Excelencia en oftálmicos

Protocol:

SOPH169-1224/I

Title: Phase I clinical study to evaluate safety by determining immunogenicity after unilateral intravitreal PRO-169 application in patients with diabetic macular edema.

Information about the molecule under study
Generic name: Bevacizumab (for ophthalmic use) 25.0 mg/mL
Distinctive name: PRO-169
Note: Treatment of diabetic macular edema

Protocol Information
Study Phase: I
Version: 4.0
Release Date: 4-nov.-25

This protocol has been carried out in accordance with the principles of the Declaration of Helsinki and will be carried out in accordance with Good Clinical Practice and in compliance with the ICH guidelines and current local legislation.

Sponsor: Laboratorios Sophia S.A. de C.V.

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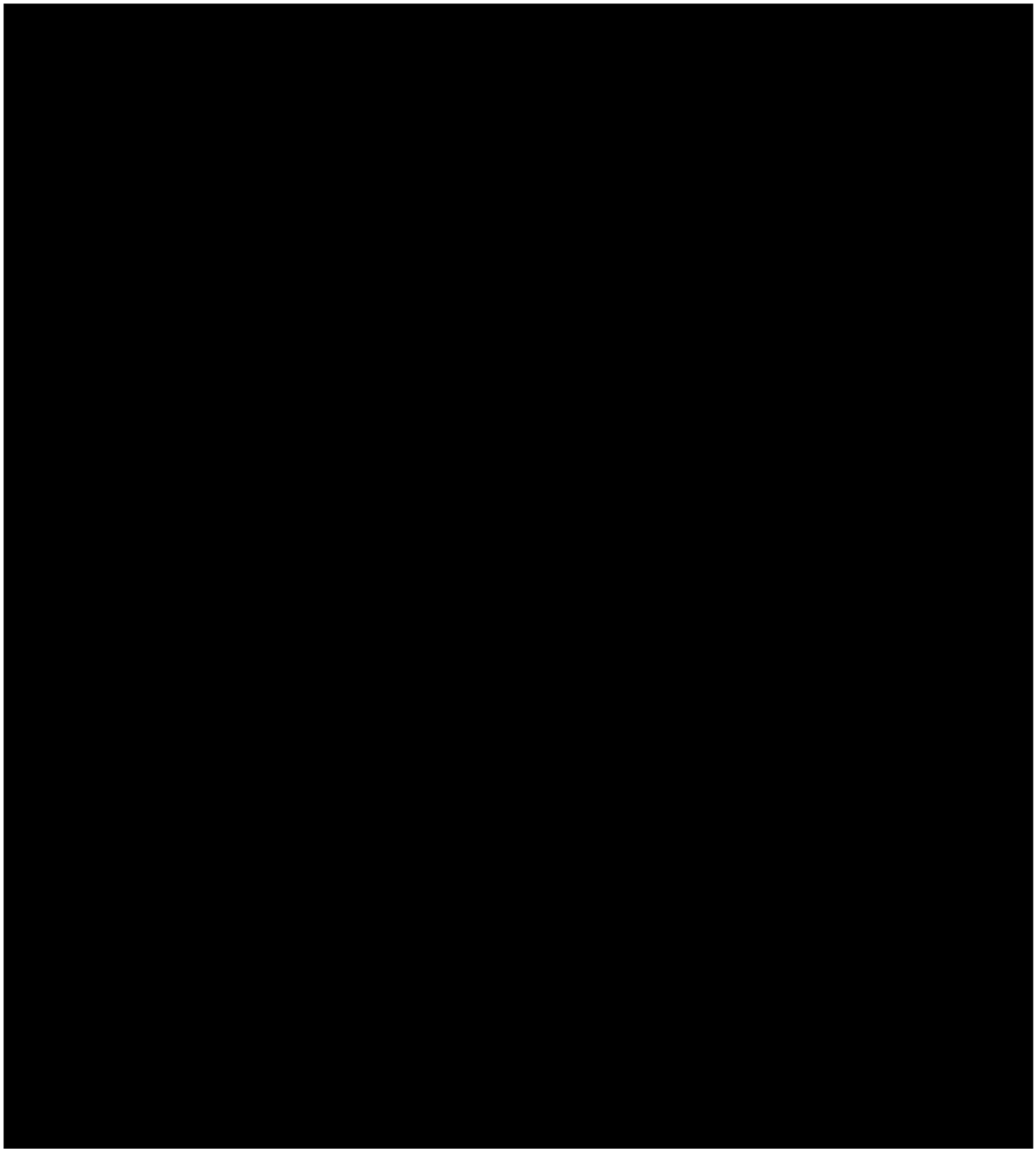
Responsible for the study

The administrative structure of the sponsoring party, corresponding to Laboratorios Sophia, S.A. de C.V., is shown in the following table:

Table 1. Responsible for the study

Signature Page

From the sponsor



Investigator Agreement

I agree to conduct this clinical study according to the design and guidelines of this protocol, in accordance with its provisions. I declare that I will conduct the study in accordance with the standards of Good Clinical Practice, and I will report all information or data in accordance with the protocol, particularly any adverse events. I will also handle clinical supplies, provided by the sponsor, strictly in accordance with this protocol. I understand that the information that identifies me may be used by the sponsor. Because the information contained in this protocol and the Investigator's Manual is confidential, I understand that it is prohibited from sharing it with any third party, which is not involved in the approval, supervision, or conduct of the study. I will ensure that I take the necessary precautions to protect the information from loss, inadvertent disclosure, or access by unauthorized third parties.

Name: *[Insert full name of researcher]*	**Signature**
Title: Principal Investigator	**Date**
Name of the center: [*Insert Name of Study Site]*	
Geographic location (city/state/country) *[Enter the geographical data of the center]*	

List of abbreviations

ADAs	Anti-drug *antibodies*
AUC	Area under *the curve*
BCVA	Best-Corrected major visual acuity
GCP	Good clinical practice
BSA	*Body Surface Area*
BUN	Blood urea *nitrogen*
CDM	Clinical data *management*
REC	Research Ethics Committee
CMPHU	Committee for Medicinal Products for Human Use
RC	Research Committee
CIOMS	Committee for the International *Organization of Medical Sciences*
CKD-EPI	*Chronic Kidney Disease Epidemiology Collaboration*
Cmax	Maximum concentration
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
CONSORT	*Consolidation Standards of Reporting Trials*
SD	Standard deviation
IUD	Intrauterine device
DRCR	*The Diabetic Retinopathy Clinical Research*
AE	Adverse Event
ECL	Electrochemiluminescence
eCRF	*Electronic Case Report Form*
EMD	Diabetic macular edema
ELISA	*Enzyme-linked Immunosorbent Assay*
RPE	Retinal Pigment Epithelium
ETDRS	*Early Treatment Diabetic Retinopathy Study*.
FA	Fluorescein angiography of the retina (*Fluorescein Angiography*)
Fab	*Fragment antigen-binding*
HR	Heart rate
ICF	Informed consent form
FDA	Food and Drug *Administration*.
RR	Respiratory rate
GFRe	Glomerular *Filtration Rate estimate*
CMT	Central macular thickness
HbA1c	Glycosylated hemoglobin
HMSA	*Homogeneous Mobility Shift Assay*
CI	Confidence interval

ICH	International *Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.*
ICMJE	International Committee of Medical Journal Editors
BMI	Body mass index
PI	Principal Investigator
ITT	Population by intent to treat
kDa	Kilodaltons
KDIGO	*Kidney Disease Improving Global Outcomes*.
LogMAR	Logarithm of the Least Resolution Angle (Logarithm *Logarithm of the Minimum Angle of Resolution*).
LOCS III	*Lens Opacities Classification System*
MedDRA	Medical *Dictionary for Regualtory Activities*.
mg	Milligrams
mL	Millilitres
Nab	Neutralizing antibodies
NOM	Official Mexican Standard
NVC	Choroidal neovascularization
OCT	Optical *Coherence Tomography.*
OCT-A	Optical Coherence *Tomography Angiography*
OD	Right eye (Latin for its acronym *oculus dexter*)
WHO	World Health Organization
OS	Left Eye (by its Latin acronym, *oculus sinister*)
IP	Investigational Product
IOP	Intraocular Pressure
PK	Pharmacokinetics
PP	Population by protocol
PS	Security population
RD	Diabetic retinopathy
RIA	Radioimmunoassay (*Radioimmunoassay*)
NCTR	National Clinical Trials Registry
SDV	*Source data verification*
SPR	Surface Plasmon Resonance Spectroscopy
BP	Blood pressure
$T_{1/2}$	Half-life
T_{max}	Time to Maximum Concentration
UFTLS	Pharmacovigilance and technovigilance unit of Laboratorios Sophia
VEGF	Vascular Endothelial *Growth Factor (Vascular Endothelial Growth Factor*).
µg	Micrograms
µL	Microliters

1. Protocol Summary

1.1 Synopsis

<table>
<tr><td colspan="2">Title of the study:

Phase I clinical study to evaluate safety by determining immunogenicity after unilateral intravitreal PRO-169 in patients with diabetic macular edema.</td></tr>
<tr><td>Study Number:

SOPH169-1224/I

Sponsor: Laboratorios Sophia, S.A. de C.V. Paseo del Norte No.5255, Col. Guadalajara Technology Park, Carretera Guadalajara-Nogales Km13.5 C.P. 45010 Zapopan, Jalisco, Mexico

The company responsible for carrying out the monitoring activities is carried out by: Visión Integral en Investigación Klini K. Cataratas No. 3, Col. Jardines del Pedregal, C.P. 01900, Alcaldía Álvaro Obregón, Mexico City.</td><td>Date of creation:

15-Jan-25</td></tr>
<tr><td>Protocol version:

4.0</td><td>Release Date:

Nov 4-25</td></tr>
<tr><td>Therapeutic indication:

Treatment of diabetic macular edema.</td><td>Application:

Diabetic macular edema.</td></tr>
<tr><td>Estimated duration of the study (from the first visit of the first subject to the preparation of the final report):

14 months</td><td>Clinical Development Phase:

Phase I Clinical Study</td></tr>
<tr><td colspan="2">Objectives:

Primary Objective:

• To evaluate the safety of intravitreal PRO-169 by the presence of serum anti-drug antibodies (ADAs) for bevacizumab.

Secondary security objectives:

• To determine the incidence of adverse events (AEs) related to the investigational product.
• Determine the incidence of serious AEs related to the investigational product.</td></tr>
</table>

Objectives for the pharmacokinetics (PK) cohort:

- Calculate the total area under the curve (AUC) of the serum concentration of PRO-169.
- To quantify the maximum serum concentration (C_{max}) of PRO-169.
- Quantify the time to maximum serum concentration (T_{max}) of PRO-169.

Exploratory objectives:

- To assess the change in best-corrected visual acuity (BCVA) compared to baseline.
- To assess the change in central macular thickness (CMG) compared to baseline.

Hypothesis:

This study does not propose any statistical hypothesis testing, so they are not considered formal statistical decisions.

Study Design:

Phase I clinical study, prospective, interventional, open-label, multicenter.

Number of subjects (planned):

Sample size is not based on statistical power calculations. It is chosen based on background and feasibility. A total of 60 subjects (60 eyes) is considered suitable to assess the primary objective of the study

In addition to the general safety variables of the study, pharmacokinetics will be evaluated in a cohort of 15 subjects.

Diagnosis and main inclusion criteria:

- Diabetic macular edema.

Selection criteria:

Inclusion criteria:

1. Age ≥ 18 years.
2. Diagnosis of type 1 or 2 diabetes.
3. Ability to give signed informed consent.
4. Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
5. All subjects (male and female) who are biologically capable of having children will be required to agree and commit to using a barrier or hormonal method of contraception

(by any route of administration) for the entire duration of the study and for 3 months after the last intravitreal injection.

6. Female subjects, biologically capable of having children, will be required to have a negative urine pregnancy test at the screening visit.
7. Best Corrected Visual Acuity (AVMC) according to the 24- to 78-letter ETDRS primer (approximate Snellen equivalent of 20/32 to 20/320)
8. Diabetic macular edema with central involvement evidenced by spectral domain optical coherence tomography (central macular thickness criterion ≥ 300 µm for men and ≥ 290 µm for women) within 8 days prior to inclusion. All measurements made on a subject should be taken with an instrument of the same make and model during the conduct of the entire study.
9. Comply with characteristics that allow an adequate examination of the fundus of the eye (transparency of media, adequate pupillary dilation).
10. Glycosylated hemoglobin < 12% of a result not older than 3 months.

Exclusion criteria:

1. Chronic kidney disease in renal failure (glomerular filtration rate [GFRe] <15 ml/min/1.73m2) requiring dialysis or transplantation; according to the 2020 Clinical Practice Guideline for the Management of Diabetes in Chronic Kidney Disease of the Organization for Improving Global Outcomes in Kidney Disease (KDIGO).
2. Active proliferative diabetic retinopathy in the study eye, including rubeosis iridis, vitreous hemorrhage, or tractional retinal detachment visible during the screening visit.
3. Individuals who have required insulin treatment within 4 months prior to the screening visit for glycemic control.
4. Previous participation in clinical studies with investigational, ocular or systemic products (at least 30 days must have elapsed between the end of your participation in a previous trial and the inclusion of the present study).
5. Known hypersensitivity or allergy to bevacizumab, or any ingredient in the investigational product.
6. Poorly controlled blood pressure (average of 3 blood pressure readings in a seated position with ≥160 mmHg systolic or ≥100 mmHg diastolic) at the screening visit.
7. Myocardial infarction or other cardiovascular event (cerebral vascular disease, transient cerebral ischemia, or hospitalization for heart failure) during the 4 months prior to the screening visit, or subjects with active myocardial ischemia.
8. Systemic treatment with VEGF-related medications within 4 months prior to the screening visit.
9. History of any rheumatological or collagen disease of autoimmune origin related to inflammatory processes such as systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, Bechet's disease, dermatomyositis, among others.
10. History of any disease that causes immunosuppression or immunosuppression, except diabetes mellitus.
11. Concomitant use of immunosuppressive agents, immunotherapy, or monoclonal antibodies by any route of administration (other than intravitreal route), within 2 years

prior to the screening visit or during the study period.

12. Subjects who have received intravitreal anti-VEGF therapy (aflibercept, ranibizumab, bevacizumab, faricimab, brolucizumab) in the study eye within 4 months prior to the screening visit.
13. Use of intraocular or periocular corticosteroids in the study eye within 4 months prior to the screening visit, or use of intravitreal corticosteroid implants at any time.
14. Use of anticoagulants or antiplatelet agents by any route of administration within 10 days prior to the screening visit or during the study period.
15. Women of childbearing potential who are pregnant, nursing, or plan to become pregnant within the study period.
16. Allergy to fluorescein (topical or intravenous) or to numbing medications used during the injection procedure.
17. Subjects with non-diabetic macular edema.
18. Lens opacities that are superior in one or more of the following criteria according to the LOCS III classification *(Lens Opacities Classification System*): nuclear component > NO3C3 (opalescence/color), cortical component > C2 and posterior subcapsular component > P1.
19. History of eye surgery (cataract removal, any intraocular surgery, aphakia, etc.) or panretinal photocoagulation within 3 months prior to the screening visit or planned within the study time.
20. Intraocular pressure greater than 21 mmHg in the selected eye, measured by Goldmann tonometry at the screening visit.
21. Presence of macular ischemia or significant peripheveal capillary loss (increase in the foveal avascular zone greater than 350 µm) demonstrated by retinal fluorescein angiography (during the screening visit or in a study conducted within 4 months prior to the screening visit).
22. History of YAG capsulotomy in the study eye, within 30 days prior to the screening visit.
23. Evidence of external eye infections, intraocular inflammation, or significant ocular surface disease in either eye, 30 days prior to the screening visit.
24. History of uveitis in the eye of study.
25. History of retinal detachment, vitrectomy, any type of filtering surgery, transplant or corneal dystrophy in the selected eye.
26. Advanced glaucoma or optic neuropathy in the selected eye.
27. Have a single functional eye (finger-counting BCVA or less in the eye with worse vision).
28. Have previously participated in this same study.
29. Be or have an immediate family member (e.g., parent/legal guardian or sibling) who is part of the research site or sponsor staff.
30. Subjects who present conditions that make it difficult or impossible to extract the blood sample, such as veins that are not visible or palpable, the presence of venous thrombosis in the sample collection area, severe edema, or any other criteria of the personnel in charge of performing this procedure.
31. Difficulty interpreting the optotypes of the ETDRS booklet.

Additional exclusion criteria for the pharmacokinetics cohort:

1. Prior intravitreal treatment with bevacizumab in the contralateral eye, within 3 months prior to the screening visit.
2. Another intravitreal anti-VEGF therapy (ranibizumab, aflibercept, brolucizumab, faricimab) in the contralateral eye, within 30 days prior to the inclusion of the study or during the conduct of the study.

NOTE: Only one eye may be selected; if both eyes are eligible for inclusion, one eye will be selected at the discretion of the PI.

Research Product (RP):

Investigational product, dosage, and route of administration:

- PRO-169. Bevacizumab (for ophthalmic use).
- Dosage: 1 intravitreal injection every 30 days.
- Route of administration: Intravitreal
- Prepared by Laboratorios Sophia, S.A de C.V., Zapopan, Jalisco, Mexico.

Duration of treatment:	**Duration of the subject in the study:**
2 months	Up to 95 days (counting from visit 1 [day 1]) or up to 103 days (counting from screening visit [day -8 to 0]).

Evaluation criteria:

Safety primary outcome variables

- Proportion of patients with the presence of bevacizumab-positive serum ADAs.

Safety Secondary Outcome Variables

- Incidence of adverse events related to RP.
- Incidence of serious adverse events related to RP.

Pharmacokinetic secondary outcome variables

- Total AUC of serum PRO-169 concentration.
- PRO-169 serum C_{max}.
- PRO-169 serum T_{max}.

Exploratory variables

- Changes in BCVA from baseline to day 90.
- Changes in the GMC, from baseline to day 90.

Statistical methodology

The statistical analysis will be carried out by personnel of Laboratorios Sophia, S.A. de C.V. A specialized statistical package will be used.

All analyses will be primarily descriptive.

The data will be expressed as means, standard deviation (SD), median, minimum value, maximum, 95% confidence interval, geometric mean.

The categorical variables will be summarized by frequency, and percentages by subjects. They will be presented with a decimal place

The data obtained will be presented in tables and graphs as appropriate.

