis of new vessels and second, it increases the permeability of the blood-retinal barrier, generating edema.

The epidemiology of DME has not been studied as much as the epidemiology of diabetic retinopathy [18]. While the prevalence of diabetic retinopathy is estimated at 35.4% and 7.5% for non-proliferative and proliferative retinopathy respectively, the prevalence for DME is estimated worldwide to be between 1.4% and 12.8%, however, local prevalences are different depending on the incidence of local diabetes and the degree of control of the affected population. [19]

Morphological changes of the retina in DME can be analyzed by optical coherence tomography (OCT). OCT is a technology that uses the capabilities of light to interfere with itself and allows images to be captured through the use of light "echoes" [20]. OCT technology therefore allows pulses of light to be used to create high-resolution images of the retina. The first studies of the eye using OCT were published between 1990 and 1996 [20]. It is now possible to capture very high-resolution images at high speed using spectral domain OCT (SD-OCT) or swept source OCT

(SS-OCT). However, most ophthalmological measurements are carried out with SD-OCT.

The first classification of DME by OCT was proposed by Otani *et al.* in 1999 [21]. This classification defined three patterns of DME: Spongiform macular edema, consisting of the generalized accumulation of fluid in the retina; cystic macular edema, with focal accumulation of fluid in some layers of the retina; and serous retinal detachment, in which fluid is under the retina. The latter classification could occur with or without vitreous traction. According to Otani, the normal values of central macular thickness by OCT are close to 130µm, rising to 1,000µm in diabetic patients (average 470 µm). Panozzo *et al.* [22] later developed a classification for DME using OCT that takes into account central macular thickness, fluid volume, morphology, and the presence of epiretinal traction. The values for central macular thickness reported by them and based on more than 1200 eyes are illustrated in Table 4. [22]

Table 4 Human central macular thickness values and definition of edema. (Adapted from Panozzo *et al.*)

Anatomical location	Normal value	Limit value	Edema
Attachment Point	150 ± 20 µm	170 - 210 µm	≥ 210 µm
Central area	170 ± 20 µm	190 - 230 µm	≥ 230 µm
Areas peripheral to the fovea	230 ± 20 µm	250 - 290 µm	≥ 290 µm

Table 5 Normal values of central macular thickness using spectral domain OCT. (Adapted from Wong *et al.*)

Anatomical location	Men	Women
Fovea	174 ± 21 µm	168 ± 23 µm
Central area	203 ± 23 µm	189 ± 20 µm

As technology has evolved, retinal measurements have changed (see Table 5) [23], and in the same way the classification of DME has been modified, apparently the therapeutic response to treatment does not seem to be predictable according to the morphological characteristics of presentation of the DME studied with OCT. However, one study [24] suggests that the accumulation of subretinal fluid responds better to treatment compared to morphological changes in which internal retinal disorganization is evidenced. In general, central macular thickness correlates with visual acuity with a correlation coefficient of 0.52 [25] and a difference

in edema of 100 μm corresponds to an average visual acuity difference of 4.4 letters [25].

However, although it is possible to detect tomographic morphological changes in DME through the use of OCT, these do not always correlate directly with improvement in visual acuity. This is why studies evaluating therapies for the treatment of DME use change in visual acuity as the primary outcome variable, not changes in central macular thickness. The EDTRS study [26] defined the methodology by which visual acuity measurements should be performed in patients with DME. The use of logarithmic visual acuity charts placed 4 meters away from the patient is required. Each booklet has 5 letters per line in 14 lines corresponding to visual acuity from 20/10 to 20/200. In this system, a doubling of the viewing angle is generated every 15 letters or 3 lines. For patients who are not able to recognize letters at a distance of 4 meters, evaluation at a distance of one meter allows the evaluation of visual acuities corresponding to 20/250 to 20/800. Currently, the EDTRS booklets are standardized for ophthalmological studies.

The main strategy for the management of DME is prevention. Aggressive glycemic control with HbA1c values below 7% reduces the risk of retinopathy onset, retinopathy progression and decreased incidence of DME by up to 58% over a 4-year follow-up period in patients with type 1 diabetes [27]. In addition, when considering the impact of the increase in HbA1c on the incidence of mortality and the presence of systemic complications such as cardiovascular alterations in general, acute myocardial infarction, cerebral vascular event, etc., it increases approximately between 11 and 25% for each percentage point of this parameter, HbA1c, from a value of 7% [28, 29]. Glycemic control in patients with type 2 diabetes is not so clearly associated with the development of DME. Brown *et al.* [30], when working with a cohort of patients with adequate glycemic control ($N = 6,888$; $\text{HbA1c} = 7.84\% \pm 1.26$), did not find a direct relationship between glycemic control and the incidence of DME, but did identify the duration of diabetes, systemic hypertension, and the presence of proliferative retinopathy as risk factors for the development of DME. A more recent study [31] has confirmed that poor glycemic control associated with poor blood pressure control increases the risk of DME ($\text{RR} = 3.6$ (95% $\text{CI} = 1.58 - 8.22$)). The association with other risk factors has not been fully elucidated.

Analyzing electronic records, Martín-Merino *et al.* [32] identified high alcohol intake, the presence

of cataracts, HbA1c levels >7%, systolic pressure >160 mmHg, total cholesterol levels >5 mmol/L, LDL levels > 3 mmol/L, and microalbuminuria as risk factors for DME. Additionally, they found that the use of diuretics, smoking, being overweight or obese and elevated triglyceride levels appear to be associated with an increased incidence of DME, but the relevance of these findings is uncertain. Treatment with insulin, sulfonylureas, or glitazones was also found to be associated with an increased risk of DME. Regardless of the risk factors for DME and preventive treatment based on adequate glycemic control and appropriate antihypertensive management, once DME appears, therapeutic management is necessary.

The therapeutic management of DME must be tailored to each patient according to their particular characteristics, however, 4 available treatment routes can be identified. Historically, DME management was performed with laser therapy, but recent advances in the understanding of inflammation and angiogenic mediators have paved the way for two additional treatment modalities: corticosteroid implants and intravitreal injections of anti-VEGF agents. The fourth treatment option, surgery, is reserved for those cases in which there is clear evidence of DME with vitreoretinal traction.

The choice of treatment depends mainly on the presentation of the DME [33], while cases of DME that do not affect the macula can be treated with either laser therapy or pharmacological agents, cases of central DME with visual impairment should be treated with pharmacological agents. The first choice for most patients with DME is the use of anti-VEGF medications [33, 34, 35, 36] as long as macular or foveal center involvement is present [37]. Three agents that neutralize the function of VEGF-A with an approved indication for the treatment of DME are currently available on the market: pegaptanib, aflibercept, and ranibizumab. In addition, bevacizumab is used off-label.

Regarding the use of corticosteroid implants, these are considered second-line medications for patients who do not respond to treatment with anti-VEGF agents due to the higher incidence of cataracts and increases in eye pressure associated with their use. Laser therapy and surgical therapies are reserved for patients who do not respond to anti-VEGF medications and are not candidates for corticosteroids.

Treatment of DME with anti-VEGF agents has been widely documented. Although pegaptanib [38]

was the first drug authorized for the treatment of DME, it has fallen into disuse because it is only able to bind to the VEGF₁₆₅ form, so its efficacy is lower than that of other anti-VEGF agents. At the present, the three main anti-VEGF treatments for DME are: ranibizumab, aflibercept, and bevacizumab (off-label use since 2006) [39].

Aflibercept is a fusion protein; ranibizumab is a humanized Fab and bevacizumab is a humanized IgG1 antibody. At least four studies [1, 4, 5, 6] comparing treatment between ranibizumab and bevacizumab have shown that the efficacy of treatment is equivalent in terms of improvement in visual acuity, but with a lower response of bevacizumab in central macular thickness measurements assessed by optical coherence tomography. With the exception of one study [1], these studies were conducted with small sample sizes or reduced treatment times.

The Protocol T demonstrated that after one year of treatment with bevacizumab and ranibizumab they were equivalent in their efficacy for the treatment of DME [1] (improvement of 9.7 ± 10.1 and 11.2 ± 9.4 letters on average, respectively [$p = 0.12$]), although both were slightly inferior to aflibercept (13.3 ± 11.1 letters on average), particularly for the treatment of patients with visual acuity deficiencies below 69 letters (11.8 ± 12.0 , 14.2 ± 10.6 and 18.9 ± 11.5 for bevacizumab, ranibizumab and aflibercept, respectively). Despite these results, there is no ophthalmic presentation of bevacizumab on the market to date.

The need for an ophthalmic presentation of bevacizumab becomes even more critical when treatment costs are taken into consideration. The approximate cost of each aflibercept injection on the market is \$1,175 USD, while the cost of each ranibizumab injection is \$1,050 USD [40]. The high cost of these drugs promotes the search for lower-cost alternative therapeutic options, this is one of the reasons why the off-label use of bevacizumab is so prevalent in present ophthalmic practice.

Additionally, the cost analysis of the Protocol T revealed that the use of aflibercept and ranibizumab was not cost-efficient with respect to bevacizumab [41]. Similarly, a meta-analysis on the cost-effectiveness of various treatments for diabetic retinopathy (laser therapy, anti-VEGF, steroids, and surgery) also found the use of bevacizumab as the most cost-effective treatment for DME [42].

The present protocol aims to demonstrate the non-inferiority of PRO-169 (bevacizumab for ophthalmic use) compared to ranibizumab for the treatment of DME.

1.2 Background research

1.2.1 Research question

Although there are a large number of studies examining the pharmacology, toxicity, tolerability, and efficacy of bevacizumab for the treatment of DME, no phase III multicenter double-blind controlled clinical studies have been conducted that clearly demonstrate the non-inferiority of bevacizumab compared to ranibizumab for the treatment of DME. The present study will answer in a clear way whether bevacizumab is non-inferior to ranibizumab for the treatment of DME.

1.2.2 Justification for research

The Protocol T demonstrated that bevacizumab (off-label) is equivalent to ranibizumab for the treatment of DME, however, the study was not designed for this purpose, but rather to compare the efficacy and safety of three agents: aflibercept, ranibizumab and bevacizumab (off-label). Additionally, studies on the cost-effectiveness of DME treatments [41, 42] have shown that the only cost-effective treatment is bevacizumab.

It is therefore necessary to develop an ophthalmologic presentation of bevacizumab that demonstrates its non-inferiority compared to ranibizumab.

1.2.3 From the development phase of the product under study

Bevacizumab was originally developed as a biopharmaceutical by Genentech for the treatment of cancer and received approval for the treatment of colon cancer in February 2004. Current indications (as of April 2021) for treatment with Avastin® (intravenous bevacizumab) are: [43]

- Treatment of metastatic colorectal cancer in combination with standard fluoropyrimidine-based chemotherapy.
- Treatment of locally recurrent or metastatic breast cancer in combination with standard cytotoxic chemotherapy as first-line treatment.
- Treatment of patients with metastatic breast cancer after failure of first-line cytotoxic chemotherapy in combination with taxanes, capecitabine, and gemcitabine.

- First-line treatment of patients with non-small, non-squamous, advanced, inoperable, metastatic, or recurrent lung cancer in addition to platinum-based chemotherapy.
- Combination therapy with erlotinib as the first-line treatment of patients with non-small, non-squamous, advanced, inoperable, metastatic, or recurrent lung cancer with activating epidermal growth factor receptor mutations.
- Treatment in combination with interferon alfa-2a as first-line treatment for patients with advanced and/or metastatic renal cell cancer.
- Treatment in combination with radiation therapy and temozolomide for the treatment of adult patients with newly diagnosed glioblastoma.
- Treatment, alone or in combination with irinotecan, for patients with recurrent glioblastoma multiforme or disease progression.
- Treatment in combination with carboplatin and paclitaxel, as the first line in patients with epithelial ovarian, fallopian tube, and primary peritoneal cancer in clinical stage FIGO III and IV.
- Treatment in combination with carboplatin and gemcitabine for platinum-sensitive patients with recurrent primary epithelial ovarian, fallopian tube, and primary peritoneal cancer.
- Treatment in combination with paclitaxel, topotecan, or pegylated liposomal doxorubicin for platinum-resistant patients with recurrent primary epithelial ovarian, fallopian tube, or peritoneal cancer who received no more than two chemotherapy regimens previously.
- Treatment in combination with paclitaxel and cisplatin or paclitaxel and topotecan for persistent, recurrent, or metastatic carcinoma of the cervix.

The original development program for anti-VEGF antibodies included ophthalmological applications [44, 45]. Since bevacizumab is a complete IgG1 antibody, the possibility of inflammatory toxicity due to the presence of the Fc fraction was considered, so the company preferred to continue with the development of ranibizumab, a Fab that lacked the potential to arouse inflammatory reactions. Ranibizumab was approved in 2006 for the treatment of wet age-related macular degeneration and its indications were expanded later.

However, direct toxicity studies in animals showed that bevacizumab does not possess direct or complement-mediated cytotoxic properties [46, 47, 48, 49]. The use of systemically administered bevacizumab was subsequently described as a potential therapy for the treatment of exudative age-related macular degeneration [50]. This study demonstrated changes in visual acuity and a decrease in central macular thickness, nonetheless, the potential for systemic adverse events precluded the development of such a therapeutic approach. Afterwards, Rosenfeld *et al.* [51] reported that the use of intravitreal bevacizumab in a patient with neovascular macular degeneration showed improvement and no associated inflammatory events. Progressively, the use of bevacizumab as a low-cost alternative to ranibizumab became popular, encompassing other VEGF-dependent ophthalmological pathologies [39, 52, 53, 54, 55, 56].

Laboratorios Sophia S.A. de C.V. has developed an ophthalmic presentation of bevacizumab (PRO-169). The molecular identity of this biopharmaceutical has been demonstrated, so the scientific literature on safety and efficacy strongly supports this biopharmaceutical, the dose administered and the administration schedule (see 1.3.1). However, since it is a different presentation (prefilled glass syringes for administration of 50mL of a solution of 25mg/mL), a phase III clinical trial must be conducted to demonstrate the efficacy and safety of PRO-169.

1.3 Background on the product under study

1.3.1 Pharmaceutical development overview

Bevacizumab is a humanized IgG1 monoclonal antibody directed against VEGF-A amino acids 81 to 92, so it is able to bind to all 4 VEGF-A isoforms (121, 165, 189, and 206).

PRO-169 (bevacizumab for ophthalmic use) has been characterized as molecularly similar to the reference biopharmaceutical (Avastin®).

Table 6 Quali-quantitative formula of PRO-169

ACTIVE INGREDIENT	QUANTITY mg/mL	FUNCTION
Bevacizumab		Active ingredient
ADDITIVES		

1.3.1.1 Preclinical Development

Bevacizumab was developed by humanizing the murine antibody A4.6.1. [57]. *In vitro* studies have demonstrated blood compatibility and lack of cross-reactivity [58], as well as no direct or complement-mediated cytotoxicity capabilities [49]. *In vitro* biological activity studies show that bevacizumab is capable of neutralizing VEGF-A with a molar efficacy of between 2.6 - 10:1 (bevacizumab: VEGF-A). [49]

The ophthalmic efficacy and safety of bevacizumab has been evaluated in *in vitro* models [48, 59, 60, 61, 62, 63, 64, 65, 66, 67] [68] as well as in *in vivo* models [46, 47, 69, 70, 71, 72, 73, 74]. These studies reveal the following findings:

- Bevacizumab's biological activity is specific against VEGF-A and is not cross-reactive with any other molecule.
- Bevacizumab does not produce direct cytotoxicity mediated by Fc receptor activation or by complement fixation.
- Bevacizumab exerts its effects on the neutralization of VEGF-A due to its binding to cell receptors.
- Bevacizumab does not produce direct toxicity or decreased viability of cell lines derived from human retina or other species.
- Bevacizumab does not alter retinal nerve conduction patterns *in vitro* or *in vivo*.

- *In vitro* biological activity is equivalent between bevacizumab and ranibizumab for inhibition of VEGF-A-dependent proliferation.
- In studies with retinal pigment epithelium (RPE) porcine cells, treatment with bevacizumab appeared to alter the permeability of monolayers of these cells, but studies with fetal human RPE cells did not replicate the effect, the claudin-dependent junctions (*tight-junctions*) were kept intact.
- Bevacizumab is able to penetrate all layers of the retina after intravitreal administration in rabbit and monkey animal models.
- A single study showed that the number and area occupied by fenestrations in the capillary lamina of choroid of rats treated with bevacizumab was lower than that demonstrated by a human IgG1 control treatment. These changes were reversible in the day 14 post-injection. The significance of this finding is not clear at the moment.

