STUDY REPORT SYNOPSIS

Prospective, randomized, multicentre, open label, phase II / III study to assess efficacy and safety of ranibizumab 0.5 mg intravitreal injections plus panretinal photocoagulation (PRP) *versus* PRP in monotherapy in the treatment of subjects with high risk proliferative diabetic retinopathy. (PROTEUS)

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Investigator(s) : João Figueira

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1 Synopsis

Sponsor-Investigator: Prof. Dr. José Cunha-Vaz, AIBILI	
Title of Study: Prospective, randomized, multicentre, open label, phase II / III study to assess efficacy and safety of ranibizumab 0.5 mg intravitreal injections plus panretinal photocoagulation (PRP) versus PRP in monotherapy in the treatment of subjects with high risk proliferative diabetic retinopathy. (PROTEUS)	
Investigators: Dr. João Figueira (Coordinating Investigator); Dr. João Nascimento; Prof. Rufino Silva; Dr. Miguel Amaro; Dr. Sarah Ayello-Scheer; Prof. Pascale Massin; Prof. Catherine Creuzot-Garcher; Dr. Geeta Menon; Dr. Haralabos Eleftheriadis, Dr. Sobha Sivaprasad; Dr. Emily Fletcher; Dr. Monica Varano; Prof. Giovanni Staurenghi; Prof. Edoardo Midena; Prof. Francesco Bandello.	
Study centre(s): AIBILI Centre for Clinical Trials (CS01); Instituto de Retina de Lisboa (CS80); Espaço Médico de Coimbra (CS82); Hospital Vila Franca de Xira (CS90); Centre National d'Ophtalmologia des Quinze-Vingts (CS06); Dept. Ophthalmology, Lariboisière Hospital (CS14); Dept. Ophthalmology, University Hospital, CHU Dijon (CS42); CTU, Dept. Oph. Gloucestershire Hosp. NHS Found. Trust (CS53); Oph. Clinical Trials Unit Frimley Park Hosp. Found. Trust (CS66); Laser and Retinal Research Unit, King's Health Partners (CS69); G.B.Bietti Eye Foundation – IRCCS, Rome (CS20); Azienda Ospedaliera Luigi Sacco Dipartamento Oftalmologia (CS34); Centre Clinical Trials, Dept. Ophth. University Pádova (CS39); Dept. Oph. Univ. Vita Salute-Scientifc Inst. Of San Raffael, Milan (CS67).	
Publication (reference): Not applicable	
Studied period (years): One year **First enrolment:** 04 April 2014 **Last completed:** 27 May 2016	**Phase of development:** Phase 2/3
Objectives: The primary objective of this study is to compare the efficacy of ranibizumab 0.5 mg intravitreal (ITV) injections plus PRP versus PRP alone in the regression of the neovascularization area in patients with High-Risk Proliferative Diabetic Retinopathy (HR-PDR) over a 12-month treatment period. The secondary objectives are to compare the following parameters between the two treatment groups: 1. Changes in Best Corrected Visual Acuity (BCVA) from baseline to Month-12. 2. Time to complete NV regression. 3. Recurrence of NV. 4. Changes in the macular retinal thickness assessed by Optical Coherent Tomography (OCT) from the baseline to Month-12. 5. Need of treatment for diabetic macular edema (DME). 6. Need of vitrectomy due to the occurrence of vitreous haemorrhage, tractional retinal detachment or other complications of DR. 7. Treatment safety profile.	
Methodology: This study was a prospective, randomized, multicentre, open label, phase II / III study to assess efficacy and safety of ranibizumab 0.5 mg intravitreal injections plus panretinal photocoagulation (PRP) versus PRP in monotherapy in the treatment of subjects with high risk proliferative diabetic retinopathy.	
Number of patients (planned and analysed): Planned: 94 patients. Included 87 patients in 13 clinical centres. Analysed (FAS): 85 patients. Analysed (PP): 57 patients.	
Diagnosis and main criteria for inclusion: **Ocular Inclusion Criteria for the Study Eye:** 1. High-risk proliferative diabetic retinopathy (HR-PDR); i. NVD ≥ 1/4 DA OR NVE ≥ 1/2 DA; ii. NVE < 1/2 DA + vitreous and/or pre-retinal haemorrhage and/or rubeosis; iii. NVD <1/4 DA + vitreous and/or pre-retinal haemorrhage and/or rubeosis;	