1.2 Study diagram

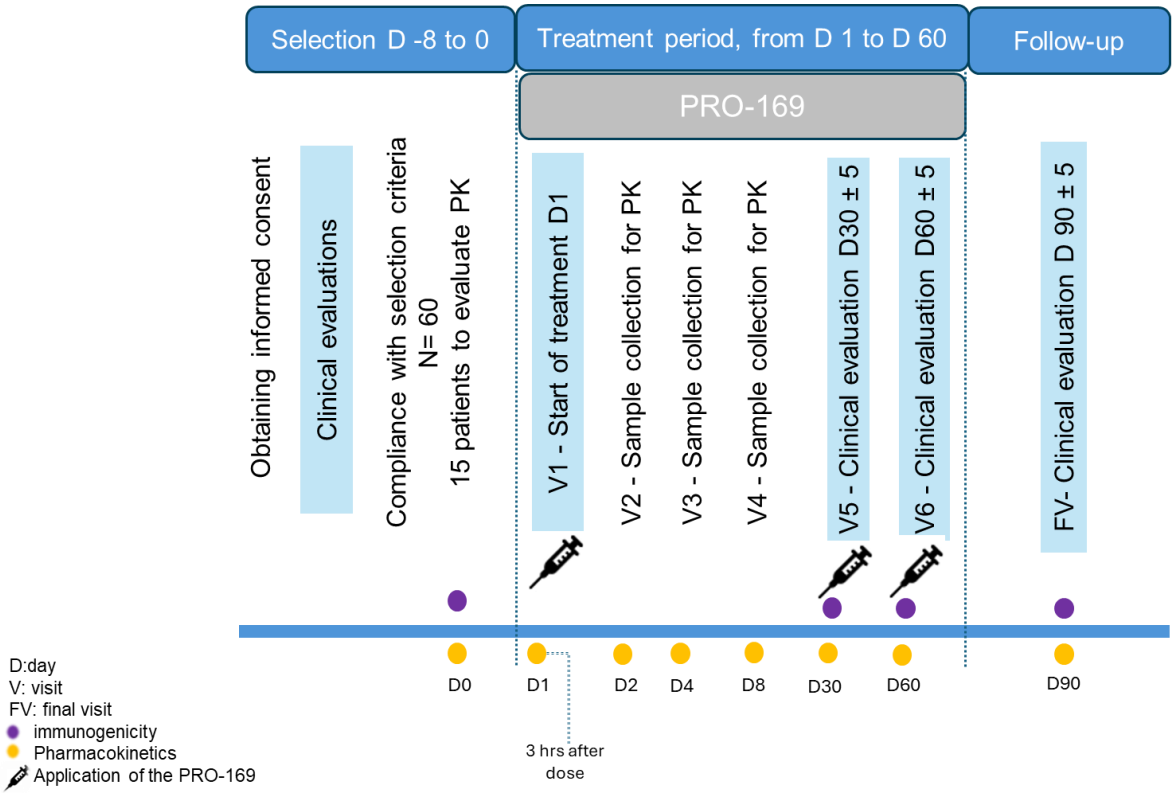


Figure 1. Study diagram

1.3 Schedule of activities

	Visit of Selection	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
		Start of treatment			Treatment Period			Final / Early Termination
Protocol Day	-8 to 0	1	2	4	8	30±5	60±5	90±5
Informed consent	X							
Evaluation of selection criteria	X							
Medical History	X							
Weight, Height, BMI and Body Mass Surface Area	X							
Vital signs (BP, HR, Temperature, RF)[Φ]	X	X				X	X	X
% HbA1c*	X							
Glomerular filtration rate (GFRe)[a]	X							
Concomitant medications	X	X	X	X	X	X	X	X
Adverse Event Registry	X	X	X	X	X	X	X	X
Pregnancy Test	X	X				X	X	X
Best-corrected visual acuity	X					X	X	X
Biomicroscopy	X					X	X	X
Indirect ophthalmoscopy	X					X	X	X
Ocular tonometry	X					X	X	X
Macular optical coherence tomography	X					X	X	X
Fluorescein angiography (AF) or optical coherence tomography angiography (OCT-A)[ж]	X							
Intravitreal injection		X				X	X	
Pharmacokinetic blood sample (applies to PK cohort)[ᵻ]	X	X	X	X	X	X¥	X¥	X¥
Blood Sample Immunogenicity	X					X¥	X¥	X¥

[Φ] Vital signs assessment should be performed prior to blood sample collection.
*If there is an HbA1c determination dated within 3 months prior to the inclusion of the study, it will not be necessary to perform it at the screening visit.
[a] If a serum creatinine result dated within 2 months prior to study inclusion is available, it will not be necessary to perform it at the screening visit and may be used to calculate GFRe.
[If] you have an AF or OCT-A performed in the 4 months prior to the inclusion of the study, it will not be necessary to perform it at the screening visit.
[ᵻ] See pharmacokinetics schedule on the following page.
¥ Blood samples will be collected prior to intravitreal application of PRO-169 (no more than 2 hours prior to the time of application at Visit 1).

Table 2. Schedule of activities

1.4 Pharmacokinetics Cohort Schedule

	Selection Visit		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
				Treatment Period					Final / Early Termination
Protocol Day	-8 to 0		1	2	4	8	30±5	60±5	90±5
Specific hours to perform procedures (when applicable)		Research Product Application	Intravitreal injection time + 3 hours (± 30 min)	Intravitreal injection time + 24 hours (± 2 hours)	Intravitreal injection time + 72 hours (± 2 hours)	Intravitreal injection time + 168 hours (± 2 hours)			
Pharmacokinetic blood sample	X		X	X	X	X	X¥	X¥	X¥

¥Blood samples will be collected prior to intravitreal application of PRO-169 (no later than 2 hours prior to the time of intravitreal application at Visit 1).

Table 3. Pharmacokinetics Cohort Timeline

2. Introduction and background

2.1 Theoretical framework

Diabetic macular edema (DME) is the leading cause of vision loss in diabetic patients of productive age. DME consists of the accumulation of fluid and serous macromolecules in the retina, both intracellular and extracellular, of the retina. This accumulation interferes with the functions of the retina, causing visual deficiencies. [1] [2] [3] [4] [1] [5]

Although DME can manifest in both types of diabetes (type 1 and type 2), its relevance as a public health problem is due to the severity of type 2 diabetes as one of the main threats to public health. Two studies published in consecutive years in 2017 and 2018, place the estimates of diabetes prevalence for 2015 and 2017, at 415 million (uncertainty range: 340 - 536 million) equivalent to 8.8% (7.2 – 11.4%) of the world 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 world population between 18 - 99 years, respectively. Predictions for 2040 and 2045 are 642 million and 693 million adults affected by the disease, respectively. [6] [7]

The ophthalmological complications that occur in patients with diabetes are multiple and include keratopathy, cataract, neovascular glaucoma, neuropathy, ischemic optic neuropathy, diabetic retinopathy (DR), and secondary macular edema. DR is the set of alterations in the retina due to damage caused by uncontrolled hyperglycemia and affects about 4 million people worldwide. This condition in turn can be subdivided into non-proliferative DR and proliferative DR, 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 decreased visual acuity in diabetic patients is DME. Historically, the first descriptions of the DR are presented at the end of the nineteenth century with descriptions by Jaeger, Mackenzie, and Noyes, among others. Mackenzie, on the other hand, is the first author to describe in detail the changes in the retina at the 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 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 would

not begin to be considered a determinant for vision loss until the studies of Klein et al. published in 1984, in which a clear relationship was demonstrated between the presence of macular edema and the impairment of visual acuity in patients with DR. mainly in adults diagnosed with diabetes after 30 years of age. [8] [9][8] [10] [11] [12] [13] [14]

DME can develop at any stage of DR, however, its prevalence increases according to the duration of diabetes and the severity of DR. In addition, there are other systemic and ocular factors that play an important role in its development, such as high blood pressure, hyperlipidemia, previous cataract surgery, and elevated levels of glycosylated hemoglobin (HbA1c). 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 edema is established, a vicious cycle of hypoxia and inflammation begins. These two elements promote the synthesis of vascular endothelial growth factor (VEGF). [15] [5] [16] [17] [18] [19] [20] [1] [5] *vascular endothelial growth factor*). VEGF values during DME are usually increased by up to 10 times their normal levels. This presence of elevated levels of VEGF has two effects within the pathogenesis of DR and the formation of DME: 1) it causes capillary damage and increased vascular permeability, which causes disruption of the blood-retinal barrier; and 2) promotes the synthesis of new vessels. [1] [5][21] [22] [23]

The prevalence of DME globally is estimated to be between 1.4% and 12.8%. However, local prevalences vary according to the incidence of diabetes in each region and the degree of disease control in the affected population. In Mexico, the estimated prevalence is between 3.1% and 6.6% of patients less than 10 years old from the diagnosis of diabetes mellitus. [24][19] [25]

The main strategy for the management of DME is prevention. Aggressive glycemic control with HbA1c values below 7%, as well as control of elevated blood pressure, have been shown to reduce the risk of DR onset and progression, as well as to decrease the incidence of DME. [26][27] [28]

Once DME occurs, its treatment is key to preventing severe visual loss. Currently, intravitreal anti-VEGF drugs are considered the first-line therapy. Currently available anti-VEGF agents with an approved indication for the treatment of DME are ranibizumab, aflibercept, brolucizumab, faricimab, and additionally, bevacizumab which is used off-label. Although all these drugs aim to inhibit the VEGF pathway, they have differences in their molecular structure, binding affinity,

mechanism of action, and pharmacodynamics, which contribute to their efficacy and duration of effect. [16] [20] [22] [23][29] [30] [31] [32] [33][34]

Although pegaptanib was the first drug authorized for the treatment of DME, its use 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. [35]

Faricimab and brolucizumab are among the newer anti-VEGF drugs. Faricimab is a humanized bispecific monoclonal antibody with dual action as it simultaneously inhibits VEGF-A and angiopoietin-2. Brolucizumab is a humanized monoclonal antibody with a monocaternary Fv fragment that inhibits all VEGF-A isoforms that bind to VEGF, VEGF-1, and VEGF-2 receptors. [23] [34]

Aflibercept, ranibizumab and bevacizumab are the most widely used medicines worldwide. Aflibercept is a fusion protein that acts as a soluble VEGF receptor; binds to VEGF-A, VEGF-B, and placental growth factor. Ranibizumab is an antigen-binding fragment (Fab, *Fragment antigen-binding*) of a humanized monoclonal antibody, developed specifically for intraocular use; binds to and inhibits all active forms of VEGF-A. Bevacizumab is a full-length (149 kDa) humanized monoclonal antibody developed for cancer therapy. At least four studies comparing treatment between aflibercept, ranibizumab, and bevacizumab have shown that the efficacy of treatment is equivalent in improving visual acuity, but with a lower response of bevacizumab on measurements of central macular thickness as assessed by optical coherence tomography (OCT). [23] [34][36] [37] [38] [39]*Optical Coherence Tomography)*. Except for the study carried out by *The Diabetic Retinopathy Clinical Research (DRCR) Retina Network (Protocol T),* These were carried out with small samples or reduced treatment times.

The T-protocol demonstrated that bevacizumab and ranibizumab were equivalent in their efficacy for the treatment of DME, after one year of treatment (improvement of 9.7 + 10.1 letters, and 11.2 + 9.4 letters on average, respectively [p = 0.12]), although both were slightly inferior to aflibercept (13.3 + 11.1 letters) particularly for the treatment of patients with visual acuity deficiencies below 69 letters (11.8 + 12.0 for bevacizumab, 14.2 + 10.6 for ranibizumab, and 18.9 + 11.5 for aflibercept). Despite these results, there is no ophthalmic presentation of bevacizumab on the market to date.[36]

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. The high cost of these drugs promotes the search for lower-cost alternative therapeutic options, one of the reasons why the off-label use of bevacizumab is so prevalent in ophthalmic practice today. [40]

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

With the advent of biological therapy, such as intravitreal anti-VEGFs, for the treatment of various retinal diseases, along with their high level of efficacy, there have also been reports of related immunogenic reactions. Biologic drugs are produced by a living organism, mostly by genetically engineering living bacterial, animal, or plant cells. These drugs have large, complex structures, and possess the potential to induce an unwanted immune response. While not all cases of immune response lead to adverse reactions, the development of immunogenicity can impact the pharmacokinetics and pharmacodynamics of the biologic drug and; therefore, decrease its effectiveness. [43] [44] [45] [44] [46]

Immunogenicity refers to a host's immune response to an external agent; in relation to biological drugs, this response is manifested through the generation of anti-drug antibodies (ADAs). *Anti-drug antibodies*) by the immune system. It has been postulated that these are generated in response to free monoclonal antibodies due to their protein structure. The development of immunogenicity against a biologic drug is influenced both by factors inherent to the patient (age, sex, ethnicity, immune status, presence of allergies, history of inflammatory processes, and HLA classification) and by product-specific factors (origin, structure and molecular weight, degradation products, post-translational modifications, impurities, formulation and closure of the container, dose level, route of administration, among others). Two types of ADAs can be generated: epitope-neutralizing ADAs (anti-idiotypic ADAs), and non-neutralizing ADAs (binding-ADAs) directed against the main structure of monoclonal antibodies. Neutralizing ADAs compete with and bind to the antigen-binding site and thus reduce the amount of free drug available, resulting in treatment failure or resistance. The

clinical consequences resulting from an immune response range from the absence of measurable effects to treatment failure and, rarely, even life-threatening events. One of the most serious effects that is postulated to be induced by these ADAs is hypersensitivity or anaphylactic reactions to biotherapeutics, which can cause effects such as sterile endophthalmitis after intravitreal injections. [46] [43] [44][47][48][43][43] [44][43]

The demonstration of ADAs in the serum of patients who received intravitreal injections of anti-VEGF has been attributed to the response to molecules entering the systemic circulation through the aqueous humor drainage pathways. [43]

There are several advanced techniques for assessing ADAs, each with advantages and limitations. Among them, antigen-binding tests stand out for using antibodies or immobilized antigens to capture ADAs in biological samples. A significant advantage of these tests is their ability to incorporate acid dissociation steps, which allows interference caused by ADAs complexes to be eliminated with the drug. However, these techniques can be expensive and require rigorous protocols to ensure reproducible results. Electrochemiluminescence (ECL) assays offer exceptional sensitivity when using luminescent labels such as tris(bipyridine)ruthenium (II). These tags generate signals through oxidation-reduction reactions, which makes them highly effective even in the presence of high concentrations of the drug in circulation. ECLs are less prone to interference and allow for a wide dynamic range. Despite their advantages, ECLs require specialized equipment and can have high upfront costs for laboratories that do not have the appropriate technology. The radioimmunoassay (RIA), based on the use of radioactive isotopes such as iodine-125, provides extremely low detection limits, making it an effective option for samples with minimal levels of ADAs. However, handling radioactive materials involves safety risks and requires specialized facilities, which has reduced its popularity compared to more modern techniques. Homogeneous mobility displacement assays (HMSAs) combine size exclusion chromatography with ligand-binding techniques, allowing ADAs of different sizes or classes to be discriminated against. This makes them ideal for identifying ADA from different classes of immunoglobulins, such as IgG and IgM. Surface plasmon resonance spectroscopy (SPR) uses resonance waves generated at the interface between a conductive metal (such as gold or silver) and a dielectric medium, allowing changes in refractive index induced by molecular binding events on the sensor surface to be detected. One of the main advantages of MMR is its ability to provide detailed kinetic information, including the rates of

association and dissociation of ADAs to drugs. However, these techniques can be complex and require advanced technical expertise to implement and analyze. [46]

The enzyme-linked immunosorbent assay (ELISA) remains the most widely used method for detecting and quantifying ADAs due to its ease of use, low cost, and ability to process a high number of samples. There is different ELISA methods used for the detection of ADAs such as direct, indirect and bridging (or sandwich). Direct and indirect variants are straightforward, but face limitations in terms of drug interference and detection of low-affinity ADAs. In contrast, the bridging format, which uses two binding points in the ADAs, is highly specific and sensitive, although it can be more complex and requires additional optimization to avoid interference under conditions of high drug concentration. [46]

2.2 Background on the investigational product

2.2.1 Preclinical study on the ocular bioavailability of two bevacizumab solutions, PRO-169 vs Avastin®, following intravitreal application in albino rabbits in New Zealand.

A preclinical comparative bioavailability study was conducted that included 96 eyes from 48 healthy New Zealand albino rabbits. The research subjects were divided into two groups; Group 1 received an intravitreal dose (50 µL) of the PRO-169 test solution (bevacizumab 1.25 mg/0.05 mL) in both eyes, while group 2 received the reference product Avastin® (bevacizumab 1.25 mg/0.05 mL). In turn, each group was divided into 6 subgroups (4 subjects per subgroup) corresponding to the slaughter time points (1 hour, 1, 2, 5, 14, and 30 days) after the application of the drug. [49]

The primary pharmacokinetic parameters that were quantified and determined were the maximum concentration (C_{max}), maximum time (T_{max}) and half-life (T1/2); and the secondary pharmacokinetic parameter was the area under the effect curve (AUC0-t). All parameters were evaluated in three tissues: aqueous humor, plasma and vitreous humor after intravitreal administration of a test solution (PRO-169) compared to Avastin®. [49]

1. **Aqueous humor**: The similarity in bioavailability (C_{max} and AUC0-t) and Schuirmann's non-inferiority test were not met. The C_{max} was 67.07 ± 70.5 µg/mL (PRO-169) vs. 80.63 ± 53.63 µg/mL (Avastin®), reached approximately one hour after injection. The half-lives were 6.59 ± 1.17 days (PRO-169) and 7.65 ± 1.98 days (Avastin®), with total exposures of 7.41% and 7.64%, respectively.

2. **Plasma**: Similarity in bioavailability was also not achieved. The C_{max} was 22.05 ± 24.5 µg/mL (PRO-169) and 15.30 ± 6.6 µg/mL (Avastin®), with times of 3.00 ± 1.73 days and 5.67 ± 7.23 days, respectively. The AUC_{0-t} was higher for PRO-169 (268.67 ± 270.45 days*µg/mL) versus Avastin® (145.28 ± 86.57 days*µg/mL). No plasma concentrations were detected beyond 24 hours (<0.250 µg/mL).

3. **Vitreous humor**: The acceptance criteria for most analytical runs were met. The C_{max} was 540.52 ± 108.95 µg/mL after one hour for PRO-169, and 563.16 ± 83.75 µg/mL after one day for the Avastin® comparator. The half-lives were similar (4,991 ± 0.89 days vs. 5,182 ± 0.88 days, respectively), with no significant differences (p=0.711). The drug was not detected beyond day 30 (< 3.33 µg/mL).

The C_{max} in vitreous it was 593.75 ± 45.63 for PRO-169 vs 644.79 ± 62.65 µg/mL for Avastin® (p=0.136). The T_{max} was 0.53 ± 0.82 vs 0.85 ± 0.73 days (p=0.330), respectively. AUC0-t was 3837.72 ± 465.91 vs 4247.31 ± 93.99 days*µg/mL (p= 0.052). [49]

In conclusion, while there were no significant differences in vitreous humor, in aqueous humor and plasma the similarity in bioavailability between PRO-169 and Avastin® was not achieved.