In a preclinical study carried out at Laboratorios Sophia, S.A. de C.V., 48 eyes of healthy New Zealand albino rabbits were evaluated, and were divided into two groups: PRO-169 and Lucentis®, where they received between one and three injections of the respective investigational product. When evaluating the primary toxicity variable, alterations in electroretinograms, and comparing the results of the treated eyes to the control eyes of both groups, no statistically significant difference was observed in the V_{max} (maximum amplitude response) or in σ (saturation constant). Similarly, there was no statistically significant difference in these variables when comparing the treated eyes of both groups. For the safety assessment, the primary endpoint was anterior chamber cellularity, and no significant differences were observed between the groups studied. The number of cases that presented some degree of intraocular inflammation were two in the Lucentis® group and three in the PRO-169 group, each case reported as an AE. The inflammation was attributed to the intravitreal injection procedure in this specie. After analyzing all the variables studied, both clinical, laboratory, histopathological and electroretinographic, it was confirmed that the safety and toxicity profile of PRO-169 is not inferior, both clinically and statistically, to that of the reference drug Lucentis® in albino New Zealand rabbits [75].

1.3.1.1.1 *Preclinical pharmacokinetics*

The ophthalmic pharmacokinetics of bevacizumab have been explored following single injections in rabbits [76]. After injection of 1.25 mg of intravitreal bevacizumab, it was observed that the concentration of bevacizumab declined mono-exponentially with a half-life of 4.32 days. Vitreous humor concentrations greater than 10 µg/mL were detectable up to day 30. The maximum concentration in aqueous humor was 37.3 µg/mL on day 3 and the maximum concentration in serum was 3.3 µg/mL on day 8. The half-lives in aqueous humor and serum were 4.88 and 6.86 days respectively. Bevacizumab was detectable in the contralateral (non-injected) eye with a maximum vitreous peak of 11.17 ng/mL at 4 weeks. The area under the curve (AUC) for vitreous humor was 3300 µg/ml/day, 295 for aqueous and 54 for serum. With these values, the percentage of the intravitreal dose that passes into the serum was calculated, reported as 1.6%. Studies using radiolabeled bevacizumab [70] placed the serum translocation figure at 2 - 4%.

A bioavailability study was also carried out in Laboratorios Sophia, S.A. de C.V. in this same species, in which the concentration in the target tissue and vitreous humor did not show any statistically significant difference for the variables C_{max} , T_{max} and AUC_{0-t} between PRO-169 and Avastin®. A detectable concentration was still found in the ocular tissues 14 days after the injection, with the highest concentration found in the vitreous humor [77].

1.3.1.1.2 *Clinical Development*

It is important to emphasize that the studies listed below have all been conducted using off-label use of Avastin® (bevacizumab for intravenous use).

1.3.1.2.1 *Phase I (ophthalmic) trials*

Due to the difficulty of conducting them, there are few studies of single and multiple dose pharmacokinetics in humans.

In 2006, Beer *et al.* [78] reported the values of free bevacizumab and VEGF-coupled bevacizumab in two patients treated with intravitreal injections who suffered complications requiring the removal of vitreous humor sometime after injection. The first patient was at 4 weeks post-injection and the second patient was 48 hours after simultaneous administration of bevacizumab with triamcinolone. The levels of bevacizumab in the first patient were 0.16% of the original dose

administered (1.25 mg). In the second patient, 53% of the administered load (1.25 mg) was detected 48 hours after injection. The levels of free VEGF detectable in both cases were below the limit of detection (41 pg/mL), suggesting adequate inhibition of VEGF for up to 4 weeks at the level of the vitreous humor. Based on these data, an intraocular half-life of between 2 and 3 days was calculated.

In 2008, Krohne *et al.* [79] conducted a study of 30 patients who were treated with intravitreal bevacizumab and underwent cataract removal surgery in the subsequent days (1 - 53), where a sample was taken of aqueous humor. After an injection of 1.5 mg of bevacizumab, the C_{max} was obtained one day after the injection, which declined with an apparent half-life of 9.82 days in aqueous humor.

In 2011, Meyer *et al.* [80] evaluated the pharmacokinetics of bevacizumab using 1.5 mg (n=13) or 3 mg (n=16) in a single dose. The C_{max} observed in both cases were given on day 1 post-injection (17.5 µg/mL and 27.9 µg/mL for the dose of 1.5 and 3 mg respectively). The half-lives obtained for both groups were 8.21 and 11.17 days, respectively, corresponding to a mono-exponential decrease.

In 2012, Talty *et al.* [81] reported intraocular values of bevacizumab 24 hours after injection into the eye cavity of a normal eye that needed to be removed due to a rapidly progressing intraorbital tumor. Using a loading dose of 2.5 mg, they reported that they were able to recover 57.2% of the administered dose at 24 hours. Bevacizumab was detectable in all ocular tissues examined.

In 2013, Moisseiev *et al.* [82] examined the pharmacokinetics of bevacizumab following topical or intravitreal administration. No bevacizumab was found inside any eye treated with topical bevacizumab. Patients who received intravitreal bevacizumab at a dose of 1.25 mg had a mean half-life of 4.9 days. In patients from whom a serum sample was obtained from, the systemic half-life of intravitreal bevacizumab was 11.3 days.

In 2017, Avery *et al.* [83] analyzed the systemic pharmacokinetics of the three main anti-VEGF agents (aflibercept, ranibizumab, and bevacizumab). 151 patients were randomized to receive 3 monthly injections of 2.0 mg aflibercept, 1.25 mg bevacizumab, or 0.5/0.3 mg ranibizumab. Systemic pharmacokinetic values were calculated for all three agents. Peak concentrations of the

biopharmaceuticals were given at day 1, 7 and 1 for aflibercept, bevacizumab, and ranibizumab, respectively. After the administration of 3 doses, the maximum concentrations of the biopharmaceuticals revealed increased systemic exposure to bevacizumab (AUC_{0-28} 14.26 nM*h AUC_{66-88} 20.39 nM*h), followed by aflibercept (AUC_{0-28} 3.41 nM*h AUC_{66-88} 4.35 nMh*) and finally ranibizumab (AUC_{0-28} 0.24 nM*h AUC_{66-88} 0.23 nMh*). This is explained by a slight bioaccumulation effect in the first two drugs mentioned. The systemic half-lives obtained were 11.4, 18.7 and 5.8 days respectively for aflibercept, bevacizumab and ranibizumab. When circulating levels of VEGF were examined, they were most markedly affected by aflibercept (below the limit of detection from 3 hours post-injection thus continuing for 1, 3, and 7 days in each of the administrations), followed by bevacizumab (significant decreases but above the level of detection during the first administration and below the limit of detection for days 1, 3, 5 and 7 of the 3rd administration in patients with wet age-related macular degeneration, but not for patients with DME or central retinal vein occlusion) and ranibizumab (no significant changes were detected at any of the points tested). The clinical significance of these findings is unclear at this time. Although the incidence of gastrointestinal systemic adverse events appears to be higher in patients treated with bevacizumab compared to ranibizumab, it is not known whether this incidence is dependent on circulating levels of VEGF.

1.3.1.3 Phase II (ophthalmic) trials

Only prospective clinical trials conducted with bevacizumab for the treatment of DME are listed below. Retrospective studies, case reports, and prospective studies for other indications will not be mentioned.

In 2007, the Diabetic Retinopathy Clinical Research Network [39] (DRCR.net) recruited 121 patients (109 analyzed) with DME and a best-corrected visual acuity from 20/32 to 20/320 for a randomized Phase II study to analyze the short-term effects of 5 possible treatments: (A) photocoagulation at recruitment (n=19), (B) intravitreal injection of 1.25 mg bevacizumab at recruitment and at 6 weeks (n=22) (C) intravitreal injection of 2.5 mg of bevacizumab at recruitment and at 6 weeks (n=24) (D) intravitreal injection of 1.25 mg bevacizumab at recruitment and placebo injection at 6 weeks (n=22) (E) intravitreal injection of 1.25 mg bevacizumab at

recruitment and photocoagulation at 3 weeks (n=22). Best-corrected visual acuity and central macular thickness were assessed by OCT at 3, 6, 9, 12, 18, and 24 weeks after recruitment. Compared to group A, groups B and C had greater reduction in central macular thickness, and an additional line of visual acuity for up to 12 weeks. The significant limit of reduction in central macular thickness at 3 weeks (>11%) was reached by 43% of patients treated with bevacizumab and by 28% of patients treated with laser. No significant differences in therapeutic responses were observed between the 1.25 and 2.5 mg doses of bevacizumab. Important adverse events reported in the population treated with at least one dose of bevacizumab included: transient increase in intraocular pressure, two myocardial infarctions in patients with a history of coronary bypass, and elevated blood pressure in three patients.

In 2007, Ahmadi *et al.* [84] recruited 115 patients with treatment-refractory DME to evaluate three treatment options: (A) 3 injections of 1.25 mg intravitreal bevacizumab every 6 weeks (n=41) (B) a combined injection of 1.25 mg bevacizumab plus 2 mg triamcinolone followed by 2 injections of bevacizumab every 6 weeks (n=37) or (C) placebo injections (n=37). Decreased central macular thickness, changes in best-corrected visual acuity, and incidence of potential adverse events were assessed. At 24 weeks, mean central macular thickness decreased by $95.7 \pm 172.5 \mu\text{m}$ and $92.1 \pm 125.3 \mu\text{m}$ in groups A and B respectively, compared to group C which presented an increase of $34.9 \mu\text{m} \pm 63.9 \mu\text{m}$. Likewise, visual acuity (LogMAR) improved 0.18 ± 0.26 and 0.21 ± 0.19 in groups A and B compared to group C: 0.03 ± 0.24 . Adverse events showed increased intraocular pressure in 3 eyes (in patients from group B only), and anterior chamber reactions in 19.5% and 18.9% of patients in groups A and B that resolved spontaneously in less than one week. One patient developed a vitreous hemorrhage and had to stop treatment.

In 2007, Soheilian *et al.* [85] recruited 103 eyes from 97 patients to assess the efficacy of three treatments: (A) a single injection of 1.25 mg bevacizumab (n=37), (B) a single injection of 1.25 mg bevacizumab plus 2 mg triamcinolone (n=33), or (C) photocoagulation (n=33). Changes in visual acuity were assessed at 12 weeks. The changes in visual acuity were: LogMAR improvement of 0.22 ± 0.23 , 0.13 ± 0.31 and 0.08 ± 0.31 respectively for groups A, B and C at 12 weeks. The changes at 6 and 12 weeks were significant. The reduction seen in groups A and B in central macular

thickness only occurred at 6 weeks, but was not significant.

In 2012, Rajendram *et al.* [86] published the results of the 2-year BOLT study. 80 patients in a single center were randomized to receive either intravitreal bevacizumab or laser therapy. The primary outcome was the difference in best-corrected visual acuity ETDRS between arms. Patients randomized to laser treatment received treatment at recruitment and evaluations every 4 months with retreatment if they met the corresponding ETDRS criteria. Patients treated with bevacizumab were treated with 1.25 mg/mL at recruitment, at week 6 and 12, and were reviewed every 6 weeks. Subsequent administration of bevacizumab was determined according to central macular thickness stability, defined as 3 visits with measurements within 20 μ m of the thinnest thickness recorded for the patient. Of 80 randomized patients, 37 (of 42) patients were analyzed in the bevacizumab group at 2 years and 28 (of 38) patients in the laser group. The baseline ETDRS scores were 55.8 ± 9.7 and 55.4 ± 7.9 for the bevacizumab and laser groups respectively. At 24 months, the ETDRS scores were 64.4 ± 13.3 and 54.8 ± 2.6 respectively. An average difference of 6.5 letters in favor of bevacizumab. Additionally, significant differences were found in the proportion of patients who improved more than 15 or more than 10 letters. At 24 months, there were no significant differences in central macular thickness between the two treatments. Patients treated with bevacizumab received an average of 13 injections (range: 11 to 15) during the 24 months of treatment, most being during the first year (9 injections on average). There were 27 ocular adverse events in the bevacizumab group and 4 in the laser group. Out of the 27 events, 22 were related to the injection, 4 related to increased intraocular pressure, and 4 related to temporary reduction in vision. A serious ocular adverse event was observed in the bevacizumab group related to increased intraocular pressure. At the systemic level, 6 adverse events related to bevacizumab were observed: two myocardial infarctions, one coronary bypass, one episode of dyspnea and chest pain, one diabetic foot ulcer, and one cholecystectomy.

In 2014, Gillies *et al.* [87] reported the results of a two-arm randomized trial of treatment: (A) bevacizumab 1.25 mg every 4 weeks (n=42) or (B) dexamethasone implant every 16 weeks. The primary endpoint was the number of patients with changes greater than 10 letters of improvement in best-corrected visual acuity, with secondary assessments of mean change in visual

acuity, change in central macular thickness, frequency of injections, and adverse events. It was reported that 17 of 42 eyes in group A (40%) and 19 of 46 in group B (41%) achieved improvement of 10 letters or more at 6 months. Central macular thickness decreased on average by 122 μ m in group A and 187 μ m in group B. Group A received an average of 8.6 injections and group B 2.7 injections. However, there were more ocular adverse events with vision loss secondary to the appearance of cataracts (11%) in group B.

1.3.1.4 Phase III or IV (ophthalmic) trials

In 2012 Soheilian *et al.* [88] published the results of a randomized phase III trial to evaluate the efficacy of 3 treatments: (A) bevacizumab 1.25 mg, (B) bevacizumab 1.25 mg plus triamcinolone 2 mg, or (C) photocoagulation. Treatments were assessed every 12 weeks. The average percentage changes in best-corrected visual acuity reported for each treatment at 6, 12, 18 and 24 months were: 30.5, 29, 21.4 and 12.8 for group A, 11.2, 12.4, 14.8 and 9.5 for group B and -11.6, -7.6, -8 and -10.9 for group C. Excess increase in eye pressure were only observed in group B, while the incidence of other adverse events and serious adverse events were equivalent between groups. [88]

In 2015, the DRCR.net published the results of Protocol T [1] in which 660 adults were assigned to receive monthly doses of (A) aflibercept 2.0 mg (n=224), (B) bevacizumab 1.25 mg (n=218) or (C) ranibizumab 0.3 mg (n=218). The average change in visual acuity at one year of treatment was assessed. From baseline measurements, average visual acuity improved by 13.3 letters with aflibercept, 9.7 letters with bevacizumab, and 11.2 letters with ranibizumab. Improvement with aflibercept was considered superior to that of the other two drugs. A sub-analysis by groups according to baseline visual acuity showed that, for patients able to read between 78 and 69 letters, the average improvement was 8.0, 7.5 and 8.3 letters for groups A, B and C, respectively. However, when evaluating patients who were not able to read up to 69 letters (visual acuity less than 20/50), the average improvement was 18.9, 11.8 and 14.2, for groups A, B and C, respectively. The average number of treatments was equivalent for all groups (9, 10, 10). The need for photocoagulation was lower in the aflibercept group, but equivalent between the bevacizumab (56%) and ranibizumab (46%) groups. Regarding the effect on central macular thickness, the

average decreases were $169 \pm 138 \mu\text{m}$, $101 \pm 121 \mu\text{m}$, and $147 \pm 134 \mu\text{m}$ for groups A, B and C, respectively. No differences were detected in the number of significant adverse events, hospitalizations, deaths, or cardiovascular events between the three groups.

In 2018, the DRCR.net [89] published a 2-year follow-up of the Protocol T seeking to define the relationship between the speed of response to treatment (response at 12 weeks) and the outcome at 2 years. In the analysis of average gains in visual acuity at week 12, the aflibercept-treated group was 2.9 letters larger than bevacizumab or ranibizumab, with no difference between bevacizumab and ranibizumab. On average, patients who gained less than 5 letters at twelve weeks had lower gains at two years than those who gained 10 letters or more, aflibercept 6.2 vs. 18.1, bevacizumab 4.6 vs. 17.4, ranibizumab 9.0 vs. 17.3. However, there was a lot of variability at the individual level, and 42%, 31%, and 47% of patients with improvement of less than 5 letters at week 12 had improvement of more than 10 letters after two years of treatment with aflibercept, bevacizumab, or ranibizumab, respectively.

1.4 Rationale for the study

Studies reported in the medical literature over the past 20 years support the premise that the use of bevacizumab for the treatment of DME is efficacious, safe, and cost-effective. However, there is no ophthalmic presentation of bevacizumab that has been shown to be comparable, in efficacy and safety, with commercially available anti-VEGF drugs licensed for ophthalmological use.

It is for this reason that a multicenter, randomized, double-blind, active-parallel control phase III study will be conducted to demonstrate non-inferiority of PRO-169 (bevacizumab for ophthalmic use) compared to ranibizumab for the treatment of DME.

2) Objectives

2.1 Primary Objective

To evaluate the efficacy, expressed as improvement in best-corrected visual acuity, and non-inferiority of PRO-169 compared to Lucentis® (ranibizumab) one year after initiation of treatment, in patients with diabetic macular edema (DME).