2. BCVA at baseline ≥ 24 ETDRS letters score (approximate Snellen equivalent 20/320).

General Inclusion Criteria:

3. Type I or Type II diabetic subjects of either gender;
4. Age ≥ 18 years;
5. Ability to provide written informed consent;
6. Ability to return for all clinical trial visits.

Test product, dose and mode of administration, batch number:

This study included the following study medication:

• 0.5 mg ranibizumab (labeled Lucentis® 0.5mg/0.05ml).

Ranibizumab is formulated as a sterile solution aseptically filled in a sterile glass vial. Each vial contains ranibizumab in an aqueous solution (pH 5.5) with histidine, trehalose, and polysorbate 20. The vial contains no preservative and is suitable for single use only. Ranibizumab has been stored according to the label instructions contained on the Summary of the Product Characteristics (SmPC) and it was kept in a secure locked facility.

Since marketed Lucentis® was used, each vial and each box was labelled with the appropriate information stating that the medication was for use in this clinical trial only. Medication labels complied with the legal requirements and has been printed in the local language. The storage conditions for study medication were described on the medication label.

Subject were randomized at Month-0 in a 1:1 ratio to one of the two treatment arms:

Study Group: The study subjects received between Month-0 and Month-2 (3-months Loading Phase) 3 ranibizumab ITV injections in Month-0, Month-1 and Month-2 combined with the standard PRP treatment, i.e., with 1 mandatory laser session 2 ± 1 weeks after the 1st ITV injection (Month-0) and a maximum of 2 laser sessions, one 2 ± 1 weeks after the 2nd ITV injection (Month-1) and another 2 ± 1 weeks after the 3rd ITV injection (Month-2) to complete the PRP treatment (according to the Study Treatment Procedure). From Month-3 to Month-11 (9-months Follow-Up/ Treatment Phase), combination treatment composed of 1 ranibizumab ITV injection plus 1 PRP session (2 ± 1 weeks after the injection) could be performed respecting always at least 1 month of interval between ITV injections. In every visit, the Investigator evaluated whether treatment should be repeated. Treatment was repeated if NV was still present (due to lack of regression or due to recurrence) and if the Investigator considered that a new treatment may bring benefit to the subject and reduce the NV area. In the Follow-up treatment PRP were performed using fill-in techniques.

Control group: The control subjects received between Month-0 and Month-2 (3-months Loading Phase) the standard PRP treatment, with 1 mandatory laser session in Month-0 and more laser sessions as needed until Month-2 to complete the PRP treatment. After completing the PRP treatment, PRP sessions could be repeated from Month-3 to Month-11 (9-months Follow-Up/ Treatment Phase) (PRP treatments were performed according to the Study Treatment Procedure).

Duration of treatment/follow-up: One year (12 months)

Criteria for evaluation: The Full Analysis Set (FAS), consistently with ICH Guideline E9, Statistical Principles for Clinical Trials, include all randomized subjects receiving at least one study treatment and having a baseline and at least one post-baseline measurements on the primary outcome (85 patients). Subjects with major entry violations likely to affect outcome were excluded by blind review. The carrying forward of the last observation, an imputation technique, was used to compensate for missing data on the primary outcome.

The Per Protocol (PP) population defines a subset of the subjects in the FAS who are more compliant with the protocol and is characterised by the availability of measurements of the primary variable and the absence of any major protocol violations (57 patients). Only observed data was used in the PP population; i.e. missing data was not imputed.

The safety analysis population consists of subjects who received at least one treatment (ITV injections plus PRP or PRP alone).