2.2.2 Preclinical study on the safety and toxicity of PRO-169 (bevacizumab) vs Lucentis® (ranibizumab) after intravitreal application in albino rabbits in New Zealand.

48 eyes of 24 healthy New Zealand albino rabbits were studied randomized into two treatment groups (PRO-169 and Lucentis®). In turn, each group was divided into three subgroups (A, B, and C) according to the intravitreal application model. Only the right eye of the research subjects was injected; subgroup A received a single intravitreal injection. Groups B and C received two and three intravitreal injections, respectively, with a lapse of 31 days between each application. Ophthalmological check-ups were carried out after injection and one month after the last application of the treatments; Each group underwent an electroretinogram, blood sampling, euthanasia, enucleation for histopathological analysis of the eyeballs, and necropsy. [50]

The safety analysis of the study showed no significant differences in the primary variable cellularity in the anterior chamber between groups (PRO-169 and Lucentis®). Isolated cases of intraocular inflammation were reported in both groups, attributable to the injection procedure and not to the drug itself. [50]

Laboratory studies indicated that all hematological parameters were within normal ranges and without significant differences. In blood chemistry, blood nitrogen (BUN), *blood urea nitrogen*) was higher in the PRO-169 group, with no clinical relevance or relationship to treatment. Alterations in liver enzymes in one subject were considered isolated events, possibly related to the use of ketamine during anesthetic induction. One death was also reported in the PRO-169 group, with no previous signs of disease or conclusive findings in the necropsy. It is suspected that stress and previous anesthesia could have played a role. [50][50]

Electroretinogram studies performed at 30, 60, and 90 days showed that there were no significant differences between treatments in terms of retinal functionality. The parameters analyzed indicated that the integrity and sensitivity of the retina were not affected.

In conclusion, the safety and toxicity profile of PRO-169 was not inferior to that of Lucentis® in albino rabbits in New Zealand. [50]

2.2.3 Comparative preclinical study on the efficacy of PRO-169 (bevacizumab) and Avastin® (bevacizumab) in laser-induced choroidal neovascularization (CNV) in rhesus monkeys.

On behalf of Sophia Laboratories, this preclinical study was conducted by the Sichuan third party Primed Shines Bio-tech Co., Ltd., to compare the efficacy of the investigational product PRO-169 versus the reference product Avastin®, after intravitreal application in a laser-induced CNV model in rhesus monkeys (a total of 18 subjects were included in the study). [51]

CNV was induced by photocoagulation around the macular area of both eyes of each subject. A revision was performed by retinal fluorescein angiography (FA) 19 days later (day -2). According to the area of fluorescein leakage points, eligible animals were enrolled for treatment and randomly divided into three groups as shown below. [51]

Table 4. Characteristics of the treatment groups

Group	Trial Drug	Number of eyes enrolled	Dosage (mg/mL)	Dose volume (µL)	Dosage Quantity (mg)	Route of administration	Variables
1	Vehicle	7	0		0		Fundus photography
2	Avastin	7	25	50	1.25	Intravitreal injection	Fluorescein angiography
3	PRO-169	7	25		1.25		OCT

The administration of the treatments was carried out 21 days after photocoagulation (day 0) for all groups. Group 1 (vehicle) received a single dose; groups 2 (Avastin®) and 3 (PRO-169) received two additional doses administered on days 28 and 56 (see Figure 2. Overall Study Design)

Evaluations by fundus photography, AF, and OCT were performed before photocoagulation (day -31); before administration of investigational products (day -2); two weeks after administration of investigational products (day 14); and four weeks after administration (day 28), see Figure 2. Overall Study Design.

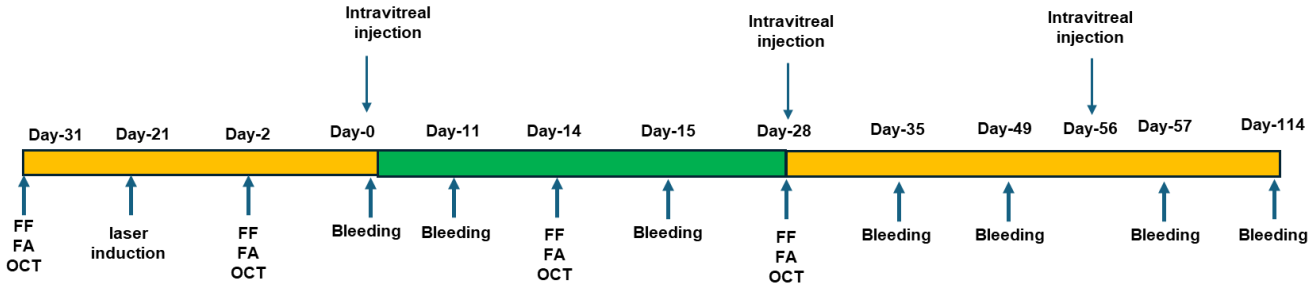


Figure 2. Overall Study Design

FF, fundus photography; AF, Fluorescein Angiography; OCT, Optical Coherence Tomography.

A total of 14 subjects (belonging to the three treatment groups) were included for the immunogenicity assessment. One mL of venous blood was extracted from animals belonging to group 1 (vehicle) on days 0, 77 and 114; and additionally, on days 11, 15, 35, 49 to subjects belonging to groups 2 (Avastin®) and 3 (PRO-169). [51]

After photocoagulation, features of CNV, such as increased retinal thickness and fluorescein leakage, were observed. Intravitreal administration of PRO-169 (1.25 mg per eye) significantly reduced both parameters on days 14 and 28, compared to the vehicle group, suggesting that this investigational product may inhibit CNV. [51]

In the immunogenicity evaluation, four samples tested positive for ADAs antibodies, but the confirmatory assays were negative, so it was not necessary to analyze neutralizing antibodies. It was concluded that the immunogenicity of PRO-169 was comparable to that of Avastin®. .[51]

2.3 Background on the investigation

2.3.1 Research Question

Is PRO-169 (bevacizumab) safe as it does not induce an unwanted immune response, after intravitreal application, in patients with MDD?

Bevacizumab is a humanized recombinant monoclonal antibody (the protein sequence corresponds to 93% human and 7% murine). For this type of medicine, the origin of the product is an important factor that can influence immunogenicity. It has been shown that murine antibodies elicit robust immune responses in humans compared to chimeric, humanized, and human monoclonal antibodies; however, it should be noted that the latter can also cause a high rate of immunogenicity depending on the dosage regimen and patient population. [52][53]

The consequences of an immune reaction to a therapeutic protein range from the transient appearance of ADAs without clinical significance, to conditions that impact the safety, pharmacokinetic properties and efficacy of the drug. [48][53][54]

A number of anti-VEGF drugs, including biosimilars, are currently marketed for ophthalmic use; however, its high cost has led to the off-label use of other drugs not authorized for this route of administration, such as Avastin® (bevacizumab). Immunogenicity profiles have been reported for anti-VEGF drugs authorized for intravitreal use; However, in the case of bevacizumab, the available information is very limited because this use is off label. The fact that bevacizumab is not available for ophthalmic use implies challenges such as differences in manufacturing standards between oncology and ophthalmic drugs, possible errors in fractionating the commercially available vial (4 mL/100 mg) into individual doses, as well as its conservation and storage in insulin syringes. All these factors can also influence an unwanted immune response. [55] [43][56] [57] [58]

Having PRO-169 in Mexico, an investigational product with the same active ingredient and in the same concentration as Avastin®, but for exclusively ophthalmological use and stored in a prefilled syringe, considerably reduces the additional risks previously mentioned. However, it is necessary to know its immunogenicity potential.

2.4 Risk-benefit assessment

2.4.1 Known potential risks

Many of the risks reported with intravitreal anti-VEGF treatments are related to the procedure itself. According to the information reported by Lytenava® (bevacizumab gamma 0.05 mL/1.25 mg, approved only in the European Union for intravitreal use), the most reported adverse reactions after its application were: conjunctival hemorrhage (5.0%), floaters (1.5%), eye pain (1.2%) and increased intraocular pressure (1.2%). Some serious but less frequent adverse reactions are transient blindness (0.3%), endophthalmitis (0.3%), intraocular inflammation (0.3%). Traumatic cataracts and retinal tear/detachment may also occur after intravitreal injection. [59] [60]

Among the adverse effects related to the drug class (so they can occur with any anti-VEGF, both intravitreal and systemic) are thromboembolic events, including stroke and acute myocardial infarction. Clinical studies in patients with neovascular age-related macular degeneration who received intravitreal bevacizumab gamma have shown a low incidence of arterial thromboembolic events. [59]

2.4.2 Known potential benefits

Preservation of visual function is the main goal with DME treatment. The importance of this lies in the growing incidence and prevalence of diabetes mellitus, as well as its complications, which continue to increase both in Mexico and in the world.

DME causes decreased central vision and therefore significantly affects patients' quality of life. The significant financial burden of treating diabetes mellitus and DME has a major impact on patients' lives. As a result, there are treatment deficiencies, changes in anti-VEGF medications, and variations in treatment intervals in routine practice. [61][62]

The evidence currently available supports the recommendation of early and intensive anti-VEGF treatment in patients with DME, as it provides greater visual acuity gains and more long-term stability; This improves patients' quality of life and opens the possibility of reducing the number of annual injections administered. [63]

In addition to the benefits shown for DME, anti-VEGF therapies have also demonstrated relevant benefits in terms of improvement and regression of DR severity, both non-proliferative and proliferative. [63]

Given the measures taken to minimize the risk to the participants in this study, the potential risks identified in association with PRO-169 are justified by the intended benefits that may be conferred on patients with diabetic macular edema.

More detailed information about the known and expected benefits, as well as risks and reasonably expected AEs of PRO-169 can be found in the Investigator's Manual.

2.5 Problem statement

DR and DME are the main ocular complications of diabetes mellitus and can lead to significant visual loss. Although the pathogenesis of these conditions is complex, VEGF overexpression plays a key role, which is why intravitreal anti-VEGF drugs are the cornerstone for the management of these diseases. [63]

Different treatment schemes have been proposed; however, in most cases, monthly injections are required to achieve anatomical and visual improvement, as well as to maintain the stability of the disease. These repeated injections cause a great economic burden for patients and can have an impact on adherence to treatment. [62][64]

The high cost of anti-VEGF drugs authorized for intravitreal use has led to the off-label use of other drugs not authorized for this route of administration, such as Avastin® (bevacizumab) and biosimilars. This involves splitting vials designed for oncology into individual doses for intraocular use, which entails safety risks, lack of standardization, and limited availability due to regulatory restrictions.

With the development of PRO-169, an investigational product with the same active ingredient and in the same concentration as Avastin®, but for exclusively ophthalmological use and stored in a prefilled syringe, it would guarantee a product designed and manufactured under standards that optimize its safety, efficacy and accessibility for the patients who need it most.

Because bevacizumab is a biopharmaceutical, clinical evidence that it does not induce immunogenicity after intravitreal administration is necessary.

2.6 Rationale for the study

Several clinical studies have validated the efficacy of bevacizumab in DME, showing it to be a comparable option to other VEGF inhibitors approved for this indication, but at a significantly lower

cost. This accessibility has meant that Avastin® (bevacizumab) or biosimilars manufactured for cancer use are widely used off-label in clinical practice.

This can present dosage, safety, stability, and asepsis risks if not performed under proper conditions; in addition to the fact that in our country this practice is not approved.

Laboratorios Sophia S.A. de C.V., has experience in preclinical studies on the bioavailability, safety and efficacy of PRO-169, and a phase III clinical study is currently being carried out in patients with DME. However, due to the nature of the drug, it is imperative to have a clinical study in this type of population that verifies that no unwanted immune responses are generated after its intravitreal application.

Additionally, it is worth mentioning that the inclusion of a pharmacokinetic (PK) cohort is intended to characterize the pharmacokinetic profile of PRO-169 in humans taking into account two important aspects: 1) the limited existing information on the systemic and ocular levels of the reference drug (Avastin®) after intravitreal administration; and 2) PRO-169 is a biopharmaceutical that shares structural characteristics with Avastin®, but corresponds to a compound specifically designed for ophthalmic use, an indication that is not approved for Avastin® so far.

It has been widely documented in the literature that the evaluation of intraocular pharmacokinetics (aqueous humor, vitreous humor, etc.) of ophthalmic drugs is not feasible in clinical studies, as it requires invasive techniques that expose participants to unnecessary risks. For these reasons, most pharmacokinetic data in ophthalmology are based on peripheral blood samples and population pharmacokinetic models that allow estimating interindividual exposure and variability from a limited number of samples per subject.[1] [2] [3]

In addition, the international guidelines for biotechnology products (FDA and EMA) mention the relevance of the study of immunogenicity and its possible effect on pharmacokinetics, particularly in repeated administration treatments.[4] [5] Therefore, the objective of including a PK cohort is to have information regarding the pharmacokinetic profile of PRO-169 after intravitreal administration in humans, as well as to evaluate the possible relationship between the presence of anti-drug antibodies (ADAs) and the influence it may have on drug elimination.

3. Objectives and hypotheses

3.1 Objectives

3.1.1 Primary security objective:

- To evaluate the safety of intravitreal PRO-169 by means of the presence of serum anti-drug antibodies (ADAs) for bevacizumab.

3.1.2 Secondary security objectives:

- To determine the incidence of adverse events (AEs) related to the investigational product.
- Determine the incidence of serious AEs related to the investigational product.

3.1.3 Objectives for the pharmacokinetics cohort:

- Calculate the total AUC of serum PRO-169 concentration.
- To quantify the serum C_{max} of PRO-169.
- To quantify the serum T_{max} of PRO-169.

3.1.4 Exploratory objectives:

- To assess the change in BCVA compared to baseline.
- To evaluate the change in GMC compared to baseline.

3.2 Hypothesis

This study does not propose any statistical hypothesis testing, so they are not considered formal statistical decisions.

4. Study design

4.1 Study Overview

Phase I clinical study, prospective, interventional, open-label, multicenter.

4.2 Rationale for the study design

The design of the study (clinical trial) is considered the highest standard of data quality when seeking to explore the effect of an intervention. The drug development phase (phase I) corresponds to the objective of the study, which is to evaluate safety. [65]

4.3 Expected duration

The total duration of the study, from the first visit of the first subject to the preparation of the final report, is estimated to be 14 months.

The approximate duration of each subject in the study is up to 95 days (counting from visit 1 [day 1]) or up to 103 days (counting from the screening visit [day -8 to 0]).

5. Study population

All subjects will be evaluated to determine if they meet the following inclusion and exclusion criteria. Prospective approval of deviations from the protocol's recruitment and enrollment criteria, also known as protocol exemptions, is not permitted.

5.1 Selection criteria

5.1.1 Inclusion criteria

To be considered eligible to participate in this study, subjects must meet all the criteria listed below:

1. Age ≥ 18 years.
2. Diagnosis of type 1 or 2 diabetes.
3. Ability to give signed informed consent.
4. Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
5. All subjects (male and female) who are biologically capable of having children will be required to agree and commit to using a barrier or hormonal method of contraception (by any route of administration) for the entire duration of the study, and for 3 months after the last intravitreal injection.
6. Female subjects, biologically capable of having children, will be required to have a negative urine pregnancy test at the screening visit.
7. Best Corrected Visual Acuity (AVMC) according to the 24- to 78-letter ETDRS primer (approximate Snellen equivalent of 20/32 to 20/320)
8. Diabetic macular edema with central involvement evidenced by spectral domain optical coherence tomography (central macular thickness criterion ≥ 300 µm for men and ≥ 290 µm for women) within 8 days prior to inclusion. All measurements made on a subject should be taken with an instrument of the same make and model during the conduct of the entire study.
9. Comply with characteristics that allow an adequate examination of the fundus of the eye (transparency of media, adequate pupillary dilation).
10. Glycosylated hemoglobin < 12% of a result not older than 3 months.

5.1.2 Exclusion Criteria

A subject will not be eligible for inclusion in this study if they meet any of the following criteria:

1. Chronic kidney disease in renal failure (glomerular filtration rate [GFRe] <15 ml/min/1.73m2) requiring dialysis or transplantation; according to the 2020 Clinical Practice Guideline for the Management of Diabetes in Chronic Kidney Disease (KDIGO).
2. Active proliferative diabetic retinopathy in the study eye, including rubeosis iridis, vitreous hemorrhage, or tractional retinal detachment visible during the screening visit.
3. Individuals who have required insulin treatment within 4 months prior to the screening visit for glycemic control.
4. Previous participation in clinical studies with investigational, ocular or systemic products (at least 30 days must have elapsed between the end of your participation in a previous trial and the inclusion of the present study).
5. Known hypersensitivity or allergy to bevacizumab, or any ingredient in the investigational product.
6. Poorly controlled blood pressure (average of 3 blood pressure readings in a seated position with ≥160 mmHg systolic or ≥100 mmHg diastolic) at the screening visit.
7. Myocardial infarction or other cardiovascular event (cerebral vascular disease, transient cerebral ischemia, or hospitalization for heart failure) during the 4 months prior to the screening visit, or subjects with active myocardial ischemia.
8. Systemic treatment with VEGF-related medications within 4 months prior to the screening visit.
9. History of any rheumatological or collagen disease of autoimmune origin related to inflammatory processes such as systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, Bechet's disease, dermatomyositis, among others.
10. History of any disease that causes immunosuppression or immunosuppression, except diabetes mellitus.
11. Concomitant use of immunosuppressive agents, immunotherapy, or monoclonal antibodies by any route of administration (other than intravitreal route), within 2 years prior to the screening visit or during the study period.
12. Subjects who have received intravitreal anti-VEGF therapy (aflibercept, ranibizumab, bevacizumab, faricimab, brolucizumab) in the study eye within 4 months prior to the

screening visit.

13. Use of intraocular or periocular corticosteroids in the study eye within 4 months prior to the screening visit, or use of intravitreal corticosteroid implants at any time.
14. Use of anticoagulants or antiplatelet agents by any route of administration within 10 days prior to the screening visit or during the study period.
15. Women of childbearing potential who are pregnant, nursing, or plan to become pregnant within the study period.
16. Allergy to fluorescein (topical or intravenous) or to numbing medications used during the injection procedure.
17. Subjects with non-diabetic macular edema.
18. Lens opacities that are superior in one or more of the following criteria according to the LOCS III classification *(Lens Opacities Classification System*): nuclear component > NO3C3 (opalescence/color), cortical component > C2 and posterior subcapsular component > P1.
19. History of eye surgery (cataract removal, any intraocular surgery, aphakia, etc.) or panretinal photocoagulation within 3 months prior to the screening visit or planned within the study time.
20. Intraocular pressure greater than 21 mmHg in the selected eye, measured by Goldmann tonometry at the screening visit.
21. Presence of macular ischemia or significant peripheveal capillary loss (increase in the foveal avascular zone greater than 350 µm) demonstrated by retinal fluorescein angiography (during the screening visit or in a study conducted within 4 months prior to the screening visit).
22. History of YAG capsulotomy in the study eye, within 30 days prior to the screening visit.
23. Evidence of external eye infections, intraocular inflammation, or significant ocular surface disease in either eye, 30 days prior to the screening visit.
24. History of uveitis in the eye of study.
25. History of detachment of
26. retina, vitrectomy, any type of filtering surgery, corneal transplant or dystrophy in the selected eye.
27. Advanced glaucoma or optic neuropathy in the selected eye.
28. Have a single functional eye (finger-counting BCVA or less in the eye with worse vision).