2.2 Secondary efficacy objectives

- To assess the efficacy, expressed as the area under the curve of improvement in best-corrected visual acuity from initiation of treatment to completion of one year of treatment, of PRO-169 compared with Lucentis® for the treatment of diabetic macular edema
- To assess the efficacy, expressed as improvement in best-corrected visual acuity at 4 months of initiation of treatment, adjusted to the baseline value, of PRO-169 compared with Lucentis® for the treatment of diabetic macular edema.
- To establish the change in central macular thickness during the treatment of diabetic macular edema with PRO-169 compared to Lucentis® at one year of treatment.
- To assess the change in central retinal volume during the treatment of diabetic macular oedema with PRO-169 compared with Lucentis® at one year of treatment.
- To determine the proportion of patients with positive responses to treatment.
- To quantify the average number of injections administered per patient during the protocol.
- To identify the frequency of laser rescue therapy during the treatment of diabetic macular edema with PRO-169 compared to Lucentis®.

2.3 Secondary security objective:

- To determine the incidence and severity of adverse events during the treatment of diabetic macular edema with PRO-169 compared to Lucentis® with an emphasis on the following adverse events of interest:
 - Injection-related: endophthalmitis, traction retinal detachment, rhegmatogenous

retinal detachment, retinal tear, cataract, vitreous hemorrhage, increased intraocular pressure.

- Related to medication at the ophthalmic level: inflammation, new or progressive traction retinal detachment, progression of extramacular to macular retinal detachment.
- Drug-related at a systemic level.

3) Study design

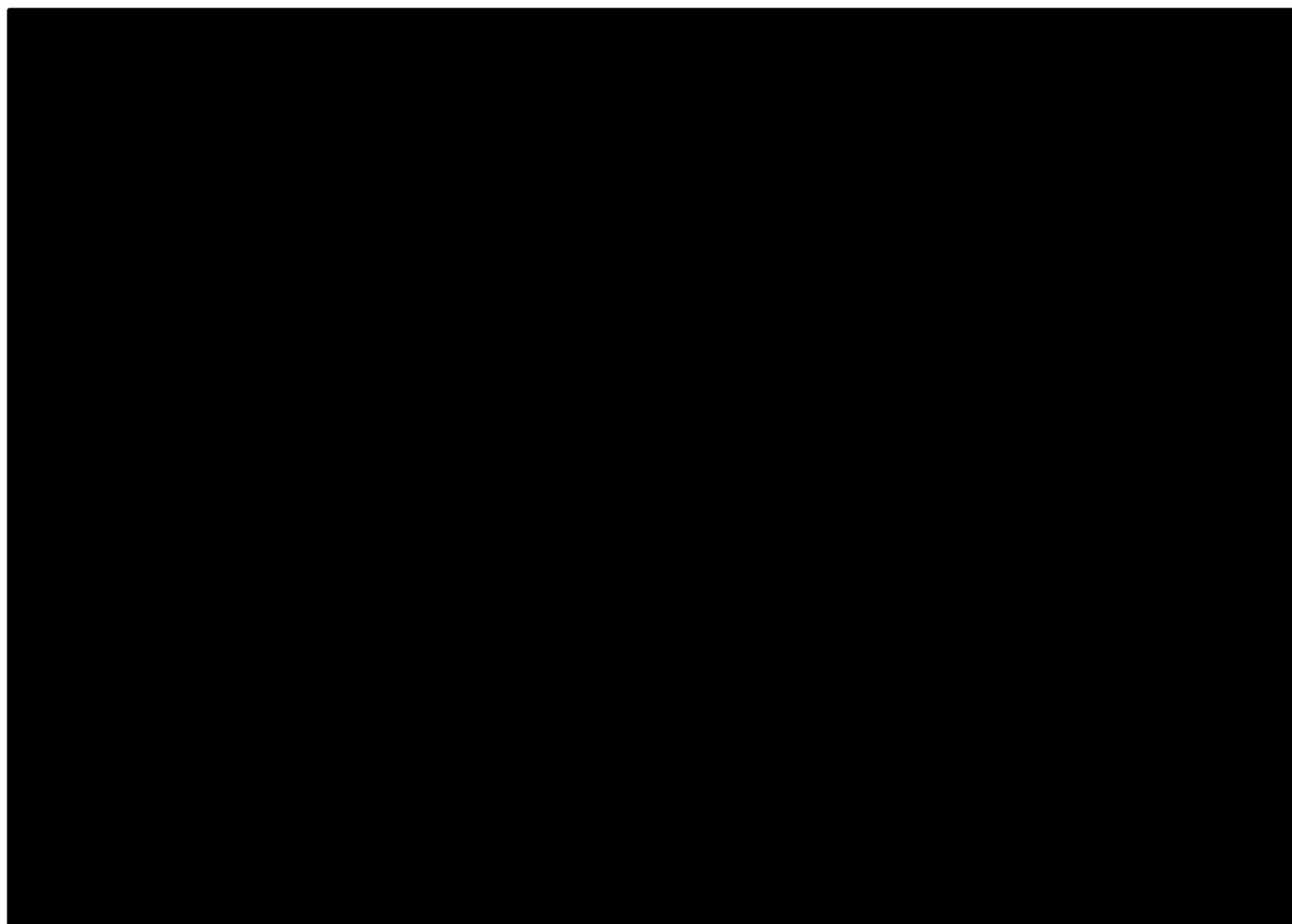
3.1 Design Overview

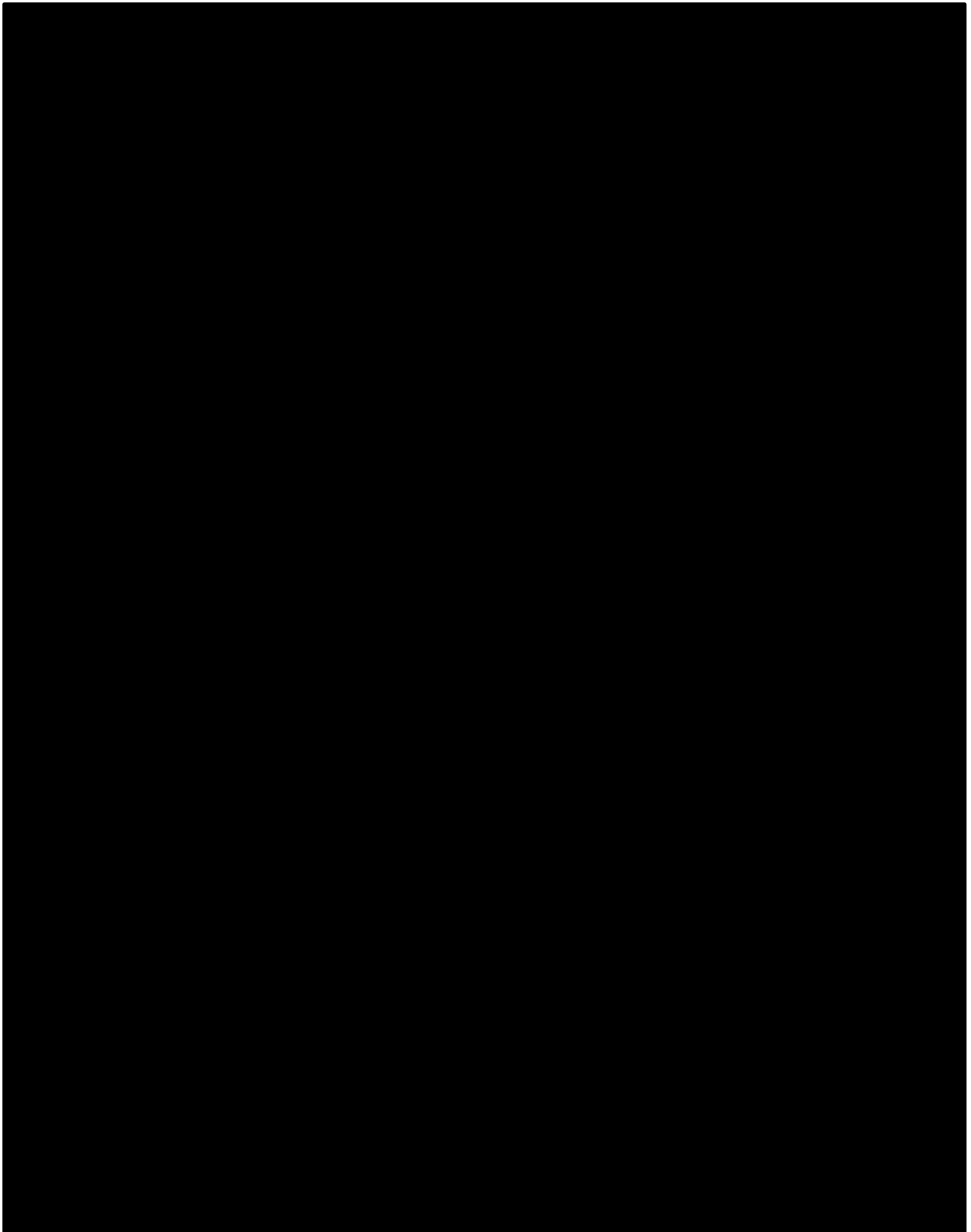
Phase III, multicenter, randomized, double-blind clinical study with parallel active control to demonstrate non-inferiority.

The basic criteria for defining the response to treatment during the study will be:

- Number of Letters read by ETDRS.
- Central Macular Thickness (CMT) by spectral domain OCT performed with the same instrument throughout the study.

Assessments are made at the beginning of EACH PATIENT'S VISIT. The values obtained define the classification of the patient's response to treatment. The response to treatment defines the next treatment.





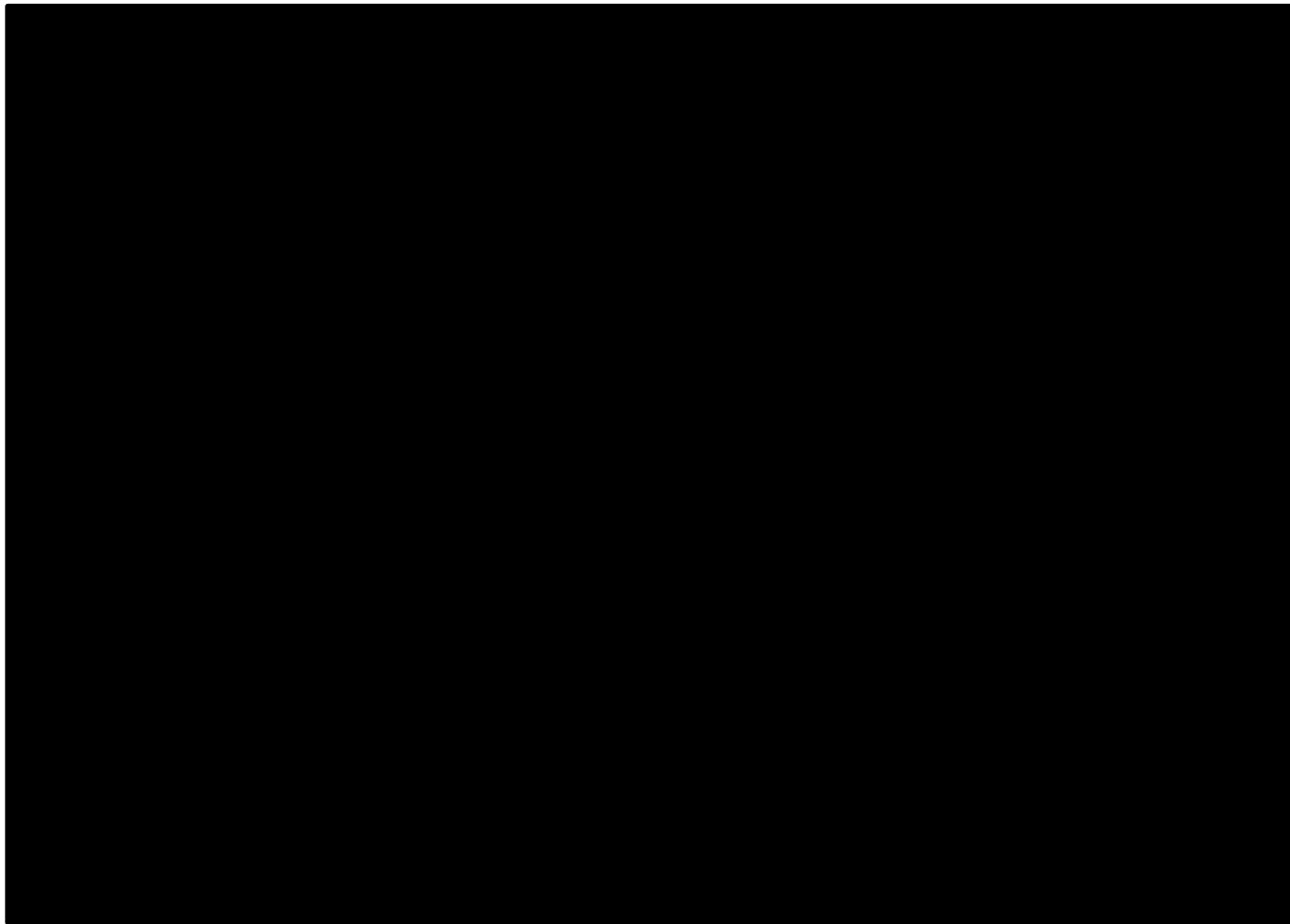
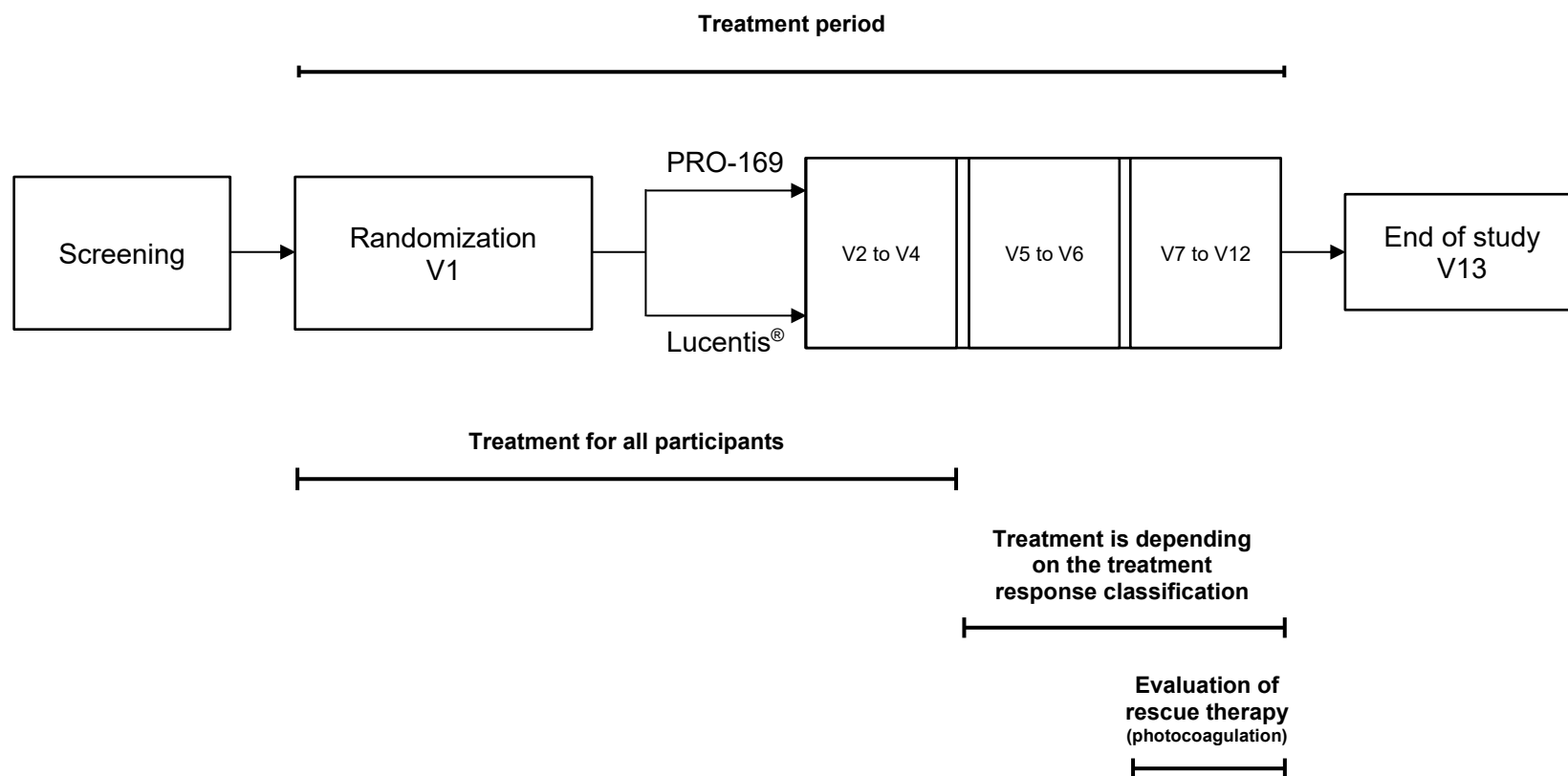


Figure 2 General outline of the protocol



3.2 Rationale for study design

The design of this study is based on:

- The biological basis of the disease and the mechanism of action of bevacizumab. The role of VEGF as the central mediator of the DME has been clearly established. Likewise, bevacizumab's neutralizing activity of VEGF-A is also clearly demonstrated.
- The ophthalmic pharmacokinetics of bevacizumab. The dose and frequency of administration of the drug are based on the best available data, both in animal and human models, to ensure an adequate therapeutic level throughout the study period and to keep the risks associated with the treatment to a minimum.
- The scientific methodology for obtaining valid data free of bias. A multicenter, randomized, double-blind, active-controlled study is the best way to ensure non-inferiority of the test treatment.
- The measurement of clinically relevant outcome variable. The best-corrected visual acuity is an outcome with a direct impact on the patient's health.
- Objective measurement of the variables in the study. Both the best-corrected visual acuity and the central macular thickness are objective measurements that have low potential for interference from the operator.
- The applicability of the results to daily clinical practice. The treatment protocol used during the study can be directly extrapolated to the treatment of patients with DME in common clinical practice.
- The experience accumulated in the world scientific literature regarding the use and usefulness of bevacizumab (currently off-label) for the treatment of ophthalmic diseases.