Efficacy: Efficacy was assessed based on the following parameters: regression of NV, BCVA, time to complete NV regression, recurrence of NV and macular retinal thickness (assessed by OCT). The Primary efficacy

variable is: Total NV area (NVT area: NVD area plus NVE area). The Secondary efficacy variables are: BCVA (letters), time to complete NV regression, recurrence of NV (NVT increase after a period of improvement), recidivism of NV (NVT reappearance after NVT complete regression) and macular retinal thickness.
Safety: Safety was assessed by evaluating: all AEs, including SAEs occurring at any time during the trial; Concomitant medications and therapies; IOP; Inflammation assessment - Anterior chamber flare, Anterior chamber cells, Vitreous and Vitreous haemorrhage density; Ophthalmoscopy - normal/abnormal and specification for Vitreous, Retina, Macula, Choroid, Optic Nerve and other; Slit lamp examination - normal/abnormal and specification for Cornea, Conjunctiva, Iris, Anterior Chamber and Lens; Biochemistry values; Haematology values and Vital signs.

Statistical methods: Tables of summary statistics for variables related with Demographics, Physical Examination, Vital Signs, Medical history, Concomitant medication/ Non-drug therapies, Laser treatment history, Study eye, Treatment group, Haematology, Biochemistry, Assessment of the Refractive Error, Slit Lamp Examination, Ophthalmoscopy, Inflammation Assessment, IOP measurement, Fluorescein Angiography, Colour fundus photography, BCVA, Macular retinal thickness assessed by SD-OCT, Treatment for DME, PRP Treatment, ITV Injections and Adverse Events are presented for the whole study population and by treatment group. Continuous variables are summarized using the following statistics: mean, standard deviation, median, 1st quartile, 3rd quartile, minimum and maximum. The frequency and percentages are reported for all categorical measures. Efficacy analysis were conducted on the FAS and PP population. The analysis on the FAS are considered as main. The null (H0) and alternative (H1) hypotheses tested in the primary efficacy analysis were:
H0: there is no difference between groups for the number of subjects with NV reduction (at Month-12).
H1: there is a difference between groups for the number of subjects with NV reduction (at Month-12).
This hypothesis were tested using Chi-square test with two-sided Alpha = 0.05.
For the secondary endpoints:

1. Best-Corrected Visual Acuity (BCVA) changes from baseline to Month-12 were tested using Student's T test; and the area under the curve (AUC).
2. Survival analysis (log-rank test) was carried out to compare Time to complete NV regression between groups.
3. Recurrence of NV was tested using Fisher's exact test.
4. Macular retinal thickness changes from baseline to Month-12 were tested using Student's T test.
5. Need of treatment for DME was tested using Fisher's exact test.
6. Need of vitrectomy due to the occurrence of vitreous haemorrhage, tractional retinal detachment or other complications of DR was tested using Fisher's exact test.

Since vitreous haemorrhage is a result of active NV, in the exploratory analysis the primary efficacy endpoint (treatment success) was re-assessed considering the regression of NV from Baseline to the 12-month visit and also the presence of significant vitreous haemorrhage or vitrectomy. Significant vitreous haemorrhage is present when it is not possible to visualize/ quantify the NV.
The combined endpoint was defined as:
• No success – No NV regression and/or significant vitreous haemorrhage and/or vitrectomy; or NV regression and significant vitreous haemorrhage; or NV regression and vitrectomy
• Success – NV regression and no significant vitreous haemorrhage and no vitrectomy
The null (H0) and alternative (H1) hypotheses tested in this exploratory analysis are:
H0: Success rate (Study group) = Success rate (Control group)
H1: Success rate (Study group) ≠ Success rate (Control group)
This hypothesis was tested using Chi-square test with two-sided Alpha = 0.05.
Secondary endpoints were re-assessed considering baseline factors and possible confounders such as age, HbA1C and number of study treatments.

1. Best-Corrected Visual Acuity (BCVA) changes from baseline to Month-12 were tested using ANCOVA adjusted for the relevant covariates.