29. Have previously participated in this same study.
30. Be or have an immediate family member (e.g., parent/legal guardian or sibling) who is part of the research site or sponsor staff.
31. Subjects who present conditions that make it difficult or impossible to extract the blood sample, such as veins that are not visible or palpable, the presence of venous thrombosis in the sample collection area, severe edema, or any other criteria of the personnel in charge of performing this procedure.
32. Difficulty interpreting the optotypes of the ETDRS booklet.

5.1.2.1 Additional exclusion criteria for the pharmacokinetics cohort

1. Prior intravitreal treatment with bevacizumab in the contralateral eye, within 3 months prior to the screening visit.
2. Another intravitreal anti-VEGF therapy (ranibizumab, aflibercept, brolucizumab, faricimab) in the contralateral eye, within 30 days prior to the inclusion of the study or during the conduct of the study.

NOTE: Only one eye may be selected; if both eyes are eligible for inclusion, one eye will be selected at the discretion of the PI.

5.2 Criteria for elimination and/or substitution of subjects

5.2.1 Elimination Criteria

- Withdrawal of the FCI charter.
- Presentation of a serious adverse event related or not to the investigational product that, in the judgment of the PI and/or sponsor, could affect the subject's ability to safely continue with study procedures.
- No tolerability or hypersensitivity to any of the compounds used during the tests (tetracaine, tropicamide/phenylephrine, or fluorescein).
- No tolerability or hypersensitivity to the investigational product.
- Need to receive any medication, ophthalmic or systemic, that may interfere with the performance of the study procedures and/or analysis of blood samples.
- Greater deviation from the protocol.

5.2.2 Substitution of subjects

In order to evaluate the objectives of the study, the sponsor will have the power to substitute subjects in the following cases:

- Loss of follow-up.
- Withdrawal of the FCI charter.
- Errors in the collection of blood samples for pharmacokinetics. In this case, the subject will be excluded from the pharmacokinetics cohort, but may continue in the evaluations for ADAs, if they comply with the other procedures and do not present major deviations from the protocol.
- When, in the opinion of the laboratory, the blood sample obtained is unfeasible for processing and analysis.

It is considered that a loss of subjects ≤ 20% does not represent a problem for the validity of the results obtained.

5.3 Selection failures

A failure of selection is defined as those participants who agree to participate in the study, giving their informed consent, but who do not enter the study. It is necessary that the following information on selection failures be reported at least in the clinical record:

1. Demographics.
2. Details of the selection failure (specify whether it is due to eligibility criteria, which one, or some other reason for the failure).
3. Presence of serious adverse events during screening.

This is necessary to comply with the CONSORT (*Consolidated Standards of Reporting Trials*) guidelines for the publication of results and to respond to possible questions from regulatory authorities.

5.4 Recruitment and retention strategies

The duration of the subject's participation in the study is approximately up to 95 days (counting from visit 1 [day 1]) or up to 103 days (counting from the screening visit [day -8 to 0]), during which time you will be required to attend five visits in total in which clinical evaluations will be carried out

(screening visit, 1, 5, 6, and final); Subjects who are included in the pharmacokinetics cohort will require three additional visits for blood sample collection (visits 2, 3, and 4).

Strategies to improve subject retention include, but are not limited to:

- Inform the subject of the objectives of the study in a clear way.
- Make calls or send text messages to remind you of appointments or activities to do.
- Systematic organization of the study procedures, so that the subject does not last longer than necessary on his visit.
- Minimize subject wait times.

All recruitment strategies implemented by the center will be submitted for approval by the relevant committees.

5.5 Procedure in case of loss of follow-up

For this protocol, loss of follow-up is defined as those subjects who at some points were active subjects of the study, but their final evaluation could not be completed.

In the event that the subject does not terminate his/her participation due to withdrawal of informed consent(s) or major deviation, he/she will be asked to come to the site to perform the ophthalmologic evaluations and procedures indicated in the final visit; In the event that you do not agree to perform these procedures, the last visit in which your withdrawal was determined will be considered your final visit. Subjects who are removed due to the presence of AEs will continue with the follow-up that is defined until the closure of their AE.

In cases where the participating subject does not attend their appointment, the research center must make a call to find out the reason and will try to make a new appointment within the established window period or an unscheduled appointment to perform the procedures that the principal investigator deems necessary (clinical evaluation, AE evaluation, early termination, etc.). In these cases, three contact attempts should be made with a separation of 1 day between each one, which should be documented in the clinical note. If it is not possible to make an appointment within the window period, you will be asked to attend the early termination visit where the same procedures as the final visit will be performed. If an appointment for the early termination visit is not achieved, the subject will be considered as loss of follow-up and will be asked about the presence of adverse events and the reason for leaving the study, as minimum data.

In relation to subjects who are part of the pharmacokinetics cohort, who cannot attend the additional visits for blood sample collection (visit 2, 3 and 4) or present deviations related to the procedures, they may continue in the study as part of the total sample; however, they will be excluded from the pharmacokinetics cohort.

5.6 Identification of the subject

Study subjects will be identified by a number and the initials of their name.

The initials of the study subject will be obtained starting with the first letter of the name, followed by the first letter of the first surname and the first letter of the second surname, obtaining a maximum of three letters, in case the person has two names or compound surname, the first letter will always be used.

Example:

A. Arieh Daniel Carrizalez Market

a. Initials: AMC

B. Juan De la Torre Orozco

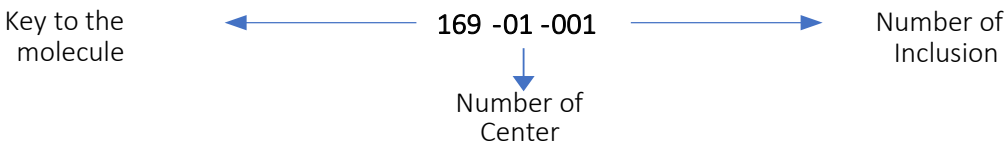
b. Initials: JDO

In the counting stage, the participant number will be assigned consecutively, using 3 consecutive digits.

Once the subject has been selected, they will be assigned a number with which they will be identified throughout the study. This code will be made up of eight numbers in the following order from left to right:

- Three digits of the molecule under study according to the name by the sponsor.
- Two digits correspond to the research centre number.
- Three digits of the number following their inclusion are assigned to the research center.

Example of assigned number:



The subjects' information will be handled confidentially by the sponsor using only the codes assigned to them. The research site will be responsible for safeguarding the information corresponding to the identity of the subjects.

6. Investigational product and treatment

6.1 Investigational product

- Product Name under investigation: PRO-169 . Bevacizumab (for ophthalmic use).
- Route of administration: Intravitreal injection.
- Pharmaceutical form: Solution for injection (25 mg/mL) for ophthalmic use.
- Solution Description: Translucent or slightly yellow particle-free solution.
- Presentation: Prefilled glass syringe for administration of 0.05 mL/dose.
- Packaging description: Type 1 glass syringe, with rubber stopper.
- Prepared by: Laboratorios Sophia S.A. de C.V., Zapopan, Jalisco, Mexico.

6.2 Investigational Product Dosage

- Dosage: 1 intravitreal injection every 30 days, for two months (3 doses will be applied in total).

Additional information on formulation is provided in the investigator's manual.

6.3 Treatment with the investigational product

The investigational product will be administered at the research site during visits 1, 5, and 6. The administration of the product will be in charge of the principal investigator or sub-investigator.

6.4 Storage and handling of the investigational product in the study center

The transport of the study drug will be carried out with continuous temperature monitoring, which must be properly recorded. The delivery will be made by means of a courier service contracted by the sponsor, expressly selected for this purpose, to the address of the research center according to the study plan.

The reception will be carried out by the assigned personnel of the research team. You will need to check that the primary packaging (box) is in good condition. If you show alterations or defects in its integrity that in your judgment could have damaged the content, you must report it to the sponsor. If the package does not show significant defects, it will proceed to open it.

Inside the shipment you must locate the acknowledgment document and the temperature meter (*data logger*). You must check that the temperature recorded complies with what is specified for transport and safekeeping. It will verify the content (PI) with what is reported in the document. In case the document corresponds to the content, you will sign the receipt and send it to the sponsor. Otherwise, it will notify the sponsor.

Storage and safeguarding are the responsibility of the research center. The medication must be stored in a secure area with restricted access and temperature control.

The storage temperature should be refrigerated between 2 and 8°C, it should be out of reach of light and in its packaging until use.

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

From its receipt at the center and until it has no stock of PI stored, the research center has the obligation to review daily (twice a day) the storage conditions of the PI and manually record, in the designated format, the temperature marked by the *data logger* (current, minimum and maximum temperature). Such data will be reviewed by the clinical monitor during their monitoring visits according to the records stored in the *data logger's memory*.

In the event of loss of the product under investigation, this must be documented in the logbook of inputs and outputs along with a clear description of the mechanism by which the loss occurred.

Once treatment/control has been administered, used syringes/vials should be returned to storage and recorded in the designated format.

Upon completion of the protocol, all study material will be retrieved by the sponsor as part of the closing visit. The final delivery of material will be made by the principal investigator or the person designated by him to deliver material at the end of the study. The refund will be made by the research center when the sponsor so indicates. Prior to the return, the research center must carry out a count of the assigned medication and the remaining medication, to create an inventory which serves for the final completion of the medication return form.

The sponsor reserves the right to initiate civil and criminal actions against the principal investigator for lack of undocumented material after the study.

6.5 Concomitant treatments and medications (permitted and prohibited)

The investigator shall instruct the subject to notify the study site of any new medications they take after signing the FCI. All major medications, procedures, and nondrug therapies (e.g., blood transfusions) administered after the patient is enrolled in the study must be recorded on the appropriate eCRF page.

Each concomitant drug or procedure must be evaluated individually against all exclusion criteria/prohibited drugs. When in doubt, the investigator should contact the sponsor's medical monitor before enrolling in a subject or allowing a new drug to be initiated.

6.5.1 Permitted Medications

6.5.1.1 Ophthalmology

The use of ophthalmic drops necessary to perform the corresponding ophthalmological examinations (tetracaine 0.5%, tropicamide 0.8% / phenylephrine hydrochloride 5%, and fluorescein), antibiotics, corticosteroids or topical medications in the study eye will be allowed.

If the contralateral eye has DME or other conditions, it may be managed with standard treatment, at the discretion of the investigator (e.g., anti-VEGF treatment other than bevacizumab), if it does not interfere with the protocol's schedule of visits. This only applies to subjects who are not part of the pharmacokinetics cohort (see section 5.1.2.1 Additional exclusion criteria for the pharmacokinetics cohort)

6.5.1.2 Systemic

Any therapy that is deemed necessary for the subject's well-being, and that does not interfere with the evaluation of the investigational product, as well as the use of hormonal contraceptives by any route of administration.

6.5.2 Prohibited Drugs

6.5.2.1 For the Study Eye

Patients will not be able to receive any additional standard or experimental treatment to treat DME in the selected eye; This includes medications given locally (intravitreal, topical, periocular, or

periorbital). They will only be able to receive the investigational product PRO-169 as specified in the protocol.

6.5.2.2 For the contralateral eye

Patients participating in the pharmacokinetics cohort may not receive any anti-VEGF treatment in the contralateral eye during their participation in the study.

7. Study methods and procedures

7.1 Research center

This study will be carried out in several research centers previously evaluated by the sponsor. The centers will be institutions or establishments where health research is carried out that complies with current regulations.

The research centers will be responsible for forming a multidisciplinary research team to execute clinical study according to the protocol. It is their prerogative to design the organization and select the personnel who will perform the functions. However, it is necessary for the sponsor that the PI and sub-investigator be ophthalmology specialists, and that at least one of them has a subspecialty in retina.

Any person who is designated, under the responsibility of the PI, as a part of the study monitoring (sub-investigator, nurse, etc.) or a specific function of participation in the study (pharmacist, administrative assistant, study coordinator, etc.) must appear in the "Delegation of Responsibilities".

The competence and training of any person who has direct participation in the activities of the study must be verified prior to the performance of any activity related to the protocol. The above must be recorded and the documents that constitute evidence of this competence and/or training must be kept in the master file of the study. The competence and training of the staff who have functions in the study, both at the central level and in the study center, is the responsibility of the sponsor.

The sponsor must ensure that all study site personnel participating in the study are adequately trained on the study (research protocol, investigator's manual, amendments, standard operating procedures, etc.) and on ICH 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 record of the study.

7.2 Clinical Study Registration

This clinical study will be registered by the sponsor in public clinical trial registries before its start (inclusion of the first subject): National Registry of Clinical Trials (RNEC) of the Federal Commission for the Protection against Health Risks (COFEPRIS) and in a WHO primary registry platform. WHO

Primary Registries meet specific criteria for content, quality and validity, accessibility, unique identification, technical capacity and administration. WHO Primary Registries meet the requirements of the International Committee of Medical Journal Editors (ICMJE).

7.3 Group assignment and blinding

As it is an open-label, non-comparative study, randomization of the subjects entering the study is not necessary.

7.4 Study variables

7.4.1 Safety Primary Outcome Variables

- Proportion of patients with the presence of bevacizumab-positive serum ADAs.

7.4.2 Safety Secondary Outcome Variables

- Incidence of adverse events related to IP.
- Incidence of serious adverse events related to IP.

7.4.3 Pharmacokinetic Secondary Outcome Variables

- Total area under the curve (AUC) of serum PRO-169 concentration.
- Serum peak (C_{max}) concentration of PRO-169.
- Maximum serum time (T_{max}) of PRO-169.

7.4.4 Exploratory variables

- Changes in BCVA from baseline to day 90.
- Changes in the GMC, from baseline to day 90.

7.4.5 Definition of variables, methods and scales for their measurement

Table 5. Operational definition of variables.

Variable	Conceptual Definition	Operational Definition	Measurement Type	Reference value	Statistical test
Presence of serum ADAs for bevacizumab	They are antibodies generated by the immune system that are directed against therapeutic drugs, in particular biological products such as monoclonal antibodies, enzymes or proteins.	Baseline result without the presence of ADAs (i.e., the one obtained in the blood sample for immunogenicity collected at the screening visit) and at least one sample with the presence of ADAs after the start of PRO-169 treatment. Baseline result with the presence of ADAs and at least one sample with a result ≥ 4 times in baseline titer values, after the start of treatment with PRO-169	Qualitative dichotomous Continuous Quantitative *	Present / Absent NA	• Frequency • Percentage
Changes in the AVMC	Visual spatial acuity is the ability to distinguish separate elements of an object and identify them as a whole. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects	Using the ETDRS booklet, the subject's best refractive correction (obtained by autokeratorrefractometer and subjective tests) will be evaluated and the examination will be repeated using the refraction obtained	Discrete quantitative	Up to 54 letters LogMAR	• Media • Medium • Standard deviation • Minimum and maximum • 95% CI
Changes in central macular thickness	It corresponds to the thickness determined in the area of the fovea taking the fovea as the central point of a 1mm circle	Change in OCT between the initial and final value	Continuous quantitative	Up to 290 µm in women and 300 µm in men	• Media • Medium • Standard deviation • Minimum and maximum • 95% CI
Incidence of IP-related adverse events	AEs are considered to be any adverse medical event that occurs in a patient or clinical research subject to whom a pharmaceutical product was administered and where the causal relationship is at least reasonably possible. [66]	EA that the PI suspects a causal relationship with the treatment	Nominal qualitative	- Incidence - Seriousness (severity) - Causation	• Frequency • Percentage

Variable	Conceptual Definition	Operational Definition	Measurement Type	Reference value	Statistical test
Incidence of serious adverse events related to IP	Serious AEs are considered to be any AEs that result in: death, threat to life, require hospitalization or prolong hospitalization, are causes of permanent or significant disability or disability, are the cause of alterations or malformations in the newborn, or other medically important conditions [66]	All AEs (according to protocol) suspected by the PI of a causal relationship to the treatment will be reported	Nominal qualitative	- Incidence - Seriousness (severity) - Causation	• Frequency • Percentage
Area Under Total Curve (AUC)	It reflects the total amount of drug that reaches the systemic circulation. It is an important measure of bioavailability and relates variations in the serum concentration of a drug as a function of time	The AUC will be reported (for details on reporting or measurement, refer to the validation protocol and/or laboratory manual provided by the designated laboratory)	Continuous quantitative	NA	• Media • Standard deviation • Geometric median • Range • Minimum and maximum
Maximum concentration (C_{max})	Maximum serum drug concentration	C max will be reported (for details on reporting or measurement, refer to the validation protocol and/or laboratory manual provided by the designated laboratory)	Continuous quantitative	NA	• Media • Standard deviation • Geometric median • Range • Minimum and maximum
Maximum Time (T_{max})	It is the time expressed in minutes that indicates the moment when the drug reaches its maximum concentration in serum	The T max will be reported (for details on reporting or measurement, refer to the validation protocol and/or laboratory manual provided by the designated laboratory)	Continuous quantitative	NA	• Media • Standard deviation • Geometric median • Range • Minimum and maximum

Abbreviations: ADAs, anti-drug antibodies; NA, not apply; ETDRS, Study on the Early Treatment of Diabetic Retinopathy; OCT, optical coherence tomography; PI, principal investigator; BCVA, best-corrected visual acuity; AE, adverse event; PI; Investigational product; 95% CI: 95% confidence interval.

7.4.6 Description of the variables, methods, and scales for their measurement

For clinical purposes, ophthalmological assessments will be carried out on the selected eye, and its record will be recorded in the clinical file and in the eCRF. Next, the description of the variables is made, which are not in order of execution.