4) Study Population

4.1 Justification and description of the study population:

As a phase III study, the participant population consists of patients (volunteers) with diabetic macular edema who meet the following criteria:

4.2 Eligibility Criteria

4.2.1 Inclusion criteria:

1) Patient-level:

- a. Age > 18 years.
- b. Diagnosis of diabetes mellitus (type 1 or 2) evidenced by:
 - i. Use of insulin to treat diabetes, or
 - ii. Use of oral hypoglycemic agents for the treatment of diabetes, or
 - iii. Diagnosis of diabetes according to WHO or ADA criteria.
- c. Meets ophthalmic inclusion criteria in at least one eye.
- d. Is capable of giving informed consent.
- e. HbA1c < 9.5% at the screening visit.
- f. All men and women who are biologically capable of having children must agree and commit to using a barrier method of contraception for the entire duration of the study and for 3 months after the last intravitreal injection of any of the anti-angiogenic drugs.

2) Eye-level:

Only one eye can be randomized per participating patient. In the event that both eyes are eligible for anti-VEGF treatment, the non-randomized eye may be treated according to the treating physician's discretion. The nature of such treatment must be described in detail. In the event that both eyes are eligible in a patient, it will be at the discretion of the investigator which eye is chosen to participate in the study.

- a. Visual acuity best corrected according to the ETDRS scale < 78 (20/32 or worse) and

> 24 (20/320 or better) within 8 days prior to randomization.

- b. Diabetic macular edema with clinical evidence of central macular thickening.
- c. Diabetic macular edema present on spectral domain optical coherence tomography (central macular thickness criterion $\geq 300 \mu\text{m}$ for men and $\geq 290 \mu\text{m}$ for women) within 8 days prior to randomization. All measurements made on a patient should be taken with the same instrument during the conduct of the entire study.
- d. Meet the requirements that allow for an adequate examination of the fundus of the eye (transparency of media, adequate pupillary dilation).

4.2.2 Exclusion criteria:

1) Patient-level:

- a. Chronic kidney disease in renal failure ($<15 \text{ ml/min/1.73m}^2$) requiring dialysis or a transplant; according to the Clinical Practice Guideline for the Management of Diabetes in Chronic Kidney Disease 2020 of the Kidney Disease Improving Global Outcomes (*KDIGO*).
- b. Individuals with conditions that may compromise participation during the entire study (unstable concomitant diseases, possibility of change of residence, etc.).
- c. Individuals who require insulin treatment for glycemic control (starting within 4 months prior to the start of the study).
- d. Participation in another clinical study with investigational products (at least 90 days must have elapsed between the end of your participation in a previous trial and the randomization of the present study).
- e. Known allergy to the treatment.
- f. Poorly controlled blood pressure (average of 3 blood pressure readings in a seated position with $\geq 160 \text{ mmHg}$ systolic or $\geq 100 \text{ mmHg}$ diastolic) at the screening visit.
- g. Myocardial infarction or other cardiovascular event (cerebrovascular disease, transient ischemic attack, or hospitalization due to heart failure) during the 4 months prior to the start of the study, or patients with active myocardial ischemia.
- h. Prior systemic treatment with VEGF-related medications within 4 months prior to

the start of the study.

- i. Women of childbearing potential who are pregnant, nursing, or plan to become pregnant within the study period.
- j. Allergy to the anesthetic medications used during the injection procedure and/or photocoagulation.

2) Eye-level:

These exclusion criteria only apply to the eye under study. The out-of-study eye has no exclusion criteria, and will receive treatment according to the criteria of the treating physician.

- a. Non-diabetic macular edema.
- b. Ophthalmologic conditions that interfere with the assessment of visual improvement in the patient (e.g., foveal atrophy, pigmentary abnormalities, dense foveal exudates, etc.).
- c. Conditions in addition to diabetes that may compromise the evaluation of edema (e.g., venous occlusions, uveitis or other inflammatory diseases, neovascular glaucoma, etc.).
- d. Lens opacities that are superior in one or more of the following criteria according to the LOCS III (*Lens Opacities Classification System*) classification: nuclear component > NO3C3 (opalescence/color), cortical component > C2 and posterior subcapsular component > P1.
- e. Positive history of prior anti-VEGF treatment for diabetic macular edema or any treatment for diabetic macular edema within 4 months prior to the start of the study (corticosteroids, photocoagulation, etc.).
- f. Anticipated need for panretinal photocoagulation (e.g., for proliferative diabetic retinopathy or any other indication) during the study period, or history of panretinal photocoagulation during the 4 months prior to the study.
- g. History of eye surgery (cataract removal, any intraocular surgery, aphakia, etc.) within 4 months prior to the start of the study or planned within the time of the study.

- h. Ocular pressure greater than 21 mmHg measured by Goldmann tonometry at the screening visit.
- i. Presence of macular ischemia or significant perifoveal capillary loss (increase in foveal avascular zone greater than 350 μ m) demonstrated by retinal fluorescein angiography during the screening visit.
- j. Evidence of macular traction and hyaloid thickening on optical coherence tomography.
- k. History of YAG capsulotomy within 2 months prior to randomization.
- l. Evidence of external eye infections or significant ocular surface disease.
- m. Vitrectomy patients.

4.3 Elimination and Replacement Criteria

"Elimination" or "withdrawal" of a patient from the study is defined when an already included and randomized participant does not complete the activities scheduled in the research protocol by decision of the site's principal investigator.

A patient's study drug may be withdrawn at any time at the discretion of the investigator. A patient may discontinue their participation in the study without giving any reason at any time during the study. Every effort should be made to perform all required procedures as part of the early withdrawal visit, even if the patient withdraws consent.

In case of removal or withdrawal of the patient, the main reason for the suspension of their participation must be recorded according to the following categories:

- a. Loss to follow-up.
- b. Voluntary retirement. The patient (or the patient's legal representative) wishes to withdraw from the study, withdrawing their informed consent. The reason for the withdrawal, if obtained, must be recorded on the eCRF.
- c. Major deviation from the protocol. The post-randomization discovery that the patient did not meet the criteria for inclusion in the protocol or did not adhere to the requirements of the protocol, and the continuation of their participation poses an unacceptable risk to the patient's health.

- d. Termination of the study. The sponsor, ethics committee, or regulatory agency cancels the study.
- e. If, according to the researcher's criteria, the patient requires ophthalmological surgery in the eye under study.

No substitution of patients will be done in losses regarding adverse events or scrutiny/screening failures.

4.4 Procedure in case of loss of follow-up or withdrawal of informed consent during the execution of the study

Loss of follow-up is defined as: patients who, once enrolled in the study, do not complete the activities of the study without having withdrawn their informed consent. For the present study, a loss of up to 10% is contemplated, the management of which is detailed below:

- 1) In the event of a missed follow-up/treatment visit, the patient will be contacted by telephone and electronic means to schedule a new visit.
- 2) If it is not possible to arrange a visit, the presence of adverse events and the reason for leaving the study will be asked as minimum data.
- 3) Data from the patient who is withdrawn prior to completion of all study visits will be used to meet the safety and efficacy objectives for which data exist. No imputation of missing data will be made.
- 4) For patients who withdraw their consent after receiving at least one dose of the study medications, every effort should be made to perform the required procedures as part of the early withdrawal visit.

The safety data obtained during the last visit will be part of the study, as well as all data obtained up to the time of withdrawal of consent. No imputation of missing data will be made.

The sponsor, with prior authorization from the research ethics committees, may substitute patients who withdraw their informed consent form, those who have lost follow-up, or who are withdrawn before completing all study visits due to a major deviation from the protocol, if it is necessary to balance the study groups to meet the primary efficacy objective of the study (398 evaluable subjects).

4.5 Subgroups

No specific subgroups are established *a priori* for the study, however, exploratory analyses are planned according to the data obtained for the values of:

- The effect of the baseline level of best-corrected visual acuity on the efficacy of the treatment will be further evaluated by analyzing subgroups of patients with visual acuity greater than or equal to or less than 69 letters.
- The effect of baseline Hb1Ac on treatment efficacy will be assessed by stratification of patients with good control ($\leq 7.0\%$ HbA1c at recruitment) or moderate control ($> 7.0\%$ HbA1c at recruitment).

5) About the drug under study:

5.1 Description of the treatments in the study:

Test treatment: PRO-169 (bevacizumab for ophthalmic use) [REDACTED] Intravitreal injectable solution. Intravitreal injection of 1.25 mg of PRO-169 in a volume of 0.05mL each month according to the administration protocol (see Annex 1).

Reference treatment: Lucentis® (ranibizumab) 10 mg/mL. Intravitreal injectable solution. Intravitreal injection of 0.5 mg of Lucentis® in a volume of 0.05 mL each month according to the administration protocol (see Annex 1).

5.2 On the storage and handling of the medication/active control in the study centers:

The transport of the study drug will be carried out with continuous temperature monitoring, which must be properly recorded.

Reception will be exclusively carried out by the non-blind staff of the research team. You will need to check that the primary packaging (box) is in good condition. In case the packaging shows alterations or defects in its integrity that in their judgment could have damaged the content, it must be reported to the sponsor. If the package does not show significant defects, it must proceed to be opened.

The treatment must be kept refrigerated between 2 and 8°C, out of the reach of light and in its packaging until use.

Throughout the study, continuous monitoring and recording of the temperature of the product under investigation must be carried out.

Access to the investigational product should be restricted. The investigator at each site is responsible for the proper storage of the study drug.

The research center is obliged to record, in the designated format, the temperature. Registration must be done twice a day, every day of the week.

Once the test treatment/reference treatment has been administered, used syringes/vials should

be returned to storage and registered in the designated format.

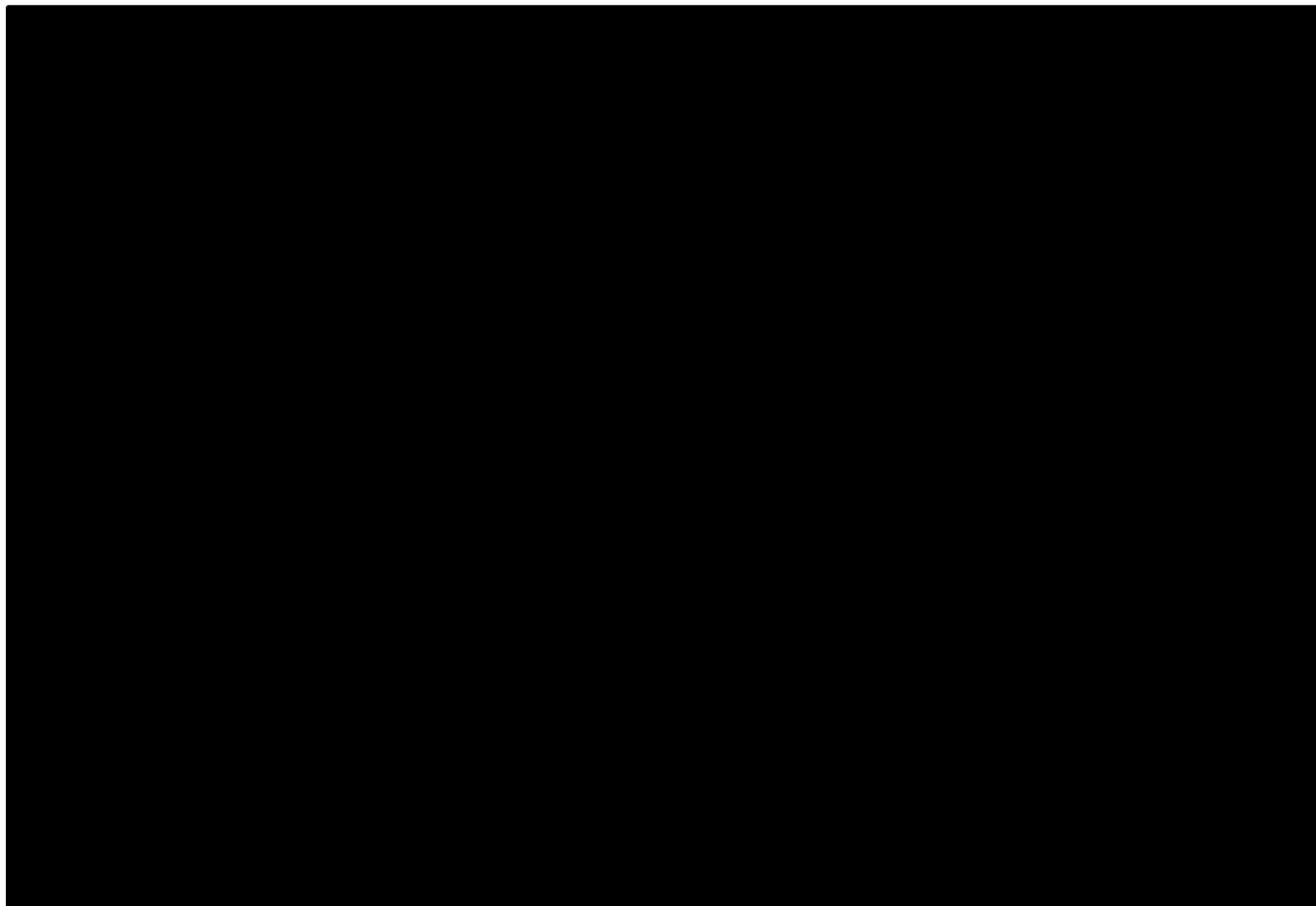
The return will be made by the research center when the sponsor so indicates. Prior to the return, the research center must carry out a recount of the assigned medication and the remaining medication, in order to create an inventory which serves for the final completion of the medication return form.

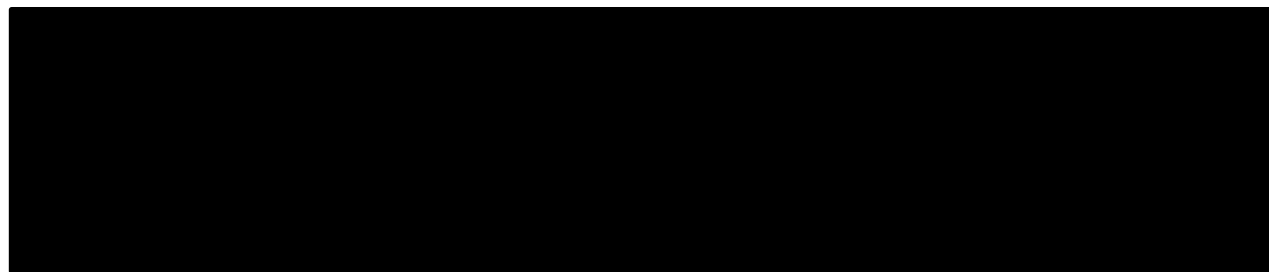
5.3 On concomitant treatments not approved during the study:

The use of other anti-VEGF agents in the eye under study is not permitted for the duration of the protocol.

The use of intravitreal steroids in the eye under study is not permitted for the duration of the protocol.

5.4 About the photocoagulation procedure:





5.5 On the justification for the study dose:

The dose of the study drug of 1.25 mg/mL with monthly administration has been defined according to:

- Retinal penetration and toxicity studies [46, 59, 60, 70, 71, 73]
- Human ophthalmic pharmacokinetic studies [80, 83]
- Phase II studies on comparative intravitreal doses of bevacizumab for DME [39]
- The demonstrated efficacy of monthly administration during Protocol T [1]

5.6 On the justification of the comparator (Lucentis® (ranibizumab)):

The active comparator for this study is Lucentis® (ranibizumab).