7.4.6.1 Anti-drug antibodies

Anti-drug antibodies (ADAs) are produced by the immune system in response to biopharmaceuticals that are recognized as foreign molecules. In the case of therapeutic antibodies, ADAs bind to the idiotope located in the Fv region, some can neutralize them (neutralizing antibodies: Nab), decreasing efficacy and increasing the clearance of the biopharmaceutical; resulting in a failure in the patient's response leading to worsening of the disease. Likewise, ADAs can induce allergic reactions such as anaphylactic shock; so, it is necessary to evaluate their presence and impact. [53]

Blood samples will be collected according to schedule (see 1.3 Schedule of activities)

1.3 Schedule of activities

		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Visit of Selection		Start of treatment	Treatment Period					Final / Early Termination
Protocol Day	-8 to 0	1	2	4	8	30±5	60±5	90±5
Informed consent	X							
Evaluation of selection criteria	X							
Medical History	X							
Weight, Height, BMI and Body Mass Surface Area	X							
Vital signs (BP, HR, Temperature, RF)ϕ	X	X				X	X	X
% HbA1c*	X							
Glomerular filtration rate (GFRe)[a]	X							
Concomitant medications	X	X	X	X	X	X	X	X
Adverse Event Registry	X	X	X	X	X	X	X	X
Pregnancy Test	X	X				X	X	X
Best-corrected visual acuity	X					X	X	X
Biomicroscopy	X					X	X	X
Indirect ophthalmoscopy	X					X	X	X
Ocular tonometry	X					X	X	X
Macular optical coherence tomography	X					X	X	X
Fluorescein angiography (AF) or optical coherence tomography angiography (OCT-A)[lf]	X							
Intravitreal injection		X				X	X	
Pharmacokinetic blood sample (applies to PK cohort)[ŧ]	X	X	X	X	X	X¥	X¥	X¥
Blood Sample Immunogenicity	X					X¥	X¥	X¥

ϕ Vital signs assessment should be performed prior to blood sample collection.
*If there is an HbA1c determination dated within 3 months prior to the inclusion of the study, it will not be necessary to perform it at the screening visit.
[a] If a serum creatinine result dated within 2 months prior to study inclusion is available, it will not be necessary to perform it at the screening visit and may be used to calculate GFRe.
[lf] you have an AF or OCT-A performed in the 4 months prior to the inclusion of the study, it will not be necessary to perform it at the screening visit.
[ŧ]See pharmacokinetics schedule on the following page.
¥Blood samples will be collected prior to intravitreal application of PRO-169 (no more than 2 hours prior to the time of application at Visit 1).

For details on the method of processing, quantification, validation and its measurement, refer to the validation protocol and/or laboratory manual provided by the designated laboratory, in charge of the analysis of samples.

7.4.6.2 Area under the total curve of serum concentration

In pharmacology, the area under the curve of the plasma or serum concentration of a drug as a function of the time elapsed since administration, called the "area under the curve" or AUC, allows us to know the degree of exposure to a drug and its rate of elimination from the body. By integrating over time rather than analyzing individual concentration measurements, a more accurate estimate of overall drug exposure is obtained. These measurements have also been found to be significant in assessing the net pharmacological response to a given dose of drug. [67]

Blood samples will be collected according to schedule (see 1.4 Pharmacokinetics Cohort Schedule).

For details on the method of processing, quantification, validation and its measurement, refer to the validation protocol and/or laboratory manual provided by the designated laboratory, in charge of the analysis of samples.

7.4.6.3 Maximum serum concentration

Plasma or serum concentrations reflect a time curve from the administration of a drug to its maximum effect and final elimination. The maximum concentration (C_{max}) of a drug in the blood serum represents the maximum effect of the drug. The C_{max} It is one of the main pharmacokinetic measures to evaluate how the body acts in the face of a drug. [68]

Blood samples will be collected according to schedule (see 1.4 Pharmacokinetics Cohort Schedule).

For details on the processing method and its validation, as well as the scales for its measurement, refer to the validation protocol and/or laboratory manual provided by the designated laboratory, in charge of the analysis of samples.

7.4.6.4 Maximum Concentration Time

Plasma or serum concentrations reflect a time curve from the administration of a drug to its maximum effect and final elimination. The time to reach maximum concentration (T_{max}) is of pragmatic importance because it varies between individuals and can be manipulated through the different routes of administration (e.g., oral, intravenous, and topical) or by modifications of the drug delivery mechanism. [69]

The T_{max} it is usually related to the half-life of a drug (T1/2). Drugs with short half-lives tend to peak and be eliminated quickly, so more frequent doses are often required to keep a drug within its clinically effective therapeutic range. [69]

Blood samples will be collected according to schedule (see 1.4 Pharmacokinetics Cohort Schedule).

For details on the processing method and its validation, as well as the scales for its measurement, refer to the validation protocol and/or laboratory manual provided by the designated laboratory in charge of the analysis of samples.

7.4.6.5 Adverse events

As described in the 8. Evaluation and management of adverse events, an adverse event is defined as Any undesirable medical event that may occur in an investigational subject during the clinical research stage of a drug or vaccine but does not necessarily have a causal relationship with it.

Management of adverse events will be performed as described in the 8. Evaluation and management of adverse events.

The Principal Investigator will record in the corresponding section of the eCRF the adverse events that the study subjects may present in addition to referring them in the clinical record.

For an adequate evaluation of adverse events, in addition to directed questioning, it is necessary to perform a comprehensive ophthalmological evaluation at each visit, which consists of: ophthalmological examination of the eyelids and adnexa; anterior and posterior segment that is performed in a routine ophthalmological check-up, whose procedures are not specifically included in the study variables. Posterior pole evaluation can be with direct or indirect ophthalmoscopy, with or without pharmacological mydriasis, at the discretion of the PI. An assessment of the fundus will be carried out in search of abnormalities that alter the result of the study. IOP will be measured in this assessment with the Goldmann tonometer and should be measured after the evaluation of stains (when applicable). The result of the assessment will be recorded in the clinical file. In the eCRF, only findings that are considered by the principal investigator will be reported as an adverse event.

7.4.6.6 Best Corrected Visual Acuity

Visual acuity (VA) is a test of visual function. Visual spatial acuity is the ability to distinguish separate elements of an object and identify them. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects. [70]

VA will be evaluated at baseline, without refractive correction, with the patient 4 meters from the backlit ETDRS panel. The panel must be calibrated to output 85 cd/m2. Tests will be done on both eyes (both the selected eye and the non-selected eye). Each eye should be tested with a chart with a different distribution of optotypes 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.

Next, the subject's best refractive correction (obtained by autokeratorrefractometer and subjective tests) will be evaluated and the examination will be repeated using the refraction obtained. The total number of letters recognized by the patient will be reported as best-corrected visual acuity (BCVA), which will be recorded in the clinical record and in the eCRF for both eyes. Forstatistical purposes, the value obtained from the AVMC in logarithmic figures (LogMAR) should also be recorded, as well as the Snellen equivalent in the clinical record and in the eCRF (see annex 16.1 ETDRS Booklet).

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

Table 6. Equivalences for 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 take the best corrected visual acuity, the patient will be asked to indicate the letters he distinguishes until he reaches the line where he cannot identify at least three optotypes, recording the number of letters that he could correctly specify.

It will also be required that in the case of vision cards that do not start from the 20/800 line of sight (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 yield.

Table 7. Adding letters to the ETDRS booklet

Start of optotypes of the primer 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

Below, some examples will be presented to minimize the possibility of errors or ambiguities.

Example 1.

Whereas one patient was able to distinguish the optotypes marked in green in this image, he was able to distinguish three optotypes from the fourth line and only two from the fifth line. The line attributed to him 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 he distinguished, we have 18 letters. However, this booklet begins at 20/200, so according to the table in section 7.4.6.6 Visual acuity best corrected

according to the ETDRS guidelines, the figure of 30 must be added to that number, 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.

Considering that one patient was able to distinguish the optotypes marked in green in this image, he was able to distinguish up to three optotypes of the fifth line. The line attributed to him 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 that he distinguished, we have 23 letters. However, this booklet begins at 20/200, so according to the table in section 7.4.6.6 Visual acuity best corrected according to the ETDRS guidelines, the figure of 30 must be added to that number, 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.

The BCVA of both eyes (right eye [OD] and left eye [OS]) will be reported, for statistical purposes, in Snellen fraction and in logarithmic figures (LogMAR) in the clinical record and in the eCRF.

7.4.6.7 Macular optical coherence tomography (OCT)

It is a diagnostic test for the evaluation of the condition of the retina, including central macular thickness (CMG) and central retinal volume (macular area volume known as cube volume in OCT). This test uses light to create high-resolution images, allowing layers of the retina to be visualized. [71]

The make and model of the instruments used will be at the discretion of the investigating physician, provided that, for each individual patient, the same equipment (brand and model) is used throughout the study. The procedure can be performed by the PI, sub-investigator, or specialized personnel with the ability to operate the delegated OCT equipment. The results of the GMC of both eyes will be reported in the clinical record and in the eCRF.

7.5 Description of procedures or assessments during the study

The different procedures that will be done during the study are described below. The list may not be in order and may be sorted in the most optimal way according to the needs of the research center.

7.5.1 Signing of informed consent

Procedure by which it is guaranteed that the subject under investigation has voluntarily expressed his approval and authorization to participate in this research, after having understood the information that has been given to him or her about the objectives of the research, as well as its benefits, discomforts, and possible risks. In this study, this procedure may be repeated since there will be a subgroup of patients (15 of the 60 total participants) who will be included for the pharmacokinetic evaluation of the investigational product, in which case two independent consents must be signed.

7.5.2 Medical history (general and ophthalmological)

It must be carried out in accordance with the provisions of NOM-004-SSA3-2012 of the clinical file. Considering that the clinical record is the unique set of information and personal data of a subject, which is integrated into any type of establishment for medical care, whether public, social or private, which consists of written, graphic, imaging, electronic, magnetic, electromagnetic, optical, magneto-optical and any other type of documents; in which, health personnel must make the

records, annotations, where appropriate, certificates and certifications corresponding to their intervention in the subject's medical care, in accordance with the applicable legal provisions.[72]

It will consist of:

Medical history: it must be prepared by medical personnel and other professionals in the health area, in accordance with the specific information needs of each of them in particular, it must have, in the order indicated, the following sections:

- Interrogation. It must have at least: identification form, if applicable, ethnic group, hereditary family history, pathological and non-pathological personal history, current condition (inquire about previous conventional, alternative and traditional treatments), and interrogation by devices and systems (especially ophthalmological).
- Physical exam. You must have at least: vital signs (temperature, systemic blood pressure, heart and respiratory rate), weight, height, BMI and body mass surface area (mandatory).
- Previous and current results of laboratory, cabinet and other studies (when applicable).
- Diagnoses or clinical problems (when applicable).
- Prognosis (when applicable).
- Therapeutic indication (when applicable).

7.5.3 Adverse events

Described in the 8. Evaluation and management of adverse events.

7.5.4 Evaluation of selection criteria

It is the evaluation that the subject meets all the inclusion criteria and none of the exclusion criteria.

7.5.5 Measuring Vital Signs

It is the measurement of heart rate, respiratory rate, temperature and systemic blood pressure. These measurements can be made with a stethoscope, digital or mercury thermometer, and with a manual or digital sphygmomanometer. This information must be contained in the patient's medical record. It is necessary that all measures be in equal circumstances.

7.5.5.1 Heart rate

Heart rate is the number of times the heart ventricles contract per unit of time, usually per minute. Its measurement will be done by direct auscultation in the chest with a stethoscope counting the

beats for one minute. In the case of women, it will be allowed to measure it in the pulse of the wrist; however, the method chosen for the subject will be the same to be used in all visits. [73]

It is necessary for the subject to be calm and in a state of rest (the subject does not have agitation because of unnecessary physical effort). For the measurement of the heart rate, it will be necessary for the subject to be calm and in a state of rest. You will be asked to rest for at least 5 minutes upon arrival at the clinic, prior to your measurement. The results will be reported in the clinical record and in the eCRF.

7.5.5.2 Respiratory rate

Respiratory rate is the number of complete breathing cycles that occur per minute. Its measurement will be done by direct auscultation in the chest with a stethoscope counting the beats for one minute. To measure the respiratory rate, it will be necessary for the subject to be calm and in a state of rest. You will be asked to rest for at least 5 minutes upon arrival at the clinic, prior to your measurement. The results will be reported in the clinical record and in the eCRF.

7.5.5.3 Temperature

The temperature will be taken through the axillary route, a digital or mercury thermometer may be used. The tip of the thermometer should be placed in the center of the armpit, making sure that the entire area responsible for recording the temperature is in contact with the skin and should be held firmly against the body, with the subject's arm close to the side of the body. The results will be reported in the clinical record and in the eCRF.

7.5.5.4 Systemic blood pressure

Blood pressure can be measured with a manual or digital sphygmomanometer, making sure that the chosen device is calibrated according to the manufacturer's recommendations. Regardless of the type of device used, it is essential that a bracelet of the right size for the reading is chosen in order to obtain an accurate measurement.

For the measurement of blood pressure, it will be necessary for the subject to be calm and in a state of rest. You will be asked to rest for at least 5 minutes upon arrival at the clinic, prior to your measurement. During the measurement, the subject should be seated with the right or left arm resting on a flat surface, with the cubital fossa at the level of the heart. The results will be reported in the clinical record and in the eCRF.

7.5.5.5 Body Mass Index (BMI)

Waist circumference, weight and height will be taken, and the BMI calculation will be made based on the Quetelet formula. The results will be reported in the clinical record and in the eCRF. [74]

$$IMC = \frac{peso\ (kg)}{altura\ (m)^2}$$

7.5.5.6 Body mass surface area

Waist circumference, weight and height will be taken, and the calculation of the body mass surface area also known as BSA (*Body Surface Area* will be made using the Mosteller formula. The results will be reported in the clinical record and in the eCRF. [75]

$$BSA = \sqrt{\frac{peso\ (kg) \times altura\ (cm)}{3600}}$$

7.5.6 Laboratory tests

The designated personnel will perform the extraction of the blood sample, by means of venipuncture. The vein will be chosen at the discretion of the personnel in charge and will perform asepsis of the skin of the area to be punctured prior to the procedure. Samples will be processed at the local laboratory or selected by the site. The following parameters will be evaluated: HbA1c and GFRe.

7.5.6.1 Glycosylated hemoglobin (HbA1c)

HbA1c is a key indicator in the evaluation of glycemic control in long-term diabetic patients. This parameter reflects the average blood glucose concentration over a period of 2-3 months. In the context of DME, monitoring of HbA1c is crucial, as elevated levels of this protein are closely linked to suboptimal glycemic control, which increases the risk of microvascular complications, such as DME. Knowledge of HbA1c levels allows healthcare professionals to adjust diabetic treatment in a timely manner, favoring the prevention of DME and preserving the patient's visual function. [16]

If there is an HbA1c determination 3 months prior to the inclusion of the study, it will not be necessary to perform it at the screening visit.

7.5.6.2 Glomerular filtration rate (GFRe)

The glomerular filtration rate is the rate in milliliters per minute at which plasma substances are filtered through the glomerulus; In other words, it refers to the removal of a substance from the blood. [76]

If there is a serum creatinine result in the 2 months prior to the inclusion of the study, it will not be necessary to perform it at the screening visit, and this can be used to calculate the GFRe. The results will be reported in the clinical record and in the eCRF.

Estimating GFRe requires equations that consider serum creatinine, age, race, and sex.
One of them is the CKD-EPI equation (*Chronic Kidney Disease Epidemiology Collaboration*) and is considered the most accurate; for this reason, and to standardize the calculation of the GFRe, we request that it be used in this clinical study. [76]
Below are suggestions for websites where you will find calculators to estimate GFRe with the CKD-EPI formula:

- https://www.merckmanuals.com/medical-calculators/GFR_CKD_EPI-es.htm
- https://www.samiuc.es/calculo-del-filtrado-glomerular-ckd-epi/
- https://www.senefro.org/modules.php?name=calcfg

7.5.7 Urine Pregnancy Test

Pregnancy testing will be performed at screening visits, 1, 5, 6, and 7 for all female research subjects of reproductive age (i.e., no natural or induced menopause, menopause defined as 12 consecutive months of amenorrhea) with hormonal contraception or intrauterine device (IUD), including bilateral tubal obstruction. For patients with bilateral hysterectomy or oophorectomy, it will not be necessary. To perform the test, the subject will be allowed to go to the bathroom, have privacy for the collection of the urine sample that will later be delivered to the personnel of the corresponding site who will perform the test, and after it is performed, the researcher must corroborate the result by observing the medical device. The results will be reported in the clinical record and in the eCRF.[77]

7.5.8 Ophthalmological evaluation

It refers to the evaluation of the eyeball, eyelids, eyelashes, and other structures of your eyes by means of inspection, slit lamp (biomicroscopy), as well as palpation (touch). As part of this

evaluation, the BCVA and the anterior segment will be assessed, intraocular pressure will be taken and the posterior segment will be evaluated (indirect ophthalmoscopy). This evaluation is intended to identify alterations that may interfere with the course of the investigation or to identify AEs. The results will be reported in the clinical record and in the eCRF.

7.5.8.1 Biomicroscopy

It is the diagnostic procedure that uses the slit lamp, also known as a biomicroscope, to evaluate the structures of the anterior segment (cornea, conjunctiva, anterior chamber, iris, pupil including pupillary diameter, lens, aqueous humor), using the specific characteristics of the light source such as the optical power of the device. This method allows for a detailed evaluation of the anterior chamber of the eye. [78]

7.5.8.2 Ocular tonometry

Tonometry is the objective measurement of IOP, based primarily on the force required to flatten the cornea or the degree of corneal indentation produced by a fixed force. Goldmann tonometry is based on the Imbert-Fick principle. [79]

The tonometry will be performed with a Goldmann tonometer, after instillation of the ophthalmic anesthetic, with fluorescein and the use of the cobalt blue filter (after the evaluation of the surface staining). 2 samples will be taken, and the average will be calculated, which will be recorded in the clinical file. The average IOP of the eye under study (OD or OS) will be recorded in the eCRF. Two measurements will be made in the study eye, the average of two shots is recorded, therefore, results will be obtained in whole numbers or means (0.5) will not be rounded.

Ocular tonometry will be performed prior to the application of intravitreal injection, when applicable.

The same measuring instrument used at the screening visit and during all subject visits should be used. The appliance must be calculated according to the manufacturer's specifications. The results will be reported in the clinical record and in the eCRF.

7.5.8.3 Indirect ophthalmoscopy

Indirect ophthalmoscopy, or fundoscopy, is the examination done with a light and a magnifying glass to observe the fundus of the eye (optic nerve and retina) through the pupil. Sometimes the pupil will need to be dilated so that the fundus of the eye can be fully evaluated. [78]

During the baseline and final visits, fundoscopy will be performed, as part of the comprehensive ophthalmological check-up, it will be at the discretion of the investigator if it is performed under pharmacological mydriasis. The results will be reported in the clinical record and in the eCRF.

7.5.9 Retinal angiography

Retinal angiography is a diagnostic procedure that allows the blood circulation of the retina and choroid to be assessed. Two techniques are currently available, fluorescein angiography and optical coherence tomography angiography; each one requires different devices, supplies and trained personnel for its realization. [80]

The angiographic technique to be used will be up to the PI and/or sub-investigator.

7.5.9.1 Fluorescein angiography

Fluorescein angiography (FA) is an essential diagnostic technique for the evaluation of diabetic retinopathy and DME. Prior to fasting for 8 hours and pupillary dilation with TP Ofteno® (tropicamide 5% / phenylephrine 0.8%), this procedure consists of the intravenous injection of a fluorescein solution, usually in one of the upper extremities. [81]

Designated personnel shall be responsible for venipuncture. The selection of the vein to be used will be determined at the discretion of the professional in charge, who must perform an adequate asepsis of the skin in the puncture area before proceeding.

Images of the retinal vasculature are then captured using a specialized camera. The technique makes it possible to identify areas of leakage, capillary non-perfusion, and other abnormalities, which is crucial for classifying diabetic retinopathy, excluding patients with macular ischemia or significant peripheveal vascular loss, and assessing the severity of DME. In addition, it plays a fundamental role in monitoring the response to treatment and appropriate therapeutic planning. [81]

It is not essential that this equipment be of a particular brand or model, as long as a device of the same characteristics is used to assess the same patient throughout the study, in case AF is necessary in subsequent visits. The procedure can be carried out by the PI, a technician or delegated personnel. You may be asked for a period of fasting prior to taking the study, which will be determined by the site's delegated staff. The results will be reported in the clinical record and in the eCRF.

7.5.9.2 Optical Coherence Tomography Angiography

Optical coherence tomography angiography (OCT-A) is a non-invasive technique that allows visualization of the retinal and choroidal circulation in vivo without the need to inject contrast media intravenously. Captures multiple repeated cross-sectional images in the same location and analyzes changes between scans; In this way, it extracts information about the volumetric movement of blood flow, generating high-contrast angiographic images of the capillary plexuses in the different layers of the retina and choroid. [82] [83] [84][85]

The patient will be placed in front of the OCT-A equipment properly aligned and will be asked to stare at the target indicated by the operator of the equipment. It may or may not require pupillary dilation with Ofteno® PT (tropicamide 5% / phenylephrine 0.8%) for the performance of this study, according to what the PI and/or sub investigator consider.

The make and model of the instrument used will be at the discretion of the investigating physician, provided that, for each individual patient, the same equipment (brand and model) is used throughout the study, in case it is necessary to perform OCT-A in subsequent visits. The procedure can be performed by the PI, sub-investigator, or specialized personnel with the ability to operate the delegated OCT-A equipment. The results will be reported in the clinical record and in the eCRF.

7.5.10 Intravitreal injection

An intravitreal injection of the study drug will be administered over a two-month period, with a total of three scheduled applications, corresponding to visits 1, 5, and 6. After each injection, a 30-minute observation period will be carried out.

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

7.5.11 Blood sample for pharmacokinetics (applies to PK cohort)

The designated personnel will be responsible for the extraction of a blood sample of approximately 5 mL by venipuncture. The selection of the vein to be used will be determined at the discretion of the professional in charge, who must perform an adequate asepsis of the skin in the puncture area before proceeding. Subsequently, the samples will be conditioned and sent to the designated laboratory for processing and analysis, in accordance with the laboratory manual.

All blood samples should be collected prior to intravitreal injection (when applicable) within the time stipulated in the pharmacokinetics cohort schedule (see section 1.4 Pharmacokinetics Cohort Schedule).

All specimens will be coded with the patient's number. These samples and any data generated can be linked to the patient only by the staff at the research site. The processing of the samples will be described in the laboratory manual.

7.5.12 Blood Sample for Immunogenicity

The designated personnel will be responsible for the extraction of a blood sample of approximately 5 mL by venipuncture. The selection of the vein to be used will be determined at the discretion of the professional in charge, who must perform an adequate asepsis of the skin in the puncture area before proceeding. Subsequently, the samples will be conditioned and sent to the designated laboratory for processing and analysis, in accordance with the laboratory manual.

All blood samples must be collected prior to intravitreal injection (when applicable) within the time stipulated in the schedule of activities (see section 1.3 Schedule of activities).

All specimens will be coded with the patient's number. These samples and any data generated can be linked to the patient only by the staff at the research site. The processing of the samples will be described in the laboratory manual.

7.5.13 Application of Medications During Visits

During the visits to the subjects, different medications or medical devices may be applied to the ocular surface for ophthalmological examination.

Examples:

- Tetracaine 0.5% ophthalmic solution, used to anesthetize the subject's ocular surface and facilitate the application of dyes, the measurement of intraocular pressure and prior to the application of intravitreal injection.
- Tropicamide 0.8% ophthalmic solution/5% phenylephrine hydrochloride, used to dilate the pupil and better assess the posterior segment.

7.5.14 Evaluation of concomitant medicinal products

It is the question about the use of medications, by any route of administration, that they are using regularly, that they have used since last month, or since their last visit.

7.6 Procedures to be carried out per visit

Listed below are the procedures that will be performed at each visit, which may not show the optimal order for the Research Center. The investigator should order it according to their needs, and the needs of the study and the sponsor, ensuring that the signing of informed consent and assent (when applicable) is the first activity to be performed.

7.6.1 Selection visit (day -8 to 0)

- Signing of informed consent
- Signature of informed consent from the PHARMACOKINETICS SUBGROUP (when applicable)
- Evaluation of selection criteria
- Medical history (general and ophthalmological)
- Measurement of weight, height, BMI, and body mass surface area
- Measurement of vital signs (temperature, blood pressure, heart rate, and breathing rate.)
- %HbA1c sampling (when applicable)
- GFRe sampling (when applicable)
- Evaluation of concomitant medications
- Adverse Event Registry
- Urine pregnancy test (where applicable)
- Best Corrected Visual Acuity Measurement (AVMC)
- Biomicroscopy
- Indirect ophthalmoscopy
- Ocular tonometry
- Macular optical coherence tomography
- Fluorescein angiography or optical coherence tomography angiography (when applicable)
- Blood sample for pharmacokinetics (in patients who will be evaluated for PK)

- Blood sample for immunogenicity

7.6.2 Visit 1 (Day 1)

- Measurement of vital signs (temperature, blood pressure, heart rate, and breathing rate)
- Evaluation of concomitant medications
- Adverse Event Registry
- Urine pregnancy test (where applicable)
- Intravitreal injection of the study drug
- Blood sample for pharmacokinetics (in patients who will be evaluated for PK)

7.6.3 Visit 2 (Day 2) – PK cohort only

- Evaluation of concomitant medications
- Adverse Event Registry
- Blood sample for pharmacokinetics (in patients who will be evaluated for PK)

7.6.4 Visit 3 (Day 4) – for PK cohort only

- Evaluation of concomitant medications
- Adverse Event Registry
- Blood sample for pharmacokinetics (in patients who will be evaluated for PK)

7.6.5 Visit 4 (Day 8) – PK cohort only

- Evaluation of concomitant medications
- Evaluation of adverse events
- Blood sample for pharmacokinetics (in patients who will be evaluated for PK)

7.6.6 Visit 5 (day 30 ± 5)

- Measurement of vital signs (temperature, blood pressure, heart rate, and breathing rate)
- Evaluation of concomitant medications
- Adverse Event Registry
- Urine pregnancy test (where applicable)
- Best Corrected Visual Acuity Measurement (AVMC)
- Biomicroscopy

- Indirect ophthalmoscopy
- Ocular tonometry
- Macular optical coherence tomography
- Intravitreal injection of the study drug
- Blood sample for pharmacokinetics (in patients who will be evaluated for PK)
- Blood sample for immunogenicity

7.6.7 Visit 6 (day 60 ± 5)

- Measurement of vital signs (temperature, blood pressure, heart rate, and breathing rate.)
- Evaluation of concomitant medications
- Adverse Event Registry
- Urine pregnancy test (where applicable)
- Best Corrected Visual Acuity Measurement (AVMC)
- Biomicroscopy
- Indirect ophthalmoscopy
- Ocular tonometry
- Macular optical coherence tomography
- Intravitreal injection of the study drug
- Blood sample for pharmacokinetics (in patients who will be evaluated for PK)
- Blood sample for immunogenicity

7.6.8 Visit 7 (End/Early Termination) (Day 90 ± 5)

- Measurement of vital signs (temperature, blood pressure, heart rate, and breathing rate.)
- Evaluation of concomitant medications
- Adverse Event Registry
- Urine pregnancy test (where applicable)
- Best Corrected Visual Acuity Measurement (AVMC)
- Biomicroscopy
- Indirect ophthalmoscopy
- Ocular tonometry
- Macular optical coherence tomography

- Blood sample for pharmacokinetics (in patients who will be evaluated for PK)
- Blood sample for immunogenicity

7.6.9 Unscheduled follow-up visits

At the request of the subject or the personnel related to the study, unscheduled follow-up visits may be carried out for the reporting of adverse events or any situation that warrants it. During these visits, all pertinent data on the adverse events reported should be collected and, where appropriate, an appropriate management plan should be established.

7.7 Data collection

7.7.1 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 which is part of the permanent records of the subject's history. Examples of source documents are medical history, clinical evolution notes, laboratory reports, cabinet study reports, nursing notes, follow-up notes, surgery records, etc.

The PI is obliged to accept the monitoring of the 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.

7.7.2 Electronic forms of data collection

All data related to the protocol will be captured through an electronic *Case Report Form* (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 the corresponding source document. This procedure allows monitoring to verify the information captured in the eCRFs. It is the responsibility of the researcher to ensure that the information is transcribed into the eCRFs in a correct, complete, and timely manner. It is understood that all data captured and sent using the eCRF to data analysis is approved by the Researcher.

7.7.3 Archiving

The data collected in this database is anonymous (it only stores the subject number together with other information of interest). The program used for data capture and storage covers the traceability requirements necessary for the execution of clinical studies. The data collected will be

stored by the sponsor or the clinical research organization designated for this purpose and its storage will have a duration of 10 years. The master file will remain in the participating institutions in charge of the PI or his work team and must be safeguarded for at least 10 years from the closure of the study.

8. Evaluation and management of adverse events

8.1 Regulation and regulations on adverse events

The registration and reporting of adverse events will be carried out in accordance with the guidelines established in NOM-220-SSA1-2016 and the international guidelines ICH E6. [86] [87][88] [89]

8.2 Definition of Adverse Event

According to the International Council for Harmonization (ICH), an adverse event (AD) is any unfavorable medical appearance in a patient under clinical investigation who is given a pharmaceutical product, regardless of causal attribution.[90]

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, x-ray) that is related to symptoms or that results in a change in study treatment or concomitant treatment or discontinuation of study medication. [87] [88] [89]

As defined in the previous paragraph, an adverse event is defined as any event that occurs during treatment with medication. However, the definition can also be applied as any unwanted event that occurs during a clinical trial, including behavioral disorders. [89]

8.3 Use of adverse events as a study safety variable

Measuring the safety of PRO-169 is paramount to the study, therefore, it is considered important to report any unwanted manifestations or diseases that occur during the course of the study, regardless of whether or not the manifestation is considered to be related to the investigational treatment. [89]

8.4 Definitions relevant to the classification of adverse events

Severity (serious/non-serious), also called seriousness (serious/non-serious). Serious or serious is defined as any event that: results in death, threatens life, requires hospitalization or prolongs

hospitalization, is a cause of permanent or significant disability or disability, is the cause of alterations or malformations in the newborn, other medically important conditions (Medically important event or reaction: That clinical manifestation or adverse event that in the opinion of the doctor may not be immediately life-threatening, result in death or hospitalization, but which could endanger the subject or require medical intervention to prevent the occurrence of any of the criteria listed in the definition of serious adverse reaction). [86]

Severity (mild, moderate, or severe). Mild is those that present with minimal symptoms, do not require treatment or suspension of the medication; moderate, when they interfere with usual activities, without threatening the subject's life, require treatment and may or may not require discontinuation of the medication; severe, those that interfere with usual activities and require pharmacological treatment and discontinuation of the medication. [86][87][88]

Causality. It is the relationship that is assigned between the pharmaceutical product and the adverse event: certainly caused by the pharmaceutical product, there is clear evidence of causality, i.e. the adverse event reappears with the administration of the pharmaceutical product; probably caused by the pharmaceutical product, there is a high suspicion of causality but no direct evidence is available or it is considered unnecessary or dangerous, i.e. the reaction disappears when the pharmaceutical product is discontinued; possibly caused by the pharmaceutical, there is additional information to suggest that the cause may be due to another pharmaceuticals or disease; unlikely to be caused by the pharmaceutical product, there is a clear explanation of the origin due to the underlying disease or the use of another pharmaceutical product; conditional, there is a lack of data to issue a clear causality; non-classifiable, those for which once all possible information has been obtained about the adverse event, it remains unclassifiable. [86][87] [88] [89]

8.5 Responsibilities of the researcher

It is the responsibility of the Investigator to verify AEs through questioning, pertinent physical examination, assessment of evolution, as well as appropriate medical and pharmacological management; as well as to follow up until the resolution or outcome and definitive discharge of the AE, following the definitions determined in national and international regulations. [86] [87] [88]

In the event of an AE or any event that puts the health and well-being of the subjects at risk, pertinent medical care will be provided, either at the research center or will be referred to the Hospital Center with the highest resolution power with which the research center has a medical

care agreement. The PI will notify the sponsor's clinical monitor, according to the times established in national and international regulations. In the case of serious adverse events, it will notify the sponsor and record the corresponding information in the eCRF and in turn, inform the IRB and IC.

The attention of the AEs will be carried out according to the event attention diagram (see Figure 3. Adverse Event Care).

The sponsor's final report will include the report of adverse events in compliance with current national and international regulations. [86] [87]

If the research subject debuts during their participation in the study with any chronic adverse event, such as diabetes or systemic arterial hypertension, they will be referred to the competent health professional for chronic treatment. The follow-up and termination of their participation will be in accordance with the provisions of NOM-012-SSA3-2012 and NOM-220-SSA1-2016. [72] [86]

8.5.1 Recording of adverse events in the electronic case report form

The adverse event registry considers:

- Subject identification information such as subject number, age, gender, and if applicable specify the eye.
- Information about the causation of the adverse event, its relationship to the investigational products, or to another cause as appropriate.
- Important date information:
    - Date on which the adverse event occurs.
    - Date on which the Principal Investigator becomes aware of it.
    - Date of resolution or outcome, as applicable.
- Information on diagnosis and clinical management.
- Record the outcome or resolution of the event:
    - Recovered/resolved without sequelae
    - Recovered/resolved with sequelae
    - Recovering/Resolving
    - Not Recovered/Unresolved
    - Fatal
    - Unknown

- Information about the investigational product or the product associated with the Adverse Event must be recorded. The information essential for registration is the generic name, distinctive name or code of the investigational product or of the product associated with the undesirable clinical manifestation; it will also be necessary to enter the data concerning the batch number, manufacturing laboratory, expiration date, dose, route of administration, start and end dates of administration and/or consumption, reason for prescription; according to whether it is an investigational product or drug (protocol in which the subject currently participates) or whether it is a drug that the research subject consumes for the treatment of underlying concomitant diseases or uses for the management of some transitory sign or symptom that does not correspond to the Natural History of the pathology that motivated its entry into the research protocol.
- Indicate whether the removal of the suspected product (of causing the event) eliminates the adverse event. Also indicate if a dose adjustment is made, if the event changes in terms of intensity or seriousness, persistence of the reaction. It is important to indicate whether in those subjects who are exposed again to the product, which had previously been suspended, the AD reappears.
- Information regarding concomitant pharmacotherapy. Indicate the generic name, dose, route of administration, start and end dates of use, as well as the reason for the prescription, regardless of whether it is in accordance with the prescribing information or technical data sheet or is used outside the regulations or what has been authorized by the local, national or international regulatory entity.
- Relevant medical history information. The analysis of the AE considers the information previously narrated, despite the clinical context in which this harmful phenomenon occurs in the participants of the clinical research protocol, is of special interest, so the information about previous conditions, hypersensitivity or allergy phenomena, previous surgical procedures, laboratory analyses or cabinet examinations that have been performed on the participant, etc., that the researcher deems it appropriate to mention may do so.

8.5.2 Adverse Event Tracking

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

8.5.2.1 Diagnosis against signs or symptoms

Whenever possible, an AE should be assessed/reported as a diagnosis and not as a sign or symptom (e.g., liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). If a diagnosis cannot be made from the signs or symptoms, then each sign or symptom should be recorded as an AE. If a diagnosis is made later, all AEs reported as signs or symptoms should be nullified and replaced by a single adverse event based on the diagnosis, with an onset date that corresponds to the onset date of the first symptom or sign of the eventual diagnosis.

8.5.2.2 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.

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

8.5.2.3 Persistent or recurrent adverse events

A persistent adverse event is one that spread continuously without resolution between different points of assessment of the subject. 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 AD 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 event, 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 and will record the date on which the event became serious, thus completing all relevant serious EA reporting data.

A recurrent AE is one that resolves between assessment points and subsequently reappears. Such events should only be recorded once in the eCRF if they are of the same nature, intensity, association with the product of the investigation and/or cause of the AE, with recurrence and duration being recorded within the narrative of the event or in the corresponding sections.

If AEs are different in nature, association with the product under investigation and/or cause of the AE, they must be registered individually in the eCRF.

8.5.2.4 About the outcomes of adverse events

The last known outcome of the adverse event should be captured. [90]

The possible outcomes of an adverse event are recovered, recovering, not recovered or in progress, recovered with sequelae, fatal and unknown.

In case of irreversible congenital anomalies, the unrecovered or ongoing option should be used. For other irreversible medical conditions, resolved outcome with sequelae should be used. [90]

8.5.2.5 Abnormal vital signs

Not all abnormal vital signs qualify as AS. For an abnormal vital sign to be reported as an AD, 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 on abnormal values should focus on obtaining a diagnosis and not just a description of the abnormality.

8.5.2.6 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 EA in the eCRF section.

8.5.2.7 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.

8.5.2.8 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 if the following are met:

    1. 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.

8.5.2.8 Adverse events exempt from reporting

Adverse events related to intravitreal injection

Intravitreal injection-related reactions are AEs that occur within 24 hours of administration of a medication by this route and are associated with puncture. These events will not be 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, regardless of whether it is related to the puncture.

Adverse events related to 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, if their use is prior to the application of the investigational product, e.g., AEs related to the application of tetracaine, povidone iodine or blepharotat placement.

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, regardless of whether it is related to the ophthalmic procedure, e.g., allergic reactions that meet severity criteria.

8.5.2.9 Pregnancies, abortions, and birth defects

Female subjects should contact their physician to report any suspected pregnancy during the study. A pregnancy report must be issued, and the sponsor must be informed immediately. Subject monitoring should continue until the outcome of pregnancy and for 6 months after the birth of the product. [56] Pregnancy is not by itself an AE.

Any abortion should be classified as a serious AE.

Any birth defect or birth defect in a product of female menarche subjects who received the study drug should be classified as a serious AE.