Ranibizumab is a VEGF-A-directed Fab that has undergone several rounds of affinity maturation.

Ranibizumab was approved by the FDA for the treatment of neovascular age-related macular degeneration in 2006 and subsequently in 2012 for the treatment of DME.

The efficacy of ranibizumab for the treatment of DME was demonstrated in two sham injection-controlled studies with 24-month observation periods that demonstrated an average difference in the number of letters of vision recovered of 8.5 (95% CI 5.4 - 11.5) and 9.6 (95% CI 6.1 - 13.0) compared to the placebo injections.

During the performance of Protocol T, ranibizumab and bevacizumab were shown to be equivalent for the treatment of patients with DME, although a small non-significant difference between ranibizumab and bevacizumab was observed for the treatment of patients with a baseline best-corrected visual acuity of less than 69 letters. [1]

For more information about Lucentis® please refer to the corresponding prescribing information.

5.7 Risks associated with the study procedures

5.7.1 Risks associated with the administration of intravitreal bevacizumab.

Bevacizumab (Avastin®) systemically (for the treatment of cancer) is associated with increased risks of wound closure failure, intestinal perforation, hemorrhage, stroke, apoplexy, myocardial infarction, hypertension, heart failure, and proteinuria. However, these risks do not appear to be reflected during intravitreal treatment.

The systemic safety of intravitreal bevacizumab has been shown to be equivalent to that of aflibercept and ranibizumab in a recent meta-analysis [90]. The rate of systemic adverse events of bevacizumab was determined to be 186 per 1000 (95% CI 146 - 238), the rate of thromboembolic events was determined to be 41 per 1000 (95% CI 15 - 117).

The ophthalmic safety of bevacizumab reported in Protocol T [1] was 33 events in 2055 injections in 218 individuals. The events of interest reported were: inflammation (2 events), retinal detachment or tear (1 event), vitreous hemorrhage (9 events), cataract (2 events), increase in eye pressure (19 events).

5.7.2 Risks associated with the administration of intravitreal ranibizumab

The systemic safety of intravitreal ranibizumab has been shown to be equivalent to that of aflibercept and ranibizumab in a recent meta-analysis [90]. The rate of systemic adverse events of ranibizumab was determined to be 194 per 1000 (95% CI 160 - 234), the rate of thromboembolic events was determined to be 48 per 1000 (95% CI 23 - 101). [90]

The ophthalmic safety of ranibizumab reported in Protocol T [1] was 33 events in 2011 injections in 218 individuals. The events of interest reported were: inflammation (2 events), retinal detachment or tear (1 event), vitreous hemorrhage (7 events), ocular hypertension (23 events).

5.7.3 Risks associated with intravitreal injections

The anesthesia used during the injection procedure can sometimes cause allergic reactions that can be serious.

The manipulation of the eyeball that occurs during treatment can be associated with eye discomfort, tearing, and eye sensations that last from one to three days.

The injection of additional volume into the eye is associated with an increase in intraocular

pressure, but this is usually transient. The risk of serious vision problems associated with this increase is less than 1%.

Other risks associated with the procedure are endophthalmitis, retinal detachment or vitreous hemorrhages. All of them occur with frequencies less than 1%, but they can be a cause of significant vision loss even when treated in time, either with antibiotics or surgery as indicated.

5.7.4 Risks associated with photocoagulation

Serious complications of photocoagulation are rare and occur at frequencies less than 1 per 1000. They include macular damage, intravitreal bleeding, increased intraocular pressure, optic nerve damage, traumatic cataract, holes in the retina, blindness, and eye loss.

Some additional risks are associated with the anesthetics and medications used during the procedure, such as allergic reactions or mechanical damage due to the volume injected. These risks are very low.

5.7.5 Risks associated with ophthalmic procedures

There are minimal risks associated with medications used for fundus study and angiographic studies. These risks are mainly allergic reactions.

Tests that use light to examine the fundus, including OCT, can be tiring and cause discomfort associated with flashes of light, but they do not cause damage to the structures of the eye.

6) Study methods and procedures

6.1 Training, communication and registration

Anyone who has direct participation in the activities of the study must be trained and their competence must be evaluated before the performance of any activity related to the protocol. The information previously stated must be recorded and the documents that constitute evidence of this training must be kept in the master file of the study. The competence of the staff who develop functions in the study, both at the central level and in the research centers, is the responsibility of the sponsor.

The sponsor must ensure that all clinical site personnel participating in the study are adequately trained on the study (research protocol, investigator's manual, amendments, etc.) and on the ICH's Good Clinical Practice, prior to the start of their participation in the study. Training must be recorded in writing and those records must be filed in the master file of the study.

This clinical study must be registered by the sponsor in public clinical trials registries in a timely manner in compliance with the laws, regulations, and guidelines applicable to each country.

6.2 Randomization and blinding

Patients will be randomized to a 1:1 ratio using a computer-based mapping system. After signing the informed consent, the patient will receive a patient number used to encode all their information during collection and completely anonymize it during the analysis.

Patients will be randomized to one of two study groups:

- a. Group treated with the PRO-169 test treatment
- b. Group treated with the reference control treatment Lucentis®

With the exception of the medication used during treatment, all other study procedures are identical for study participants.

The study is double-blind, so both the doctor in charge of evaluating the patient and the patient will be blinded to the treatment.

Since the study drugs contained in prefilled syringes (bevacizumab) and ampoules (Lucentis®) are clearly identifiable, the personnel in charge of administering the drug must be dedicated

exclusively to this task and will not be able to participate in the evaluation of patients. The personnel in charge of administering the drug are not blind to the treatment.

Any person in the Research team can carry out the following activities:

- Screening visit processes:
 - Share information about the study and discuss the study design with the subject
 - Recruiting Subjects
 - Obtain and document informed consent
 - Get your medical history
 - Record previous and concomitant medications
 - Check the inclusion and exclusion criteria
- Perform pregnancy tests
- Obtain and/or process blood samples
- Randomly assign subjects using the computer-based assignment system through the appropriate eCRF provider and receive notification of the randomization.
- Assign subsequent doses using the eCRF provider's system at follow-up visits

Tasks exclusive to non-blind personnel

- Receive and acknowledge receipt of investigational products and notify the applicable eCRF provider.
- Prepare and manage the investigational product
- Maintain optimal storage conditions for investigational products and notify the unblinded CRA if there are cold chain interruptions
- Perform product accounting under investigation and monitor supplies
- Document and maintain records of the investigational product

Non-blind staff may include:

- Investigator or sub-investigator (ophthalmologist)
- Pharmacist

Blind personnel may include:

- Blind investigator or sub-investigator (ophthalmologist with a **retina specialty required**)
- Coordinator
- Nurse
- Laboratory Staff

During the conduct of the study as detailed below, the investigator in charge of the assessment of the patients of the study will be in charge of determining if the patient should receive a subsequent injection of medication, if they should suspend or restart the treatment or if they are a candidate for photocoagulation. Once this decision has been recorded according to the classification derived from the values observed during the monthly visit, the patient will proceed to receive the

treatment, which will be recorded in the file only as an intravitreal injection administered, delayed injection or indication for photocoagulation, together with the temporal data and the record of adverse events associated with the procedure.

6.2.1 Un-blinding

The investigator should not break the study blind unless information about the study drug is necessary for the patient's medical treatment. The sponsor must be notified before breaking the blind unless there is a medical emergency requiring breaking the blind. In such a case, the investigator (or their delegate) at the site must contact the sponsor immediately and follow the instructions given.

In the event that the blind breaks for one of the patients, the medication must be immediately discontinued and the patient will be withdrawn. The patient will need to undergo the procedures of the final visit.

The analysis of the data will be carried out in a non-blind manner only when the database has been closed and defined as suitable for analysis, at which point none of the values can be altered or excluded from the analysis.

6.3 Study visit and activities program

The study comprises 14 visits, below is a table with the study activities in relation to each visit:

Table 7 Study activities

	Screening visit	Visit 1 Randomization	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13 Final/Completion Early
Day	-8 to -1	1	30±3	60±3	90±3	120±3	150±3	180±3	210±3	240±3	270±3	300±3	330±3	360±3
Informed consent	✓													
Medical history	✓													**
Vital Signs	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
% HbA1c	✓													✓
Glomerular filtration rate (eGFR)	✓													
Concomitant medication	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Adverse event registration	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pregnancy test	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Best-corrected visual acuity	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Biomicroscopy	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Indirect ophthalmoscopy	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ocular tonometry	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Macular optical coherence tomography	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Angiography with fluorescein staining	✓							✓						✓
Randomization		✓												
Intravitreal injection		✓	✓	✓	✓	✓*	✓*	✓*	✓*	✓*	✓*	✓*	✓*	
Photocoagulation								✓*	✓*	✓*	✓*	✓*	✓*	

* According to injection assessment algorithms and rescue therapy assessment algorithms (See Annex 1)

** Waist circumference, weight and height measurements will be taken, and a BMI calculation will be done.

6.3.1 Description of activities per visit

1) *Screening*

The screening visit will be conducted as follows:

Obtaining and documenting Informed Consent.

Once participation in the study has been confirmed, the patient will receive a study identification number. The recording of the patient's identification data and its relationship to the identification number should be maintained in the investigator's records. The investigator must collect contact information from the patients (telephone number and address) to ensure follow-up in case of loss. This data will be available only to the investigator and is not part of the data collected on the eCRF. This distinction should be clearly explained to the patient during the visit. From the assignment of the identification number, all captures made in the eCRF will be identified with this number. The data captured and available for analysis will therefore be anonymized and will not contain elements that allow the identification of the patient's identity. The identification number implies a randomization to one of the treatment groups, but this information will only be available to the medical personnel designated for the administration of the study treatments.

Once informed consent has been obtained, the results of the following tests should be performed or recorded:

- 1) Medical history: Complete medical history, including a directed interrogation about cardiovascular history, to ensure compliance with the criteria for inclusion in the study, as well as the absence of exclusion criteria.
- 2) Vital signs assessment.
- 3) Laboratory tests: % HbA1c and eGFR. The sample may be taken one day after the visit, only if both respect the established time window (day -8 to -1) of the screening visit.
- 4) Record of previous and concomitant medications including dose and frequency of administration.
- 5) Adverse event Registry.
- 6) Pregnancy test.
- 7) Best-corrected visual acuity test under ETDRS standards.

- 8) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 9) Indirect ophthalmoscopy for a detailed examination of the retina.
- 10) Ocular tonometry (Goldmann) to define the basal ocular tone.
- 11) Optical coherence tomography with spectral domain equipment to evaluate the condition of the retina. This study may be carried out one day after the visit, only if both respect the time window (days -8 to -1) of the screening visit.
- 12) Fluorescein angiography for classification of diabetic retinopathy and exclusion of patients with macular ischemia or significant perifoveal vascular loss. This study may be taken one day after the visit, only if both respect the established time window (day -8 to -1) of the screening visit.

Visit 1 must be completed within 8 days.

2) Visit 1 (Randomization).

Visit 1 (randomization) will take place on day 1.

- 1) Vital signs assessment.
- 2) Randomization.
- 3) Ocular tonometry.
- 4) Adverse event registry.
- 5) Registry of concomitant medications.
- 6) Application of the injection.
- 7) Observation period of 30 min after injection.
- 8) Indicate instructions on ophthalmic and systemic warning signs, and means of contact.

3) Visit 2

Visit 2 will be conducted 30 ± 3 days after randomization. During this visit, the following activities will be carried out:

- 1) Vital signs assessment.
- 2) Registration of concomitant medications.
- 3) Adverse event registry.

- 4) Pregnancy test.
- 5) Best-corrected visual acuity test under ETDRS standards.
- 6) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 7) Indirect ophthalmoscopy for detailed examination of the retina.
- 8) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.
- 9) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 27 to 33) of said visit.
- 10) Application of the injection.
- 11) Observation period of 30 min after injection.
- 12) Indicate instructions on ophthalmic and systemic warning signs, and means of contact.

4) Visit 3

Visit 3 will be conducted 60 ± 3 days after randomization. During this visit, the following activities will be carried out:

- 1) Vital Signs Assessment.
- 2) Registration of concomitant medications.
- 3) Adverse Event Registry.
- 4) Pregnancy test.
- 5) Best-corrected visual acuity test under ETDRS standards.
- 6) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 7) Indirect ophthalmoscopy for detailed examination of the retina.
- 8) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.
- 9) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 57 to 63) of said visit.

- 10) Application of the injection.
- 11) Observation period of 30 min after injection.
- 12) Indicate instructions on ophthalmic and systemic warning signs, and means of contact.

5) Visit 4

Visit 4 will be conducted 90 ± 3 days post-randomization. During this visit, the following activities will be carried out:

- 1) Vital Signs Assessment.
- 2) Registration of concomitant medications.
- 3) Adverse Event Registry.
- 4) Pregnancy test.
- 5) Best-corrected visual acuity test under ETDRS standards.
- 6) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 7) Indirect ophthalmoscopy for detailed examination of the retina.
- 8) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.
- 9) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 87 to 93) of said visit.
- 10) Application of the injection.
- 11) Observation period of 30 min after injection.
- 12) Indicate instructions on ophthalmic and systemic warning signs, and means of contact.

6) Visit 5

Visit 5 will be conducted 120 ± 3 days after randomization. During this visit, the following activities will be carried out:

- 1) Vital Signs Assessment.
- 2) Registration of concomitant medications.
- 3) Adverse Event Registry.

- 4) Pregnancy test.
- 5) Best-corrected visual acuity test under ETDRS standards.
- 6) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 7) Indirect ophthalmoscopy for detailed examination of the retina.
- 8) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.
- 9) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 117 to 123) of said visit.
- 10) Classification of response to treatment according to Annex 1 of this protocol.
- 11) Application of the injection in accordance with the criteria of section 6.4.11 of the protocol.
- 12) If the injection was applied:
 - a. 30-minute post-injection observation period.
 - b. Indicate instructions on ophthalmic and systemic warning signs, and means of contact.

7) Visit 6

Visit 6 will be conducted 150 ± 3 days post-randomization. During this visit, the following activities will be carried out:

- 1) Vital Signs Assessment.
- 2) Registration of concomitant medications.
- 3) Adverse Event Registry.
- 4) Pregnancy test.
- 5) Best-corrected visual acuity test under ETDRS standards.
- 6) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 7) Indirect ophthalmoscopy for detailed examination of the retina.
- 8) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.

- 9) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 117 to 123) of said visit.
- 10) Classification of response to treatment according to Annex 1 of this protocol.
- 11) Application of the injection in accordance with the criteria of section 6.4.11 of the protocol.
- 12) If the injection was applied:
 - a. 30-minute post-injection observation period.
 - b. Indicate instructions on ophthalmic and systemic warning signs, and means of contact.

8) Visit 7

Visit 7 will be conducted 180 ± 3 days post-randomization. During this visit, the following activities will be carried out:

- 1) Vital Signs Assessment.
- 2) Registration of concomitant medications.
- 3) Adverse Event Registry.
- 4) Pregnancy test.
- 5) Best-corrected visual acuity test under ETDRS standards.
- 6) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 7) Indirect ophthalmoscopy for detailed examination of the retina.
- 8) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.
- 9) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 117 to 123) of said visit.
- 10) Fluorescein angiography for assessment of diabetic retinopathy. This study may be carried out one day prior to the visit, only if both respect the time window (days 177 to 183) of said visit.
- 11) Classification of response to treatment according to Annex 1 of this protocol.

12) Application of the injection in accordance with the criteria of section 6.4.11 of the protocol.

13) If the injection was applied:

- a. 30-minute post-injection observation period.
- b. Indicate instructions on ophthalmic and systemic warning signs, and means of contact.

14) Photocoagulation in accordance with the criteria in sections 6.4.11 and 6.4.13 of the protocol.

This procedure may be carried out one day after the visit, only if both respect the time window (days 177 to 183) of said visit.

9) Visit 8

Visit 8 will be conducted 210 ± 3 days post-randomization. During this visit, the following activities will be carried out:

- 1) Vital Signs Assessment.
- 2) Registration of concomitant medications.
- 3) Adverse Event Registry.
- 4) Pregnancy test.
- 5) Best-corrected visual acuity test under ETDRS standards.
- 6) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 7) Indirect ophthalmoscopy for detailed examination of the retina.
- 8) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.
- 9) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 207 to 213) of said visit.
- 10) Classification of response to treatment according to Annex 1 of this protocol.
- 11) Application of the injection in accordance with the criteria of section 6.4.11 of the protocol.
- 12) If the injection was applied:
 - a. 30-minute post-injection observation period.
 - b. Indicate instructions on ophthalmic and systemic warning signs, and means of

contact.

13) Photocoagulation in accordance with the criteria in sections 6.4.11 and 6.4.13 of the protocol.