8.5.3 Procedures for a serious adverse event

The process of attention to the adverse event considers the following stages:

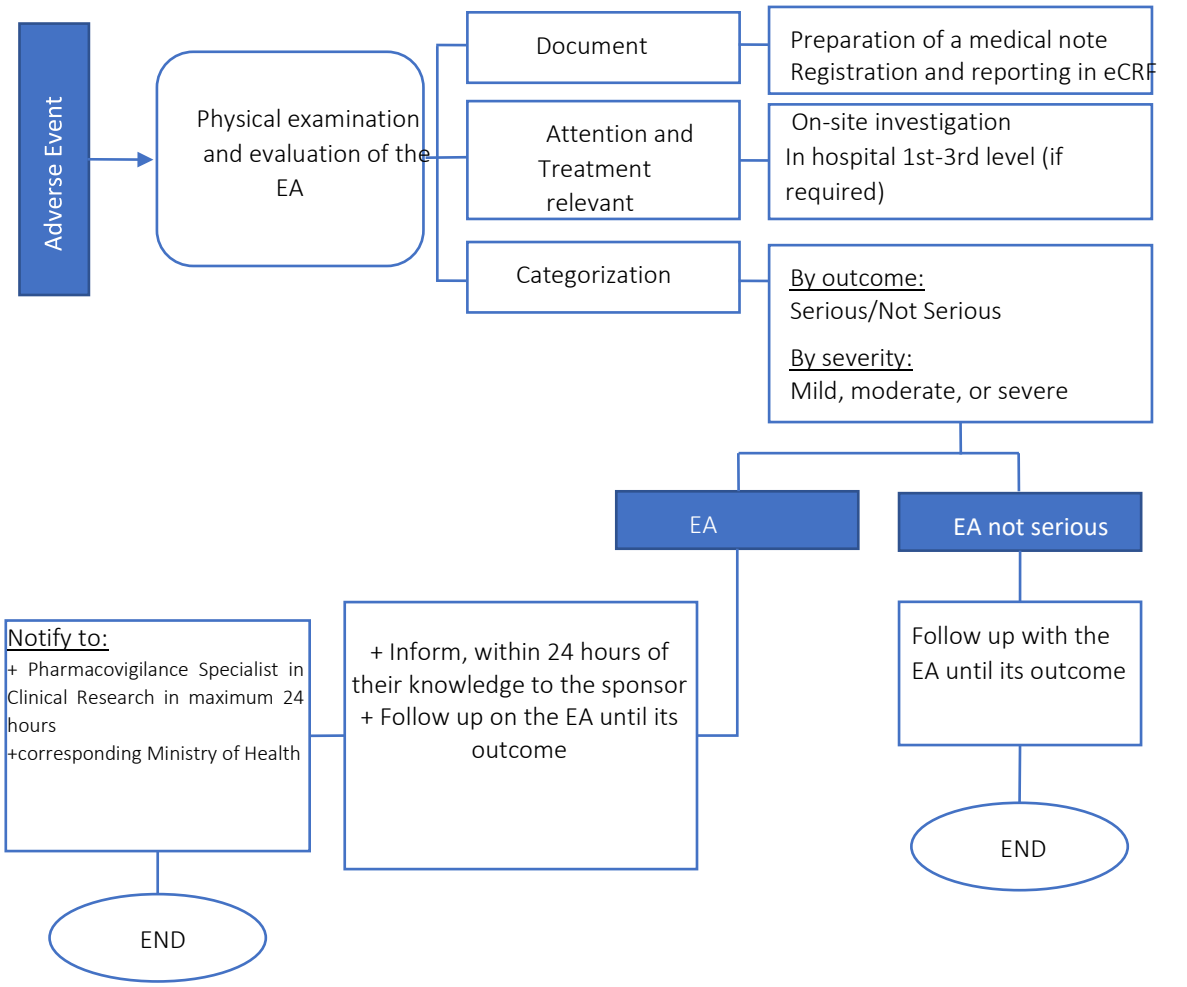


Figure 3. Adverse Event Care

During the development and conduct of this study, undesirable harmful events or adverse reactions/incidents, of medical implication, may occur in the research subject, which do not necessarily have a causal relationship with the investigational products. These harmful phenomena can occur during the use of investigational pharmaceutical products at doses authorized for use in humans by a local, national or international regulatory entity. However, it may be suspected that the investigational product causes some unwanted clinical manifestation. AEs, Incidents, Adverse Incidents, ADRs or SRAMs to one or more pharmaceutical products can occur during the systematic evaluation of the participants (on the days when the clinical review is scheduled, according to the schedule of activities) or suddenly, in such a way that:

- The investigator must be the first person to be notified that the subject has developed or presented any harmful phenomenon of a clinical nature during his or her participation in this study.

- According to his clinical judgment, Based on the pertinent physical examination, interrogation, etc., as well as the analysis of the information available in the medical literature and what is referred to in the Investigator's Manual, Information to prescribe or Summary of the Comparator Drug Label, the principal investigator determines the pertinent care of the harmful event/reaction.
- Such care can be in the research center or in the hospital with the greatest resolution power. In such a way that, if the subject is sent by the PI to a hospital, he or she attends through a referral system. The reference can be with a card that identifies the subject as a study participant and links him or her to the pre-established agreement with the institution, or through a medical reference note issued by the Principal Investigator. Laboratorios Sophia, S.A. de C.V., will pay the expenses for the medical care of the participating subject, when the adverse event is associated with or is related to the product under investigation.
- Taking the clinical information collected, either during the care provided at the research center or that provided by the treating physician(s) in the hospital, the PI will record the AE in its clinical note, stating the seriousness, intensity (mild, moderate or severe) and relationship with the investigational product.
- The PI must migrate the relevant data to the eCRF and its respective adverse event section. By, in cases of serious adverse events, the clinical monitor of the study must be notified within 24 hours after learning of it, so that in turn it informs the Clinical Team and the UTFLS, and that the CEI/CI is subsequently notified. Non-serious adverse events will be recorded and treated appropriately, and the safety profile of the investigational drug or PI will be reported to the appropriate regulatory entity in the final report of the clinical trial.

The recording of the outcome of the AE depends substantially on the follow-up that the Principal Investigator performs on the subject, since it is expected that most of the harmful phenomena (Consult Subsection 2.4 Risk-benefit assessment and consult the Investigator's Manual) are ophthalmic in nature, however, there may be systemic alterations. Therefore, in the opinion of the researcher, the withdrawal of the participant or their permanence will be considered.

8.5.4 Causation assessment

Causality assessment is the methodology used to estimate the probability of attributing the observed adverse event to a pharmaceutical product. It considers probabilistic categories according to the available evidence and the quality of the information, based on the national regulations of pharmacovigilance and technovigilance, the World Health Organization and the Uppsala Monitoring Center. [86][91]

An adverse event may or may not be related to the clinical trial. A causal relationship means that the intervention caused (or is reasonably likely to have caused) the adverse event. This usually implies a relationship between the time of the intervention and the adverse event (e.g., the adverse event occurred shortly after the subject under investigation received the intervention). [89]

For all adverse events, the Principal Investigator is responsible for examining and evaluating the subject to determine the association of the event with the clinical study and the intervention, whether related to the experimental treatment, concomitant, surgical procedure, or diagnostic procedures performed during the study. [89]

Accepting that the adverse event is related to the clinical study requires a plausible mechanism of action—that is, that there is a logical sequence between the event that occurred and the intervention that caused it. In some cases, it is helpful to know the opinion of other doctors directly or indirectly involved in the research; as well as whether the subject considers that a relationship exists or not. [89]

The Pharmacovigilance and Technovigilance Unit of Sophia Laboratories (UFTLS) may use the categories of causality described by *the Uppsala Monitoring Centre*, to categorize the likelihood of adverse event to the investigational product or concomitant treatments or treatments used during visits: [86] [91]

- Definitive (certain): a clinical event, including alterations in laboratory tests, that manifests itself with a plausible temporal sequence in relation to the administration of the drug, and that cannot be explained by concurrent disease, or by other drugs or substances. The response to drug withdrawal (withdrawal) must be clinically plausible. The event must be definitive from a pharmacological or phenomenological point of view, using, if necessary, a conclusive re-exposure (challenge) procedure. [86] [91]

- Probable: A clinical event, including alterations in laboratory tests, that manifests itself with a reasonable time sequence in relation to the administration of the drug, that is unlikely to be attributed to concurrent disease, or to other drugs or substances, and that a clinically reasonable response is presented upon withdrawal. Reexposure (challenge) information is not required to assign this definition [86] [91]
- Possible: A clinical event, including alterations in laboratory tests, that manifests itself with a reasonable time sequence in relation to the administration of the medicinal product, but which can also be explained by concurrent disease, or by other drugs or substances. Information regarding the withdrawal of the drug may be missing or unclear. [86] [91]
- Unlikely: A clinical event, including alterations in laboratory tests, that manifests itself with an unlikely temporal sequence in relation to the administration of the drug, and that can be more plausibly explained by concurrent disease, or by other drugs or substances. [86] [91]
- Conditional/Unclassified: A clinical event, including alterations in laboratory tests, reported as an adverse reaction, for which further data are essential for appropriate evaluation, or additional data are under review. [86] [91]
- Non-assessable/Unclassifiable: A notification that suggests an adverse reaction but cannot be judged because the information is insufficient or contradictory, and that cannot be verified or completed in its data. [86] [91]

Thus, the degree of certainty to establish the investigational product as the causal agent of the harmful phenomenon that occurs to the subject of the clinical study, can be indicated directly by the Principal Investigator based on his clinical experience or by applying the categories of causality described by *the Uppsala Monitoring Centre*. It is important that the researcher and the UFTLS consider the following arguments in favor of the causal relationship:

1. Strength of association, which refers to the number of cases in relation to those exposed.
2. The consistency of the data, i.e. the presence of a common characteristic or pattern.
3. The exposure-effect pattern, which determines the relationship with the site of appearance, time, dose and reversibility after deletion.

4. Biological plausibility, which refers to the possible pharmacological or pathophysiological mechanisms involved in the development or presentation of the adverse event.
5. Experimental findings, e.g., the appearance of abnormal **metabolites** or high levels of the drug or its biotransformation product.
6. Analogy, which refers to the experience gained with other related drugs, adverse reactions frequently produced by the same family of pharmacological agents.
7. Nature and characteristics of the data, i.e. objectivity, accuracy and validity of the relevant documentation. [92]

9. Study Monitoring

The sponsor will contract with an external provider, who will be 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.

The details of the monitoring activities are specified in a separate document from this protocol in a Monitoring Plan.

9.1 Monitoring the Study Site

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

The initial 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(s), 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 that adverse event reports are being conducted appropriately. At each visit, the monitor will discuss the findings with the researcher and define the actions to be taken.

The closure 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 analyzed, that all IP (used and unused) has been returned to the sponsor, and that all unused material has been recovered.

Details on monitoring are set out in the relevant monitoring plan.

9.2 Audit and quality assurance

To ensure compliance with GCPs and all applicable regulatory requirements, Laboratorios Sophia, S.A. de C.V. may conduct quality assurance audits. Regulatory agencies could also conduct a regulatory inspection of this study.

Details about the audit process are set out separately in the Audit Plan.

They may take place at any time before, during, or after the conclusion of the 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. If the audit has not been scheduled by the sponsor, the facility must notify Laboratorios Sophia, S.A. de C.V. immediately.

10. Sample size calculation and statistical analysis

10.1 Sample Size Calculation

A sample size of 60 subjects, which includes a 20% increase for possible losses, was considered to be sufficient to answer the main objective of the study.

10.1.1 Calculation methodology

The evaluation of immunogenicity and its potential clinical effect play an important role in the evaluation of the safety and efficacy of a biologic. In this sense, the immunogenicity caused by using Bevacizumab in healthy volunteers after the application of a single dose has been evaluated. In a study with three groups of 38 participants, they found that the three groups developed ADAs in different proportions: 12/38, 9/38 and 14/38 respectively [93]. In another study, also with intravenous administration in two treatment groups (42 subjects per group), the formation of ADAs was reported in 7/42 and 10/42 participants, respectively [94].

Whereas, in cancer patients, where bevacizumab is administered in combination with chemotherapy, an incidence of ADAs of ≤ 5% (approximately 17 subjects) and 16–17% in other groups (53/315 and 50/312 patients, respectively) has been documented)[95] [96]

The available literature on intravitreal bevacizumab-induced immunogenicity in patients with diabetic macular edema is very limited. Most of the evidence comes from studies conducted in healthy volunteers or in cancer populations, with different routes of administration, different clinical conditions, or different anti-GFR drugs.

The European Medicines Agency through the Committee for Medicinal Products for Human Use (CHMP) published an Evaluation Report on the use of bevacizumab for age-related macular degeneration. It cites a phase 1 pharmacokinetic study that was conducted in 135 healthy subjects, with the aim of comparing a new formulation (CHDR1427_ONS-1045-001) with two reference products (U.S.-Avastin and E.U-Avastin), only one subject (1%) was observed to be positive for ADAs.[97]

The immunogenicity of other anti-GFR drugs, such as aflibercept, has been evaluated in patients with macular edema. The aim of the studies was to determine the bioequivalence between a biosimilar and the reference products. The proportion of subjects who developed ADAs ranged from 0 to 10.6% [98] [99][100][101][102][103].

Based on the above, it is expected that a proportion ≤10% of patients could develop ADAs after receiving PRO-169 treatment to characterize the immune response to the drug. However, it should be taken into account that The response to the drug can be affected by factors that are associated with the drug such as: the structure of the drug, the dose and place of administration; and factors related to the subject such as age, sex, general medical condition [104] and given that most of the studies cited above have been carried out in different populations and pathologies, the present study seeks to characterize the immunogenicity and pharmacokinetic profile of PRO-169, to provide and generate more evidence for the design of future studies.

The ICH points out, in terms of sample size, that "The number of subjects should always be large enough to provide a reliable answer to the questions posed. This number is usually determined by the primary objective of the study. In case the sample size is determined on some other basis, it must be clear and justified." The FDA and Muglia & Di Giovanna recommend for phase 1 clinical studies, participation of 20 to 100 healthy volunteers or subjects with some condition. For its part, the Association of the British Pharmaceutical Industry (ABPI) recommends a participation of 50 to 200 healthy volunteers or subjects with some condition. [105] [106][107][108]

While there is no minimum sample size for phase 1 clinical studies, the FDA mentions that The safety profile must be characterized in a reasonable time and considering the duration of exposure to the drug and its relationship with adverse events. In these studies, it is important to consider the ethical principle of beneficence, which Fisher, J. and Walker, R describe by emphasizing that "researchers should seek to minimize the risk of affecting participants and compensate for any remaining potential risks so that individuals or society benefit from the research." However, even if these risks are mitigated in phase I studies, there will always be a probability that subjects will experience an adverse event in the process of generating data on the safety and tolerability of the new drug.[109]

As for the sample for pharmacokinetic evaluation, there are a limited number of published reports on the pharmacokinetic profile of bevacizumab in animals and/or human eyes. However, Avery et al. evaluated the systemic pharmacokinetics of three drugs: Bevacizumab (n=15), Ranizumab (n=20), and Aflibercept (n=21) in patients with age-related macular degeneration. They observed differences in pharmacokinetics between the three treatments. For this study, a sample size was not formally calculated, arguing that it was chosen to ensure the completion of the study. Moisseeiev et al. studied the pharmacokinetics of an application of bevacizumab in 22 patients (22

eyes) observing that the half-life of the intravitreal injection of bevacizumab obtained in the study is comparable to that reported in previous studies.

In phase 3 clinical studies, where the objective is to evaluate the efficacy of intravitreal Aflibercept biosimilars in patients with age-related macular edema, the evaluation of the pharmacokinetic profile is included as a secondary measure, the sample size used in these studies per treatment group is in a range of 11 to 47 participants (Bressler et al., 2024; Wolf et al., 2024; Bordón et al., 2024; Sadda et al., 2023).

Considering that the objective of a Phase 1 clinical study is to determine safety and provide initial pharmacokinetic results, as well as the references cited above, the sample size for the pharmacokinetics cohort is not based on a statistical calculation, but rather on a reasonable number of participants to provide such data to characterize and generate useful data about the investigational drug. According to the EMA and FDA, at least 12 participants are needed to carry out bioequivalence studies. Meanwhile, Lanser et al. observed that the sample sizes of nine studies in which pharmacokinetics were evaluated ranged from 10 to 29 patients.

The sample size proposed in the present study is consistent with the usual practice of exploration PK studies in phase 1 and is considered adequate for the objectives of the study.

10.2 Group Assignment and Blinding

As it is an open-label, non-comparative study, randomization of the subjects entering the study is not necessary.

10.3 Statistical methodology

The statistical analysis will be carried out by personnel of Laboratorios Sophia, S.A. de C.V. A specialized statistical package SPSS version 19.0 (IBM Corporation, Armonk, NY, USA) or R (*The R Foundation for Statistical Computing*; http://www.R-project.org) for Windows. The coding of the subjects will be performed using consecutive numbers. The data will be collected and sorted in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the platform of the selected statistical package.

Continuous variables will be summarized and described using classical measures of central tendency: mean, standard deviation (SD), median, minimum value, and maximum, 95% confidence interval (95% CI), geometric mean.

The categorical variables will be summarized by frequency, and percentages by subjects. For this, tables of frequencies p x q will be constructed. All percentages will be presented with decimal.

The operational definition of the variables is described in the Table 5. Operational definition of variables.

10.4 Study populations

10.4.1 Security Population

The safety population (HCP) will include all subjects who have been administered at least one dose of IP, regardless of whether they have completed the study.

10.4.2 Intention-to-Treat (ITT) Population

The ITT population includes all subjects who have received at least one dose of PI, who do not present major deviations from the protocol during the conduction of the protocol and who have a minimum of 2 blood samples for immunogenicity evaluation.

10.4.3 Population by Protocol (PP)

The per-protocol population will include all those subjects who complete the study without major deviations from it.

10.4.4 Deviations from the Protocol

Deviations from the protocol will be recorded throughout the study. They must be classified as minor, major or critical, and will be presented in the Monitoring report.

10.5 Lost Data

10.5.1 Procedure for Handling Missing Data

There is no imputation or plan for the management of missing data.

10.5.2 Visitor windows

Patients who have recurrent out-of-window visits and/or who are life-threatening by omitting to perform a critical procedure, or significantly compromising the study data will be considered major deviations from the protocol and will be part of the SP and ITT.

Table 8. Visit windows

Visit	Day of the visit	Window
Selection visit	Day 0	Day -8
V1	Day 1	NA
V2	Day 2	NA
V3	Day 4	NA
V4	Day 8	NA
V5	Day 30	Day 25 to Day 35
V6	Day 60	Day 55 to Day 65
V7 Final/Early Termination	Day 90	Day 85 to Day 95

10.5.3 Subgroup Analysis

If the sponsor deems it appropriate, subjects may be stratified for exploratory analysis by age range, by sex (females *versus* males), or some other characteristic.