This procedure may be carried out one day after the visit, only if both respect the time window (days 207 to 213) of said visit.

10) Visit 9

Visit 9 will be conducted 240 ± 3 days post-randomization. During this visit, the following activities will be carried out:

- 1) Medical History Update.
- 2) Vital Signs Assessment.
- 3) Registration of concomitant medications.
- 4) Adverse Event Registry.
- 5) Pregnancy test.
- 6) Best-corrected visual acuity test under ETDRS standards.
- 7) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 8) Indirect ophthalmoscopy for detailed examination of the retina.
- 9) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.
- 10) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 237 to 243) of said visit.
- 11) Classification of response to treatment according to Annex 1 of this protocol.
- 12) Application of the injection in accordance with the criteria of section 6.4.11 of the protocol.
- 13) If the injection was applied:
 - a. 30-minute post-injection observation period.
 - b. Indicate instructions on ophthalmic and systemic warning signs, and means of contact.
- 14) Photocoagulation in accordance with the criteria in sections 6.4.11 and 6.4.13 of the protocol.

This procedure may be carried out one day after the visit, only if both respect the time window

(days 237 to 243) of said visit.

11) Visit 10

Visit 10 will be conducted 270 ± 3 days post-randomization. During this visit, the following activities will be carried out:

- 1) Vital Signs Assessment.
- 2) Registration of concomitant medications.
- 3) Adverse Event Registry.
- 4) Pregnancy test.
- 5) Best-corrected visual acuity test under ETDRS standards.
- 6) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 7) Indirect ophthalmoscopy for detailed examination of the retina.
- 8) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.
- 9) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 267 to 273) of said visit.
- 10) Classification of response to treatment according to Annex 1 of this protocol.
- 11) Application of the injection in accordance with the criteria of section 6.4.11 of the protocol.
- 12) If the injection was applied:
 - a. 30-minute post-injection observation period.
 - b. Indicate instructions on ophthalmic and systemic warning signs, and means of contact.
- 13) Photocoagulation in accordance with the criteria in sections 6.4.11 and 6.4.13 of the protocol. This procedure may be carried out one day after the visit, only if both respect the time window (days 267 to 273) of said visit.

12) Visit 11

Visit 11 will be conducted 300 ± 3 days after randomization. During this visit, the following activities will be carried out:

- 1) Vital Signs Assessment.
- 2) Registration of concomitant medications.
- 3) Adverse Event Registry.
- 4) Pregnancy test.
- 5) Best-corrected visual acuity test under ETDRS standards.
- 6) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 7) Indirect ophthalmoscopy for detailed examination of the retina.
- 8) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.
- 9) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 297 to 303) of said visit.
- 10) Classification of response to treatment according to Annex 1 of this protocol.
- 11) Application of the injection in accordance with the criteria of section 6.4.11 of the protocol.
- 12) If the injection was applied:
 - a. 30-minute post-injection observation period.
 - b. Indicate instructions on ophthalmic and systemic warning signs, and means of contact.
- 13) Photocoagulation in accordance with the criteria in sections 6.4.11 and 6.4.13 of the protocol.

This procedure may be carried out one day after the visit, only if both respect the time window (days 297 to 303) of said visit.

13) Visit 12

Visit 12 will take place 330 ± 3 days after Randomization. During this visit, the following activities will be carried out:

- 1) Vital Signs Assessment.

- 2) Registration of concomitant medications.
- 3) Adverse Event Registry.
- 4) Pregnancy test.
- 5) Best-corrected visual acuity test under ETDRS standards.
- 6) Biomicroscopy for detailed examination of ocular structures and transparency of media analysis.
- 7) Indirect ophthalmoscopy for detailed examination of the retina.
- 8) Ocular tonometry (Goldmann) to define the basal ocular tone prior to the administration of intravitreal treatment.
- 9) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the time window (days 327 to 333) of said visit.
- 10) Classification of response to treatment according to Annex 1 of this protocol.
- 11) Application of the injection in accordance with the criteria of section 6.4.11 of the protocol.
- 12) If the injection was applied:
 - a. 30-minute post-injection observation period.
 - b. Indicate instructions on ophthalmic and systemic warning signs, and means of contact.
- 13) Photocoagulation in accordance with the criteria in sections 6.4.11 and 6.4.13 of the protocol.

This procedure may be carried out one day after the visit, only if both respect the time window (days 327 to 333) of said visit.

14) Visit 13 (End of Study/Early Termination)

Visit 13 will be conducted 360 ± 3 days after randomization, or at any time in case of early termination (see section 4.3 and 4.4, as well as treatment failure criteria described in section 6. of Annex 1). During this visit, the following activities will be carried out:

- 1) Vital Signs Assessment.
- 2) Weight, size and waist circumference
- 3) Registration of concomitant medications.
- 4) Adverse Event Registry.

- 5) Laboratory tests: % HbA1c. The sample may be taken one day after the visit, only if both respect the established time window (day -8 to -1).
- 6) Pregnancy Test.
- 7) Best-corrected visual acuity test under ETDRS standards.
- 8) Biomicroscopy for detailed examination of ocular structures and media transparency analysis.
- 9) Indirect ophthalmoscopy for detailed examination of the retina.
- 10) Ocular tonometry (Goldmann).
- 11) Optical coherence tomography with spectral domain equipment to assess the condition of the retina. This study may be carried out one day prior to the visit, only if both respect the established time window (± 3 days).
- 12) Fluorescein angiography for assessment of diabetic retinopathy. This study may be carried out one day after the visit, provided that both respect the time window (± 3 days).
- 13) Classification of response to treatment according to Annex 1 of this protocol.
- 14) At the end of this visit, the patient's participation in the study will be finished.

6.3.2 Unscheduled follow-up visits

Patients may return to the facility for an unscheduled visit as needed. If the patient goes to the study site for an unscheduled visit, the date and reason for the visit must be documented on the eCRF's "Unscheduled Visit" section and in the patient's record.

6.4 Study evaluations (clinical and laboratory)

The following data will be collected during the duration of the study:

6.4.1 Medical history

Including, but not limited to: age, sex, height, weight, waist circumference, body mass index, previous hospitalizations and history of cardiovascular events, smoking, type of diabetes, time of evolution of diabetes, treatment for diabetes including dosage, duration and adherence to treatment, complications of diabetes, concomitant diseases and medications including dose and duration of treatment.

6.4.2 Ophthalmological medical history

Previous treatments for DME or any other medical and/or surgical treatment previously performed to treat any ophthalmologic condition.

6.4.3 Vital Signs

Temperature, heart rate, respiratory rate, systolic and diastolic blood pressure.

Vital signs may be measured by an assistant duly designated in the organization of the center and the delegation of responsibilities, the technique to be used for Heart Rate and Respiratory Rate will be with the count of repetitions in one minute assessed by direct auscultation with a stethoscope.

Blood pressure should be measured in the left arm with 5 minutes of rest beforehand. The instrument may be manual or automatic. It is necessary that all measures be in equal circumstances.

6.4.4 Laboratory tests

The designated personnel will perform the extraction of a blood sample by venipuncture. The vein will be chosen at the discretion of the personnel in charge and asepsis of the skin of the area to be punctured prior to the procedure will be performed. Samples will be processed at a local laboratory or by one selected by the site. The following parameter will be evaluated: % of HbA1c and eGFR.

6.4.5 Best-corrected visual acuity according to ETDRS guidelines

The test should be performed with the patient 4 meters away from the backlit panel. The panel must be calibrated to output 85 cd/m². Tests will be done on both eyes (both the eye under study and the out-of-study eye). Each eye should be tested with a chart with different character distribution to avoid memorization by the patient when it is possible to change the character chart, otherwise this evaluation should be started with the eye that is included in the study. The total number of letters recognized by the patient should be recorded, as well as the Snellen equivalent and the LogMAR score. The patient should perform the test under the conditions that ensure a best-corrected vision.

The following equivalence table will be used for the recording of the best corrected visual acuity.

LogMAR Value	Snellen (feet)	Snellen (meters)
1.6	20/800	20/240
1.5	20/640	6/190
1.4	20/500	6/150
1.3	20/400	6/120
1.2	20/320	6/96
1.1	20/250	6/75
1.0	20/200	6/60
0.9	20/160	6/48
0.8	20/125	6/38
0.7	20/100	6/30
0.6	20/80	6/24
0.5	20/63	6/19
0.4	20/50	6/15
0.3	20/40	6/12
0.2	20/32	6/9.5
0.1	20/25	6/7.5
0.0	20/20	6/6
-0.1	20/15	6/4.5
-0.2	20/12	6/3.6

To measure the best-corrected visual acuity, the patient will be asked to indicate the letters they distinguish until they reach the line where they cannot identify at least three optotypes, recording the number of letters that could be correctly specified.

It will also be required that in the case of vision cards that do not start from the 20/800 line (the point from which the letter count for ETDRS equivalence begins), the following adaptation is made by adding the following number of letters to what the examination of a patient yields.

Starting point of the optotypes to be used	Addition to the number of letters obtained
20/800	0
20/640	5
20/500	10
20/400	15
20/320	20
20/250	25
20/200	30
20/160	35

Examples:

Example 1.



In the scenario where one patient was able to distinguish the optotypes marked in green in this image, as well as distinguish three optotypes from the fourth line and only two from the fifth line, the line attributed will be the last one in which he was able to read 3 or more optotypes, in this case 20/100 or 6/30. If we add up the letters that were distinguished, we have 18 letters. However, this booklet begins at 20/200, so according to the table in section 6.4.5 *Best-corrected visual acuity according to ETDRS guidelines*, a value of 30 must be added to the letters distinguished, so the amount to be reported is 48. The equivalence to be reported in LogMAR and Snellen (feet) would be 0.7 and 30, respectively.

Example 2.



In the scenario where one patient was able to distinguish the optotypes marked in green in this image, and up to three optotypes of the fifth line. The line attributed will be the last one in which he could read 3 or more optotypes, in this case 20/80 or 6/24. If we add up the letters distinguished, we have 23 letters, at 20/200, so according to the table in section 6.4.5 *Best-corrected visual acuity according to ETDRS guidelines*, a value of 30 must be added to the letters distinguished, so the amount to be reported is 53. The equivalence to be reported in LogMAR and Snellen (feet) would be 0.6 and 24, respectively.

6.4.6 Biomicroscopy

Detailed examination of ocular structures and media transparency analysis will be performed by the blinded team at all visits. Using a slit lamp, the anterior structures of the eye will be evaluated including: eyelids, conjunctiva, cornea, anterior chamber, pupil, lens, etc. Any lighting technique(s) that suits the ophthalmologist will be used to perform the evaluation.

This study will be performed at all visits, except for visit 1.

6.4.7 Indirect ophthalmoscopy

Detailed examination of the retina. Prior to pupillary dilation of the patient with TP Ofteno® (tropicamide 5%/phenylephrine 0.8%), an indirect ophthalmoscope and a 20-D magnifying glass will be used to carry out a detailed examination of the retina.

The make and model of the instruments used will be at the discretion of the investigator. The structures that will be evaluated will be: the optic nerve, the retina, its vascularization, the transparency of the media in the posterior segment, etc.

This study will be performed at all visits, except for visit 1. This will be performed by the blinded team.

6.4.8 Ocular tonometry (Goldmann).

Define the basal ocular tone prior to the administration of the intravitreal treatment. Special attention should be paid to eye pressure at each visit.

The ocular tone will be determined with a Goldmann tonometer, after applying a topical ophthalmic solution of Ponti Ofteno® (tetracaine 0.5%) and a sterile fluorescein strip. This will be carried out prior to the application of intravitreal injection, when applicable.

Special attention should be paid to eye pressure at each visit (safety objective).

This study will be performed at all visits, except for visit 1.

6.4.9 Optical coherence tomography with spectral domain equipment.

The make and model of the instruments used will be at the discretion of the investigator, and for each individual patient the same equipment (brand and model) must be used during all visits.

This study will be performed at all visits, except for visit 1.

Assessment of retinal condition including central macular thickness and central retinal volume (volume of the macular area known as cube volume in OCT).

6.4.10 Fluorescein angiography

Assessment of diabetic retinopathy. After fasting for 8 hours and pupillary dilation with TP Ofteno® (tropicamide 5% / phenylephrine 0.8%), the patient will be prepared by cleaning the area for an intravenous injection of a fluorescein solution into one of the upper extremities, and then photographs will be taken with a fundus camera.

It is not essential for this equipment to be of a particular make or model, as long as a device with the same characteristics is used to assess the same patient throughout the study.

The procedure can be carried out by a technician or delegated personnel.

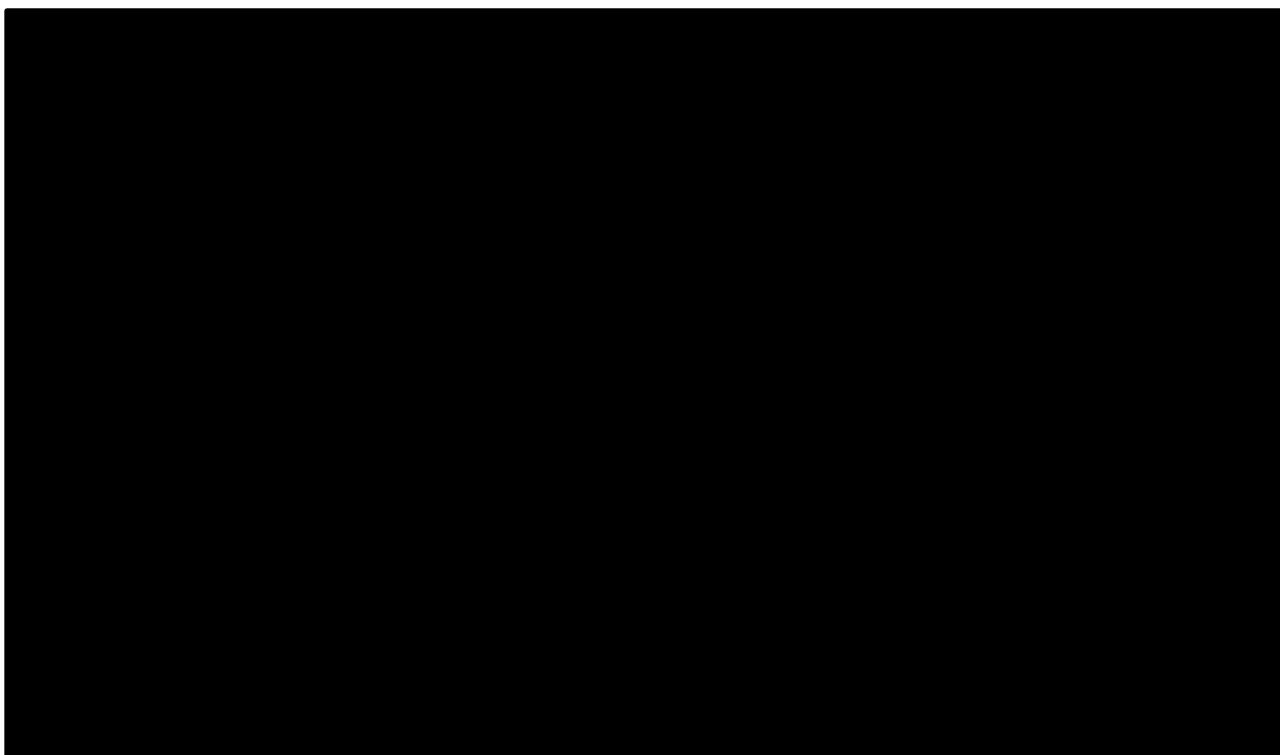
This procedure will be performed:

- In visits 1, 7 and 13.
- A blind researcher and/or sub-investigator must interpret this study.

6.4.11 Intravitreal Injection

The schedule for intravitreal injections is described below:

The treatment decision for each visit depends on the patient's visit number and the classification obtained at the visit in which they are (see section 3.1 and Annex 1):



The indication and characteristics of the administration of a prophylactic topical antibiotic regimen before and/or after each intravitreal injection will be at the discretion of the investigator and/or sub-investigator.

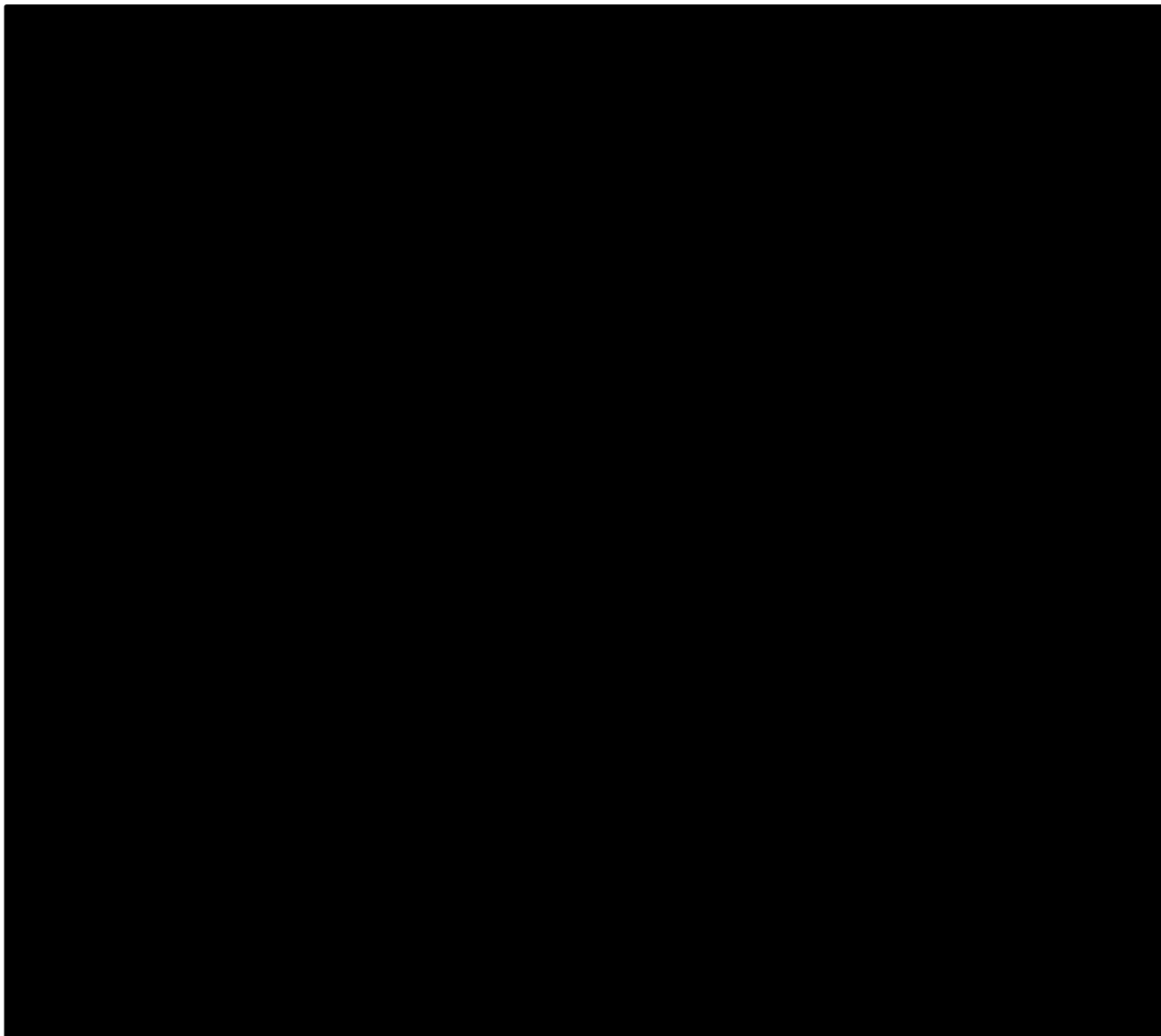
6.4.12 Urine pregnancy test

This refers to a rapid pregnancy test to be performed on all women of childbearing potential who wish to enter the study. By “fertile age” we mean women who have had their menarche and have not presented their menopause. Menopause is defined as 12 months from the last menstruation in women over 40 years of age; or who have had a bilateral hysterectomy or oophorectomy.

Women of childbearing potential with contraceptive methods including bilateral tubal obstruction should be tested for pregnancy. This test will be performed by the principal investigator or designated team member according to the instructions on the device provided by the sponsor.

6.4.13 Photocoagulation

After pupillary dilation with TP Ofteno® (tropicamide 5%/phenylephrine 0.8%) and application of topical anesthetic Ponti Ofteno® (tetracaine 0.5%), a contact lens will be placed according to the doctor's preference to perform this procedure using Meticel Ofteno® (hypromellose 2%). This therapy will be applied with the parameters and technique of the doctor's preference for each particular case.



6.5 Endpoints

6.5.1 Primary endpoint:

Visual acuity will be assessed with a standardized ETDRS best-corrected visual acuity test. To determine the efficacy of the treatments, the average value of the change in best-corrected visual acuity between the baseline value and the value at one year of treatment will be used. A correction of the values will be made due to a covariance analysis, adjusting according to the best-corrected visual acuity at baseline.

6.5.2 Secondary efficacy endpoints:

- The 4-month best-corrected visual acuity assessment will use the average values of the best-corrected visual acuity change between baseline and the value at visit 4 (after 4 months of treatment).
- The evaluation of the area under the curve of the change in the best corrected visual acuity will be carried out taking into account all the values obtained by each patient during all the visits.
- The evaluation of central macular thickness will be performed by optical coherence tomography. Efficacy will be determined as the mean change between the baseline value and the value at one year of treatment.
- The evaluation of the central volume of the retina will be performed by optical coherence tomography. Efficacy will be determined as the mean change in volume value between the baseline measurement and the measurement at one year of treatment.
- The assessment of the proportion of patients who responded to treatment will be carried out by determining the proportion of patients with improvement of more than 15 lines, more than 10 lines, or loss of more than 10 lines after one year of treatment.
- The assessment of the number of treatments will be quantified as the average number of injections received during the study.
- The assessment of the number of patients who required photocoagulation treatment will be quantified as a proportion of patients per treatment group.

6.5.3 Demographic and exploratory endpoints:

- The effect of the baseline Hb1Ac level on treatment efficacy will be evaluated by patient stratification. This stratification will be made as follows with respect to the basal value:
 - Controlled: HbA1c levels $\leq 7.0\%$
 - Uncontrolled: HbA1c levels $> 7.0\%$
- The effect of the baseline level of best-corrected visual acuity on treatment efficacy will be further evaluated by patient stratification. This stratification will be done as follows:
 - Patients at recruitment with a best-corrected visual acuity >69 ETDRS letters.
 - Patients at recruitment with a best-corrected visual acuity ≤ 69 ETDRS letters.

6.5.4 Safety endpoints

The safety assessment will be carried out by analyzing the frequency and severity of adverse events.

6.6 Contraception and management of new pregnancies in study patients or in partners of study patients

Women of childbearing potential who meet the study's inclusion and none of the exclusion criteria will be required to take a monthly pregnancy test before receiving the next treatment within the protocol. All men and women who are biologically capable of having children must agree and commit to using a barrier method of contraception for the entire duration of the study and for 3 months after the last intravitreal injection of any of the antiangiogenic drugs. Study patients who become pregnant will be discontinued from the study.

If pregnancy occurs during the study treatment period (e.g., after screening or within 30 days of the last intravitreal injection), the sponsor should be immediately informed of the pregnancy.

The investigator should inform the pregnant patient of her right to receive information about treatment in case her treating physician (gynecologist) deems it necessary for the subsequent management of her pregnancy. If the patient agrees to be informed by her particular physician, the investigator must notify the physician that she was participating in a clinical trial at the time of becoming pregnant and must provide information relevant to the treatment arms. If the patient decides to receive the treatment information in a non-blind manner in order to suit her pregnancy,

the investigator must request that the study blinding be broken at the time of delivering the information to the patient. All appropriate measures should be taken to ensure that the investigator remains blind. The investigator will not be the person responsible for breaking the blind.

All reported pregnancies will be followed up until term and six months after birth. The outcome, including any premature termination, must be reported to the sponsor.

Both the pregnancies of patients in the study as well as the pregnancies of the partners of the participating men will be followed.

6.7 Handling of biological samples

Samples will be taken in accordance with the provisions of the laboratory that carries them out. The collection of these samples must be carried out directly by venipuncture following sterile techniques.

6.7.1 Identification and labelling of biological samples

All tubes for sample collection must be labeled according to the procedures of each site or laboratory that will perform the collection/processing of the samples.

6.7.2 Handling, packaging and transport of biological samples

In accordance with the requirements of the laboratories of the participating sites.

6.8 Information management and records archiving

6.8.1 Confidentiality

All patient information in the study will be considered confidential, and will be treated and archived as such. The information will be handled in accordance with current national regulations, institutional research guidelines and sponsor procedures.

6.8.2 About the data collection periods:

The periods of interest, in terms of data collection for efficacy analysis, include: study visits.

The period of interest, in terms of data collection for safety analysis, includes: from randomization to 30 days after the date of the last visit.

6.8.3 Source documents

Source documents are all written or printed records derived from automated processes (e.g., printouts of laboratory results issued by automated analysis equipment) where the information is recorded for the first time and that is part of the permanent records of the patient's history. Source documents are considered to be the medical history, clinical notes, laboratory reports, reports of diagnostic studies, nursing notes, follow-up notes, surgery records, etc.

Researchers from participating sites and institutions are obliged to accept the monitoring of information related to the study, audits, review by ethics and research committees, and inspections by the health authority, this obligation implies direct access to the source documents.

6.8.4 Regarding the collection on the electronic Case Report Form (eCRF)

All data related to the protocol will be captured through an eCRF by the investigation team staff. The data related to the protocol should NOT be captured directly in the eCRF, but should be transcribed from their corresponding source document. This procedure allows monitoring to verify the information captured in the eCRF. It is the responsibility of the researcher to ensure that the information is transcribed into the eCRF in a correct, complete, and timely manner. It is understood that all eCRF forms captured and submitted for data analysis are approved by the Investigator.

6.8.5 Archive

The data collected in the study database are anonymized (it is only stored with the patient's number, which may or may not contain the patient's initials, along with other information of interest). The program used for data capture and storage covers the traceability requirements necessary for the execution of clinical protocols. The data collected will be stored by the sponsor or the clinical research organization designated for this purpose and its storage will be in accordance with current regulations. The patient number assignment records will remain in the participating institutions under the responsibility of the principal investigator or their work team and must be safeguarded in accordance with current regulations.

7) Evaluation and management of adverse events

7.1 Regulation and registration on adverse events

The registration and reporting of adverse events will be carried out in accordance with the guidelines established in the local regulations in force and in accordance with the international guidelines ICH E6.

7.2 Definition of Adverse Event

According to the International Council for Harmonization (ICH), an adverse event (AE) is any unfavorable medical appearance in a patient under clinical investigation who is given a pharmaceutical product, regardless of causal attribution.

Therefore, an AE can be any of the following: any unfavorable and unintentional disease, symptom, or sign (including an abnormal laboratory finding) that is temporally related to the use of a medical product, whether or not it is considered related to such a product; any new illness or exacerbation of an existing disease (worsening of the nature, frequency, or severity of a known condition); relapse of an intermittent medical condition (e.g., headache) not present at baseline; any deterioration in a laboratory value or other clinical test (e.g., electrocardiogram [ECG], x-ray) that is related to symptoms or that results in a change in study treatment or concomitant treatment or discontinuation of study medication.

7.3 Definitions relevant to the classification of adverse events

Seriousness (serious/not serious). An event defined as **serious** is one that: results in death, threatens life, requires or prolongs hospitalization, is a cause of permanent or significant disability or disability, is the cause of alterations or malformations in the newborn, or other medically important conditions.

Severity (mild, moderate, or severe). **Mild** events are those that present minimal symptoms, do not require treatment or suspension of the medication; Events are considered **moderate** when they interfere with usual activities, without threatening the patient's life, require treatment and

may or may not require discontinuation of the medication; **Severe** events are those that interfere with usual activities and require pharmacological treatment and discontinuation of the medication.

Causality is the relationship that is assigned between the drug and the adverse event: **certainly caused by the drug**: there is clear evidence of causality (i.e. the adverse event reappears with the administration of the drug); **probably caused by the drug**: there is a high suspicion of causality but there is no direct evidence or it is considered unnecessary or dangerous (i.e. the reaction disappears when the drug is stopped); **possibly caused by the drug**: there is additional information to suggest that the cause may be due to another drug or disease; **unlikely to be caused by the drug**: there is a clear explanation of the origin due to the underlying disease or the use of another drug; **conditional**: there is a lack of data to issue a clear causality; **non-classifiable**: those that once all the possible information has been obtained about the adverse event, it remains unclassifiable. The causality assessment may be supported by the application of the Naranjo criteria [91].

7.4 Data required during the capture and reporting of adverse events

For all adverse events, the following should be captured and reported:

Patient identification data (patient number), data of the person reporting the adverse event (the investigator or designated and trained person), the reported adverse event (diagnosis and ancillary findings, start date of the adverse event, treatment and management of the adverse event, importance/seriousness, severity, causality, outcome, end date of the event), dose administered, concomitant medications (distinctive and generic name, doses administered).

7.4.1 About Adverse Event Tracking

All adverse events should be followed up until the outcome of the event.

7.4.2 About the management of adverse events

The site investigator is in charge of ensuring the management and follow-up until the outcome of the adverse events that occur during the course of the protocol. Costs of participant care arising from adverse events attributable to the treatment under study will be covered by the sponsor. The sponsor will be covered by a liability policy obtained by the sponsor.

7.4.3 About Adverse Event Outcomes

Possible outcomes of an adverse event are: spontaneous resolution, resolution with additional treatment, resolution with discontinuation of treatment, resolution with sequelae, disability, unresolved, unknown, and death.

7.4.4 Adverse events of interest

The main concerns of ophthalmic bevacizumab therapy stem from the ability of anti-VEGF agents to redistribute into the bloodstream. Both aflibercept, bevacizumab, and ranibizumab have been shown to cause alterations in systemic VEGF levels. The greatest magnitude of this effect occurs with aflibercept, followed by bevacizumab and finally ranibizumab [83]. However, the rate of adverse events for different treatment modalities does not appear to differ in safety according to the most recent meta-analysis [90]. However, active monitoring of cardiovascular events detected during therapy with anti-VEGF agents is important. Systemic adverse events of interest are: nonfatal myocardial infarction, nonfatal stroke, death from vascular causes. In addition, the incidence of serious systemic events of importance to the study are death from any cause, hospitalization from any cause, severe gastrointestinal event, serious renal event, and hypertension.

At an ophthalmological level, sudden loss of vision, increased intraocular pressure, retinal detachment, intravitreal hemorrhage and endophthalmitis are considered adverse events of interest.

7.5 Adverse Event Reporting

7.5.1 Investigator's report to sponsor

The investigator and their team are responsible for the identification and reporting of adverse events that occurred during the course of the protocol. The notification of serious adverse events by the team of investigators to the clinical monitor must be made within a maximum time of 24 hours after becoming aware of the event. Sponsor is responsible for reporting adverse event reports to the appropriate regulatory authority in each country. The sponsor may delegate this activity to its local affiliates or to whom it applies, and must be documented in a letter of

delegation of responsibilities.

7.5.2 Report to the regulatory authority

The reporting of serious adverse events to the authority will be made through the means specified in each country within the times set by the local regulations in force.

Reports of non-serious adverse events will be reported at the end of the study within the final report unless otherwise requested by local regulation.

7.5.3 Activity reporting and follow-up information

All follow-up activities obtained after the initial report of a serious adverse event must be reported to the appropriate monitor within 24 hours of obtaining the information.

7.6 Assessment of adverse events

Events should be reported using appropriate medical terms and the MedDRA coding system whenever possible.

7.6.1 Intravitreal injection-related reactions

Intravitreal injection-related reactions are AEs that occur within 24 hours of administration of a medication by this route and are associated with puncture. A clear diagnosis is preferred when injection-related reactions are reported with the signs and symptoms documented as additional information. In the event that local and systemic AEs occur at the same time, each event must be reported individually in the eCRF.

"Transient pain at the time of injection" AEs will not be managed or reported as AEs.

If there are doubts regarding the association of AEs and the puncture process, it should be reported as AE. In the event of an AE that meets severity criteria, it should be reported as an AE, whether it is related to the puncture or not.

7.6.2 Risks associated with ophthalmic procedures

AEs related to ophthalmic procedures necessary for the exploration and/or application of the investigational product will not be handled or reported as AEs, as long as their use is prior to the application of the investigational product (i.e. AEs related to the application of tetracaine, povidone iodine or the placement of a blepharostat).

If there are doubts regarding the association of AEs and the ophthalmic procedure, it should be reported as AE.

In the event of an AE that meets severity criteria, it should be reported as an AE, whether it is related to the ophthalmic procedure or not (i.e. allergic reactions that meet severity criteria).

7.6.3 Adverse events secondary to other adverse events

In general, AEs secondary to other AEs (cascading events or sequelae) should be identified according to the primary cause, with the exception of serious events. A medically important AE secondary to an AE that is separated in time from the initial event should be reported as a separate event in the AE section of the eCRF.

For example:

- If vomiting results in mild dehydration without additional treatment in a normal adult, vomiting should only be reported on the eCRF.
- If vomiting results in severe dehydration, both events should be reported separately on the eCRF.
- If severe gastrointestinal bleeding leads to renal failure, both events should be reported separately on the eCRF.
- If a dizziness event leads to a fall and subsequent fracture, then all three events should be reported separately in the eCRF.
- If neutropenia is accompanied by infection, both events should be captured independently in the eCRF.