10.6 Demographic and baseline characteristics

A descriptive analysis of the demographic and baseline variables of the security population will be carried out.

10.6.1 Disposition of Subjects and Losses

The sponsor may decide to replace subjects who present deviations related to the procedures (sampling and application of the PI) of the study until the sample size necessary to meet the primary objective of the study is completed.

It is considered that a loss of subjects <20% does not represent a problem for the validity of the results obtained.

10.7 Analysis of variables

10.7.1 Primary Variable

For the descriptive analysis of the primary variable, it will be carried out in the ITT population, only one positive result for ADAs per subject will be considered. That is, if a subject tests positive at visit 1 and subsequently tests positive again at visit 2, it will be considered only once for the calculation of the proportion.

A positive result will be considered in the following cases:

- Baseline result without the presence of ADAs (i.e., the result obtained in the blood sample for immunogenicity collected at the screening visit) and at least one sample with the presence of ADAs, after the initiation of PRO-169 treatment.
- Baseline result with the presence of ADAs and at least one sample with a value ≥ 4 times the baseline result, after the start of treatment with PRO-169

10.7.3 Secondary Variables

Analysis of adverse events will be performed in the PS. The results will be expressed as subjects and proportions. The causality, intensity, severity, and outcome of the AEs manifested during the conduct of the study will be collected through the electronic case report form (eCRF), where the clinical team of the research center will carry out the emptying of the information obtained from the source documents (medical history, clinical notes). These documents will be reviewed by the clinical monitoring team. The AEs reported in the eCRF will be subsequently reviewed by the sponsor's Clinical Pharmacovigilance Specialist, to ensure the veracity and concordance of the information, based on the stipulations of the PI investigator's manual (PRO-169), as well as its agreement with the study protocol.

The general summary of AEs will be reported in tables or figures using the preferred term (PT) for AEs (based on the Medical Dictionary for Regulatory Activities [MedDRA]), according to their classification by organs and systems (SOCs).

The results shall be presented in tables or figures, indicating the number of observations in each measurement.

10.7.4 Pharmacokinetic variables

Pharmacokinetic variables will be described by measures of central tendency; mean, standard deviation, minimum, maximum, geometric mean, median and range. They shall be presented in graphs or tables, as appropriate.

10.7.5 Exploratory variables

The analysis of the exploratory variables will be carried out in the PP population. To observe changes in BCVA and CMG from baseline at 12 weeks of treatment.

10.8 Internal Analysis

10.8.1 Preliminary Analysis

No preliminary analyses are contemplated for this study.

10.9 Clinical Data Management

Clinical data management (CDM) allows the generation of high-quality, reliable data with statistical value. CDM is the process of collecting, cleaning, and managing the information of the subjects of a study in accordance with regulatory standards (guidelines 21 CFR Part 11, ICH and GCP). It covers eCRF design, eCRF feedback, database design, *data entry*, *source data verification* (SDV), *queries*, *medical coding*, *soft lock*, and *hard lock*.

In accordance with roles and responsibilities, multiple users can be created, whose types of access to the eCRF can be limited to capture (IP or authorized site personnel), medical coding, database design, or quality control (*Quality Check*). The [110] [111]Figure 7 indicates the flow for the management of discrepancies that may arise.

The CDM team will include the following roles (assigned to site staff, sponsor, or third party authorized by the sponsor in charge of programming and maintaining the eCRF):

- Data Manager
- Database Designer/Programmer
- Medical *Coder*
- Clinical Data Coordinator
- Quality Control
- Capturist (*data entry associate*)

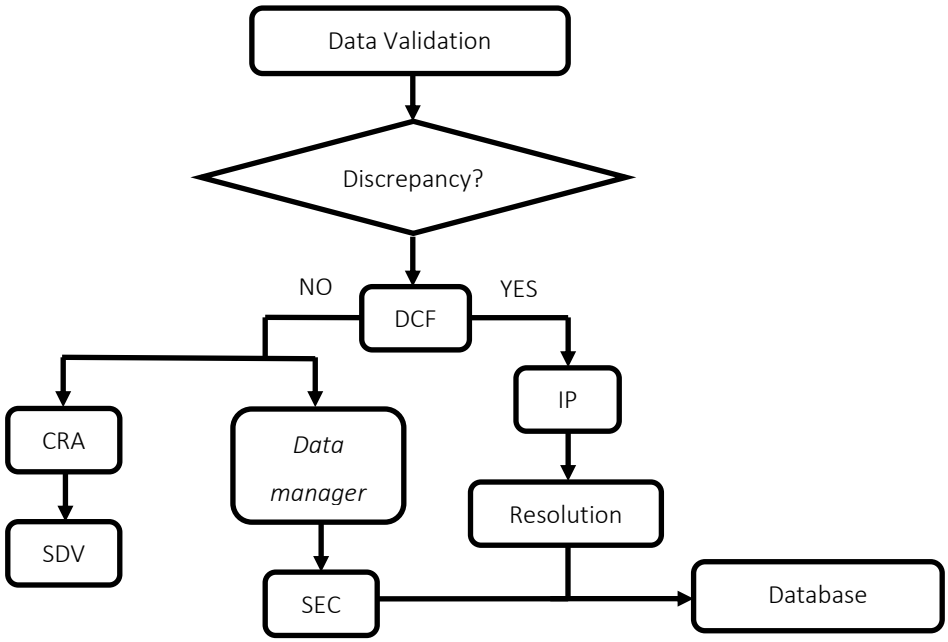


Figure 4. Handling discrepancies.

(DCF, medical note; CRA, clinical monitor; SEC, self-evident correction) *[110]*

The CDM will be carried out by the designated personnel of the biostatistics area. It will be reviewed in a subset of the total sample, equivalent to 25% of it (15 subjects). These will be selected randomly when 60% of the sample is available, reviewing only the following variables:

- Presence of serum ADAs.
- AVMC.
- GMC.
- Incidence of Adverse Events.

Only those data entries that have SDV will be reviewed in the CDM. All discrepancies detected will be shared via email to the Regional Clinical Studies Leader, Clinical Research Pharmacovigilance Specialist and/or the Therapeutic Area Specialist responsible for the study for resolution, according to the Sponsor's Current Standard Operating Procedure.

If discrepancies are detected in more than 50% of the subjects reviewed in the CDM, this percentage will be increased by 10%, up to a maximum of 80% of the total sample.

11. Ethical considerations

11.1 Approval of the committees

The present study will be conducted in accordance with the standards of the Declaration of Helsinki, World Medical Association 2013. Nuremberg Code; Nuremberg Trial by the International Tribunal of Nuremberg, 1947. Belmont Report, National Commission for the Protection of Biomedical and Behavioral Research Subjects, 1979. It shall be conducted in accordance with the scientific and technical requirements necessary for the registration of medicinal products for human use by the International Council for Harmonization ([112] [113][114]*The International Council for Harmonisation*, ICH for its acronym in English) Guide to Good Clinical Practices. International Ethical Guidelines for Biomedical Research in Human Subjects of the Council for International Organizations of Medical Sciences ([89]*Council for International Organizations of Medical Sciences*, CIOMS, 2016). The Research Ethics Committee (REC) and the Research Committee (IC) will evaluate the protocol before conducting the study and will issue their approval or possible modifications for its performance, these Committees must be notified of any significant changes to the protocol. In addition to the above, the current regulations of the regulatory authority will also be complied with. [115]

The staff will submit to evaluation by the Research Ethics Committees, Research Committees, and when applicable to the Biosafety Committee, the essential documentation of the research project: research protocol, forms of informed consent, researcher's manual, subject material, as well as other documents additionally requested, in accordance with the local, national or international requirements applicable by the regulatory entities.

The study will not begin at the research center if there are not the confidentiality agreements and economic proposal of each of the principal investigators, duly signed and without having previously obtained the favorable opinion and/or approval of the RECs, CIs, and when applicable, by the Biosafety Committee, the corresponding ones.

The study will not begin without having complied with the relevant local, national or international regulatory requirements and without having the corresponding health authorization.

The study is a study with a greater than minimum risk, in accordance with the Regulations of the General Health Law on Health Research, Title Two, Chapter I, Article 17, Section III, published in the Official Gazette of January 6, 1987. [116]

11.2 Amendments to the protocol

The amendment procedure will be pertinent when there is a need to make any change to a document that is part of the research project or protocol, derived from variations in the methodological structure, substitution of the principal investigator or in the face of the identification of risks in the research subjects. The documents subject to amendment will be protocol, forms of informed consent, researcher's manual, documents for the subject, measurement scales and schedule of activities.

Any amendment must be approved by the sponsor and/or the principal investigator, the amended document(s), once reviewed and approved by the CEI and the IC or, when applicable, by the Biosafety Committee, (entities that issued the initial favorable opinion for the conduct of the research) will be sent for authorization by COFEPRIS.

Amendments that substantially modify the protocol confer an additional or different risk on the research subjects must be approved by the Committees. It is the investigator's responsibility to act in situations that require immediate action to avoid unnecessary harm to study participants.

The principal investigator is responsible for communicating to the IRB any amendments to the protocol that could eventually affect the rights, safety, or welfare of research participants. Likewise, it must inform of any situation or new knowledge that will show a greater risk for the participants, the premature termination or suspension of the study, the reasons and the results obtained so far. It must also report on the conclusion of the study upon completion of the research protocol.

11.3 Early Study Termination

The study may be suspended or terminated prematurely if there is a sufficiently reasonable cause. Written notice, documenting the reason for the suspension or early termination, must be delivered by the party executing the suspension. The PI should promptly inform the study participants, the IC, and the IRC providing the reasons.

Situations in which study suspension or early termination will be contemplated include, but are not limited to:

- The regulatory authority (COFEPRIS) considered it for security alerts.
- The Sponsor determined it for its convenience or eventualities such as financial support, manufacturing errors, etc.
- The identification of unexpected risks to the participants, which are significant or unacceptable.
- Obtaining new relevant safety information.
- Insufficient adherence to the requirements of the protocol.
- The data obtained is not assessable or is not sufficiently complete.
- The determination that the primary objective has been achieved.
- The determination of futility.

In the event of suspension, the study may be resumed once the situations that led to the suspension have been corrected; if this justification is sufficient for the sponsor, CI, CEI and regulatory authorities.

11.4 Informed Consent

The FCI contains complete and understandable information on the study and the products under investigation, in accordance with the applicable regulations in force and the GCP.

The FCI will be considered as a source document and will be filed as such. The principal investigator of the site is responsible for ensuring that all new versions of the informed consent are submitted to the appropriate approvals (the same to which the original informed consent form was submitted) and that the most current approved version is the one that is presented to the study subjects after authorization from COFEPRIS.

For this study, there will be two different FCIs. One will be considered the main FCI as it will apply to all participants included. The second FCI will be complementary and additional to this main FCI and will be applicable only to participants who meet the eligibility criteria and who wish to be part of the pharmacokinetics subgroup. This subgroup will be made up of 15 of the total 60 participants.

11.4.1 Obtaining

Informed consent must be obtained before the subject undergoes any procedure indicated in the protocol. Patients must be informed that their participation is voluntary. For this purpose, the informed consent form must be signed.

Written consent documents will incorporate the elements of informed consent described in the Declaration of Helsinki and the ICH Guide to GCPs and will be following all applicable laws and regulations.

The PI, or the study staff delegated by him/her, will provide the prospective participant with all the information regarding the characteristics of the study, its potential benefits, risks, objectives, and procedures.

This information will be in a language understandable to the subject, it will be explained to the subject that he or she has the right to interrupt his or her participation in the study at any stage, without this affecting the relationship with the researcher and/or his or her future assistance. Informed consent will be put into consideration of the potential participant, which must have enough time to analyze each and every one of the aspects mentioned above and, if they have doubts, these will be clarified by the person in charge of obtaining consent. The PI will be able to explain the study prior to the surgical procedure, obtaining the signature until the baseline visit.

Once the participant agrees to participate in the study, he/she must sign and date the informed consent form in the presence of two witnesses, whether they are related to the study subject, who will participate during the informed consent process and sign guarantees that the process was carried out prior to any study procedure. that the information of the study was clearly explained and doubts were clarified if any.

If a subject is illiterate, acceptance will be with his or her fingerprint, and if the subject is unable to give adequate written informed consent, a representative of the "legally authorized" subject may provide such consent for the subject in compliance with applicable laws and regulations.

In the same way, the PI, or the study staff delegated by him, must sign and date this consent.

The FCI must be signed in duplicate by all those involved, one copy will be filed in the researcher's folder, and the other will be given to the participant. The PI or delegated personnel must document the process of obtaining informed consent by means of a detailed, precise and contemporary medical note, specifying the signed version, the date on which the documents were signed and how the process was carried out.

In cases where the participant is going to be part of the pharmacokinetics subgroup, he/she must replicate all the above steps and considerations to, in addition to the main FCI, establish his/her acceptance of participation by signing the FCI of the PHARMACOKINETICS SUBGROUP.

11.4.2 Special considerations

The procedures that will be performed during the conduct of the study do not pose an additional risk that should be considered apart from the listed informed consent procedures.

11.4.3 Modifications to Consent

Any changes to these documents constitute an amendment and must be submitted for approval to the CEIs and COFEPRIS.

Such amendments may be implemented only after written approval from the IRB and the Regulatory Entity (as applicable), except for an amendment that is required to eliminate an immediate hazard to the study subjects.

A process of re-consent of each subject affected by the amendment must be carried out under the same conditions as those described above, to communicate the new information contained in the document to them in a timely manner. The subject will be given a signed original of the amendment, and the researcher will keep the second original.

11.5 Confidentiality

All documents and information provided to the research center by the sponsor are strictly confidential. PI expressly agrees that data about your professional and clinical experience, provided to the sponsor on paper and stored in electronic form, is solely for use related to your activities with the clinical trial sponsor, in accordance with Good Clinical Practice.

The PI agrees that he and his team members will use the information only within the framework of this study, to carry out the protocol. This agreement is binding if the confidential information has not been disclosed to the public by the sponsor.

The clinical study protocol provided to the PI may be used by the PI and his team to obtain informed consent(s) from the subjects for the study. The clinical trial protocol, as well as any information taken from it, should not be disclosed to other parties without the written permission of the sponsor.

The PI will not disclose any information without the prior written consent of Laboratorios Sophia, S.A. de C.V., except to the representatives of the Competent Authorities, and only at their request. In the latter case, the investigator undertakes to inform Laboratorios Sophia, S.A. de C.V. before disclosing the information to these authorities.

The PI will fill out and maintain a record of the selection of the subjects, as well as the identification and enrollment list of each of the subjects participating in the study. The investigator agrees to give on-site access to the auditor and/or representatives of the Competent Authorities. The information will be processed in compliance with professional secrecy.

In the eCRF and all communications related to study subjects, they will identify them only by the study subject's identification number, either by the scrutiny number or the assignment number. The information collected in this study will be exchanged between the sponsor and the research center and must be treated confidentially. The Health Authority, the CEI, the IC, 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 subjects. If the results of the study are published, no personal information on the study subjects will be revealed.

The protection of personal data will be carried out in accordance with the corresponding regulations in force.

11.6 Conflict of interest

The independence of the conduct of the study and its results from any current or perceived external influences is critical. For this reason, any current conflict of interest of any person who has a role in design, conduct, analysis, publication or any aspect of this study will be declared. Furthermore, those who have a perceived conflict of interest will be asked to handle it in a manner appropriate to their participation in the study.

11.6.1 Declaration of Interests

The PI undertakes to make a declaration of financial interests as well as conflict of interest prior to the start of the study.

11.7 Access to Information

The final database of the study will be the property of Laboratorios Sophia, S.A. de C.V. and its access will be restricted. The PI will not have access to it, unless it has prior written authorization from the sponsor.

The information obtained, which is relevant to the safety of the subjects participating in the study, must be immediately shared with the research center, so that it can be notified to the study subjects.

11.8 Ancillary and post-study care

Once the study was completed and adverse events were closed according to the 8. Evaluation and management of adverse events, the sponsor will not extend care to the research subject.

12. Biosecurity aspects

This protocol, entitled: "Phase I clinical study to evaluate safety by determining immunogenicity after the unilateral application of intravitreal PRO-169 in patients with diabetic macular edema", and number: SOPH169-1224/I HAS NO BIOSAFETY IMPLICATIONS, since infectious and contagious biological material will NOT be used; pathogenic strains of bacteria or parasites; viruses of any kind; radioactive material of any kind; genetically modified animals and/or cells and/or plants; toxic, dangerous or explosive substances; any other material that endangers the health or physical integrity of the research center's staff or research subjects or affects the environment. It is also declared that this project will not carry out cell, tissue or organ transplant procedures, or cell therapy, nor will laboratory, farm or wildlife animals be used.

13. Posting Policy

13.1 Final Report

Once the statistical analysis is completed, the final report will be written with the results obtained, by the Team of the Regional Management Department of Medical Affairs of Laboratorios Sophia, S.A. de C.V. This report will be prepared following the recommendations of the E3 *Step 4 Guide* of the ICH.

13.2 Communication of results

Regardless of the results of the study, Laboratorios Sophia, S.A. de C.V., is committed to communicating the final report of the study to the principal investigators and COFEPRIS. These results will also be shared with the IC and the IEC. It will be the responsibility of the PI to communicate it to the research subjects.

Laboratorios Sophia, S.A. de C.V. will always maintain the rights over the publication and disclosure of the information contained.

13.3 Publication of results

Laboratorios Sophia, S.A. de C.V., acting as the sponsor of the study, assumes full responsibility for its function and retains exclusive ownership rights over the results of the study, which it may use in any way it deems appropriate.

The PI undertakes not to publish or communicate data collected from the study, unless there is the prior written agreement of Laboratorios Sophia, S.A. de C.V. 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.

However, if the sponsor is in the process of filing a patent application on the results of the study, the sponsor may delay its publication or communication of the results of the study until the date of registration or whenever it deems appropriate.

The assignment of authors for publications derived from this research will be the sole responsibility of the sponsor. Researchers invited to participate as authors must express their authorization. All researchers who are going to participate as authors can review the article prior to its submission and will be free to make comments and suggestions about it within the first 15 calendar days from the receipt of the document.

14. Financing and Insurance

14.1 Compensation to Study Participants

Participation in this study is completely voluntary and non-profit, so patients will not receive any payment directly for participating in the study. However, patients may receive financial support at each study visit for travel expenses (transportation and food expenses).

14.2 Insurance for Study Participants

In accordance with current local regulations, Laboratorios Sophia S.A. de C.V. has contracted a civil liability policy, 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.

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16. Annexes

16.1 ETDRS Booklet

The above annex is a representation of the ETDRS booklet, it is possible that the booklet designated for the study has variations in format and translation.