All EAs must be registered separately if there are concerns regarding the association of events.

7.6.4 Persistent or recurrent adverse events

A persistent adverse event is one that spreads continuously without resolution between different patient assessment points. Such events only need to be registered once in the eCRF. The initial severity (intensity or degree) of the event will be recorded at the time of the first AE record. If a persistent AE becomes acute, the maximum severity should be recorded in the appropriate section of the eCRF. If the event becomes a serious, it must be reported no later than 24 hours after the knowledge of the change in the status of the event. The eCRF section should be updated to reflect

the serious status, recording the date on which the event became serious, thus completing all relevant serious AE reporting data.

A recurrent AE is one that resolves between assessment points and subsequently reappears. Each recurrence of the AE must be recorded separately in the eCRF.

7.6.5 Abnormal lab values

Not all abnormal lab values qualify as AEs. For a laboratory value to be reported as an AE, it must meet any of the following criteria:

Be accompanied by clinical symptoms. It results in a change in treatment (dose modification, treatment interruption, etc.). It results in medical intervention (e.g., potassium replacement for hypokalemia) or a change in concomitant therapy.

As with signs and symptoms, reporting abnormal laboratory values should focus on getting a diagnosis and not just a description of the abnormality.

7.6.6 Abnormal vital signs

Not all abnormal vital signs qualify as AE. For an abnormal vital sign to be reported as an AE, it must meet any of the following criteria:

Be accompanied by clinical symptoms. It results in a change in treatment (dose modification, treatment interruption, etc.).

The report of abnormal values should focus on obtaining a diagnosis and not just a description of the abnormality.

7.6.7 Impaired liver function tests

The finding of elevated ALT or AST (>3x ULN) along with elevations in total bilirubin (>2x ULN) or jaundice in the absence of cholestasis or another cause of hyperbilirubinemia is considered an indicator of severe liver damage. Therefore, physicians should report as AEs the occurrence of any of the following:

Elevation of ALT or AST >3x ULN in combination with total bilirubin >2x ULN. ALT or AST elevation >3x ULN in combination with jaundice.

Proper diagnosis or laboratory abnormalities (if no diagnosis can be established) should be recorded in the appropriate section of the eCRF and promptly reported to the sponsor.

7.6.8 Death

Death should be considered an outcome and not an event. The event or condition that caused or contributed to the fatal outcome should be recorded as the AE in the eCRF section.

7.6.9 Pre-existing medical conditions

A pre-existing medical condition should be recorded as AE only if the frequency, severity, or characteristics of the condition worsen during the study.

7.6.10 Hospitalization or prolonged hospitalization

Any AE that results in hospitalization or prolongation of hospitalization must be documented or reported as a serious AE with the following exceptions:

Hospitalization for pre-existing conditions as long as the following are met:

- Hospitalization was planned before the study.

An AE will be considered serious under the criterion of "hospitalization" when there is a stay of more than 24 hours in a hospital care area, where the need for hospitalization is also confirmed.

7.6.11 Pregnancies, miscarriages, and birth defects

Fertile women should contact their doctor to report any suspected pregnancy during the study. A pregnancy report must be issued and the sponsor must be informed immediately. Monitoring of the patient should continue until the outcome of the pregnancy. Pregnancy is not in itself an AE.

Any abortion should be classified as a serious AE.

Any birth defect or birth defect in a product from a woman who received the study drug should be classified as a serious AE.

8) Information management and quality control

The information obtained from patients will be recorded in source documents. The information will then be registered through the eCRF.

The researcher or a person trained and authorized to do so will be in charge of transcribing the data from the source documents to the eCRF.

The monitor will review the information recorded in the eCRF against the source documents to determine the fidelity of the transcript and ensure that the data is accurate, original, and corresponds to the attributed source document.

In the event that missing or incorrect data is detected in the eCRF, the site researcher will be asked to provide the correct data from the source documents.

Once the data handling staff and quality control staff are satisfied with the information contained in the database, it will be closed and data must not be entered or modified in it. The data will then be available for statistical analysis.

9) Statistical analysis

9.1 Hypothesis

9.1.1 Null hypothesis:

The efficacy and safety of PRO-169 are inferior to Lucentis® for the treatment of diabetic macular edema.

9.1.2 Alternative hypothesis:

The efficacy and safety of PRO-169 are not inferior to Lucentis® for the treatment of diabetic macular edema.

9.2 Populations

9.2.1 Intent to Treat population.

The analysis of the primary outcome, the average change in the improvement of the best-corrected visual acuity, will be performed according to the principles of *intention to treat*, where all eyes that have been randomized and that have no more than two missed continuous visits will be analyzed.

9.2.2 Per protocol population.

In addition, a *per protocol* analysis will be carried out on all patients who have completed the year of follow-up, excluding all patients that presented deviations from the protocol.

This is done under the principle that there is a balance between the study sites. If not, one factor to include in the analysis of variance will be the sites. In the event that there are discrepancies in the results, an exploratory analysis will be carried out to evaluate the factors that could generate these discrepancies.

9.2.3 Safety population.

Analysis of safety outcomes will be conducted in the safety population, defined as any patient who has received at least one dose of the study drug or active comparator.

9.3 Data analysis

The statistical analysis will be performed using specialized statistical software (SPSS version available [IBM Corporation, Armonk, NY, USA] or R [*The R Foundation for Statistical Computing*, <http://www.R-project.org>]). The data obtained from the eCRF will be captured in an Excel sheet (Microsoft® Office). The analysis will be performed blinded.

9.3.1 Statistical analysis

The statistical analysis will be presented to give an overview of the patients under study and an overview of the efficacy and safety of the results. The data provided by the sites will be summarized for this purpose. For qualitative variables, tables of absolute and relative frequencies will be constructed. All percentages will be presented with one decimal. The quantitative variables will be summarized with number of patients participating (n), mean, standard deviation (SD), median, maximum and minimum. Changes in best-corrected visual acuity will be expressed as continuous variables. In the event that normal distributions are not presented, appropriate analyses will be used for this purpose.

9.3.2 Analysis for the primary variable

For the primary variable, the Student's t-test statistic will be estimated for the mean difference once the values have been adjusted with respect to their baseline value within each individual. In the event that assumptions regarding normality and homogeneity of variances are not met, the non-parametric Mann-Whitney U test will be presented.

That same difference within groups will be made for each of the values observed over time and estimated least squares (LSM) averages will be compared between groups over time with an repeated measurements Analysis of Variance (ANOVA) adjusted for the baseline value of each patient. In case of an heterogeneity of variance, the Welch test will be presented.

A second analysis will be performed and the confounding variables will be included in an Analysis of Covariance (ANCOVA), to adjust for any differences that would have occurred in the random assignment procedure as long as they have been significant individually. The confounding variables determined in the protocol are baseline HbA1c, waist circumference, body mass index (BMI), concomitant medication, time of evolution of diabetes and associated comorbidities. Confidence

intervals for the difference between treatments of the difference within treatments will be estimated to measure the magnitude of the effect.

According to the limits defined during the sample size calculation, -4 will be considered as the non-inferiority limit. If the lower limit of the confidence interval of the difference between the control treatment and the experimental treatment is less than -4, the test treatment will be considered as non-inferior.

9.3.3 Analysis for secondary variables

For the analysis of these variables, the same primary analysis will be carried out as long as the variables are continuous and have the necessary measurements to do so. In the case of discrete variables, the analysis will compare the proportions of patients who present improvement or loss of visual acuity best corrected by means of CMH tests between groups. 95% confidence intervals of the difference will be presented in the analysis to observe the variation in the magnitude for the difference.

9.3.4 Exploratory Analysis

The primary efficacy analysis will be performed for the baseline control groups and for the variable of improvement or loss of the best-corrected visual acuity stratified in different types of response presented in section 6.5.3.

9.3.5 Security Analysis

The assessment of adverse events will be monitored throughout the study, by direct observation at each visit.

All adverse events (in accordance with the protocol) will be recorded and not only those in which the investigator suspects a causal relationship with the treatments.

Incidence rates for adverse events will be summarized by number of patients with at least one event by System Organ Classification (SOC)/Preferred Term (PT) in MedDRA version 24.0 or higher. Similar tables will also be presented involving severity (seriousness) and intensity. According to their causality or attributability to any of the treatments under investigation:

- Serious Adverse Events
- Non-serious adverse events
- Treatment-related adverse events.

- Serious treatment-related adverse events.
- Discontinuations due to an adverse event

9.3.6 Procedure for handling missing data

There is no imputation procedure for missing data.

9.3.7 Deviations from the statistical analysis plan

Any deviation from the statistical plan must be approved and justified in the statistical analysis plan and in the final report of the study.

9.4 Sample size calculation

9.4.1 Sample size estimation:

442 patients (398 evaluable) randomized 1:1 into two groups of 221 individuals each (one eye per patient).

9.4.2 Justification for the sample calculation:

The sample size has been calculated to determine non-inferiority for the primary endpoint (improvement in adjusted best-corrected visual acuity with respect to baseline) between treatments according to the following premises:

A previous study evaluating the improvement in visual acuity through the use of aflibercept, bevacizumab, or ranibizumab [1] showed that the efficiency with treatment using ranibizumab consisted of an average improvement of 11.2 ± 9.4 letters. This study derived its sample size ($n=220$ eyes per group) from a previous study with a total n of 173 in which the standard deviation in the improvement in number of letters was 11.2 (95% CI 10.0, 12.5) and the SD adjusted according to baseline visual acuity was 10.2 (95% CI 9.2, 11.4). Using the CI values as a guide, a simulation was carried out to establish an average difference of -4 letters as non-significant:

POWER Procedure (SAS Program): t-test of two independent samples for the mean difference:

Table 8 Assumptions for sample estimation

Distribution	Normal
Method	Exact
Number of sides	1, Upper limit
Null Difference	-4
Alpha	0.05
Nominal Power	0.8
Weighting for Group 1	1
Weighting for Group 2	1

Table 9 Estimated Sample Size

Number	Difference of Means	Standard Deviation	Estimated Power	Estimated sample size
1	-3.0	9	0.800	2006
2	-3.0	10	0.800	2476
3	-3.0	11	0.800	2994
4	-2.5	9	0.800	892
5	-2.5	10	0.800	1102
6	-2.5	11	0.800	1332
7	-2.0	9	0.801	504
8	-2.0	10	0.800	620
9	-2.0	11	0.800	750
10	-1.5	9	0.800	322
11	-1.5	10	0.801	398
12	-1.5	11	0.801	482
13	-1.0	9	0.800	224
14	-1.0	10	0.802	278
15	-1.0	11	0.800	334
16	-0.5	9	0.802	166
17	-0.5	10	0.801	204
18	-0.5	11	0.801	246
19	0.0	9	0.804	128
20	0.0	10	0.800	156

Considering that the average difference between ranibizumab and bevacizumab in the study used as the basis for sample calculation [1] was 1.4 (95% CI -0.4 to +3.2 $p = 0.12$) letters, the sample size considered for this study is 398 evaluable patients (a difference of -1.5, an expected standard deviation of 10, with a non-inferiority limit of -4 (number of letters) and a nominal power of 0.8). If a 10% dropout rate is considered, the total number of eyes in the study should be 442.

10) Quality control and assurance

In this research, independent and systematic reviews of the activities and documents related to the study will be implemented, to ensure the quality of its design, conduction, analysis and reporting; all in accordance with GCPs, current national regulations and the sponsor's standard operating procedures.

10.1 Clinical site monitoring

The study sponsor is responsible for monitoring the study. Monitoring activities include, but are not limited to: general safety monitoring, general study quality monitoring, monitoring by study site, detection monitoring, reporting and tracking of adverse events, monitoring for resolution of discrepancies in data capture, etc. Responsibility for monitoring activities and ultimate responsibility for monitoring rests with the sponsor. The details of the monitoring activities are specified in a separate document from this protocol in a Monitoring Plan.

The research centers participating in the study will be monitored. For each center, at least one initiation visit and one close-out visit must be carried out, which does not exclude the possibility of carrying out one or more follow-up visits between these two mandatory visits.

The initiation visit must be carried out before the inclusion of the first participant in that center; In it, the monitor will verify that the material to be used during the study has been received and that the personnel who will participate in the study activities have been trained on the study, as well as verify that the regulatory requirements and applicable standard operating procedures are met.

At the follow-up visit, the monitor will conduct a review of the study documents to confirm that: the applicable research protocol and standard operating procedures are being followed, data completion is complete and timely, and adverse event reports are being conducted appropriately. At each visit, the monitor will discuss the findings with the investigator and define the actions to be taken.

The close-out visit will take place at the end of the study, once the last site participant has been discharged from follow-up. On this visit, the monitor will verify that the site has all the necessary documents for archiving, that all biological samples have been sent for analysis, that all the drug under study (used and unused) has been recovered and sent to the sponsor, and that all unused

material has been recovered.

10.2 Audit of the study and its information

To ensure compliance with GCPs and all applicable regulatory requirements, Laboratorios Sophia, S.A. de C.V. may conduct quality assurance audits. Research ethics committees and research committees, as well as regulatory agencies, may also conduct reviews and/or inspections of this study.

If any audit or inspection is conducted, the investigator and the institution shall agree to allow the auditor/inspector direct access to all relevant documents, and shall allocate their time and that of their staff to the auditor/inspector to discuss the findings and any pertinent problems. In the event that the audit has not been scheduled by the sponsor, the facility must notify Laboratorios Sophia, S.A. de C.V. immediately.

10.3 Procedure for modification of the protocol

The only way to implement a modification to the research protocol is through an amendment, which must be approved by the sponsor and researchers, approved by the research and research ethics committees and authorized by the health authority, before it can be implemented.

In the event that the change to the protocol aims to eliminate an immediate risk to patients under investigation, it may be implemented immediately, and then constituted as an amendment and follow the corresponding approval processes.

11) Ethical considerations

This study will be conducted with the utmost respect for the participants (that is, the patients) in accordance with the protocol, the ethical principles originating from the Declaration of Helsinki, and the Harmonized Tripartite Guideline for GCPs of the ICH [92]. Each investigator shall conduct the study in accordance with applicable local or regional regulatory requirements and shall be conducted in accordance with the Investigator's Responsibilities [93].

11.1 Ethical review

An ethics committee and others that apply according to local regulation must review and approve this protocol, the informed consent form, any form of dissemination or publicity of the study (posters, flyers, graphic or audiovisual advertisements, etc.), the researcher's monograph, any information to be provided to the study patients and any other information requested in the local regulation in force prior to the conduct of the study.

After this, the regulatory authority must also approve this protocol prior to its implementation.

11.2 Informed consent

The informed consent form contains complete and understandable information about the study and the investigational product, in accordance with current applicable regulations and GCP. Patients who agree to participate in the study will sign the informed consent form and must be given a duplicate. The signature sheet must include what is specified in the local regulation. The signing of the document may require the signature of at least one witness independent of the study. Once the process of obtaining the signature of the informed consent is completed, an original will be given and the duplicate will be placed in the patient's file on site. The informed consent form will be considered as a source document and will be filed as such. The site's principal investigator is responsible for ensuring that all new versions of the informed consent are submitted to appropriate approvals (the same as the original informed consent form was submitted to) and that the most current approved version is presented to study patients.

11.3 Confidentiality

All eCRFs and communications related to study patients will identify them only by the study patient identification number. The information collected in this study will be treated confidentially and exchanged between the sponsor and the research site. The health authority, the ethics and research committees, the sponsor, the monitors/auditors and third-party auditors will be the only bodies authorized to review the study documentation. If publications arise from this research project, in no case will they contain information on the identification of the study patients. If the results of the study are published, no personal information of the study patients will be revealed. The protection of personal data will be carried out in accordance with current regulations.

11.4 Researcher Conflict of Interest

All principal investigators of the sites must declare their conflicts of interest in writing prior to the start of their activity in the study.

12) Publication Policy

Any manuscript derived from the data obtained with this protocol must be submitted to review by the sponsor before any attempt to submit it for publication in any scientific journal or congress.

13) Financing and insurance

13.1 Compensation to study participants

Subjects who participate in the study will not receive financial compensation for their participation in the study. However, the subjects will receive financial support for travel expenses on each scheduled visit to which they attend punctually. Said support, as well as the amount, may be specified in the informed consent form.

13.2 Insurance for study participants

In accordance with current local regulations, Laboratorios Sophia S.A. de C.V. has obtained a civil liability insurance policy, in order to comply with the responsibility of providing medical treatment and compensation to which a subject would be legally entitled, in the case of damages directly caused by this research.

In the event of a medical emergency, the research center must have personnel, material, equipment and procedures for its immediate management.

14) References

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15) Annex 1