2. Survival analysis (Cox regression method adjusted for the relevant explanatory variables) was carried out to compare Time to complete NV regression between groups.
3. Recurrence of NV was tested using logistic regression.
4. Macular retinal thickness changes from baseline to Month-12 were tested using ANCOVA adjusted for the relevant covariates.
5. Need of treatment for DME was tested using logistic regression.
6. Need of vitrectomy due to the occurrence of vitreous haemorrhage, tractional retinal detachment or other complications of DR was tested using logistic regression or exact logistic regression.

Number and percentage of subjects reporting any AEs/SAEs and/or need for rescue treatment are presented.
AEs are summarized by presenting the number and percentage of patients having at least one occurrence of any AE during the study, having at least one occurrence of an AE within each system organ class and a list of AEs by patients, i.e., the following is provided: Frequency distribution of AEs; Frequency distribution of AEs by body system and preferred term; Listing of AEs by patients.
All Adverse Events (including serious) are tabulated using frequency tables including - Number of AE, Number of Subjects and Percentage of Subjects by: Severity; Site; Relationship with study drug; Action taken with study drug; Action taken with the AE; Outcome and Seriousness.

SUMMARY - CONCLUSIONS

Efficacy: The number of patients with regression of neovascularisation area in HR-PDR eyes during the one year follow-up was significantly higher in the study group (PRP + ranibizumab) in comparison with the control group (PRP) considering both the FAS and PP populations (FAS: 92.68% and 70.45%, respectively, p=0.009; PP: 93.1% and 64.29%, respectively, p=0.008).

The primary efficacy analysis with the FAS and PP population yields very similar results. Thus, the analysis on the FAS was considered as main to avoid any bias.

The average NVT area is higher in the PRP group and this difference is statistically significant at months 3, 7 and 12 (month-3: 0.12±0.38 and 2.40±5.00; p=0.005; month-7: 0.81±1.83 and 2.16±3.24; p=0.021; month-12: 0.52±1.04 and 2.20±4.92; p=0.035). Regarding NVD and NVE, the number of subjects with NVD reduction and NVE reduction at month-12 is higher in the study group in comparison with the control group but this difference is significant only for the NVE reduction (NVD: 93.33% and 68.75%, respectively, p=0.083; NVE: 91.43% and 73.68%, respectively, p=0.048). Concerning neovascularization area, there are no statistically significant differences between groups for the NVD area at all visits. However, the average NVD area is consistently higher in PRP group at all visits. Similarly, average NVE area is also consistently higher in PRP group and reaches statistically significant differences at month 3 (month-3: 0.12±0.37 and 2.06±3.17; p=0.001).

There are no statistically significant differences between groups for the best corrected visual acuity at all visits. Although the average BCVA difference from baseline to month-12 is greater in the study group than in the control group, this difference is not statistically significant (-0.90±12.08 and -5.81±15.08; p=0.104). Considering the area under the curve the letter score loss per month is -1.23 in the PRP + ranibizumab group and -3.34 in the PRP group (p=0.295).

Correcting for age, BCVA is statistically higher in the PRP+ ranibizumab group at Month-7 (p=0.027) and at Month-12 (p=0.031).

The number of subjects with NVT complete remission in the study group is higher than in the control group (43.9% and 25%, respectively) and this difference is, statistically, borderline significant (p=0.066).

Regarding time to complete NV regression, there is a significant difference in the survival time in the study group compared to the control group (p=0.002). In the study group the time to complete NV regression is shorter (3.64 months and 7 months, respectively).

The number of subjects with NV recurrence in the control group and in the study group is not significantly

different although the number is higher in the study group (67.5% and 57.14%, respectively). The number of subjects with recidivism of NV is significantly higher in the study group (66.61% and 0%, respectively; p<0.001).

Macular retinal thickness presents significant differences at month-3, month-7 and month-12 between the control group and the study group, being thinner in the study group. Considering the retinal thickness changes from baseline to month-12, both groups seem to progress in a similar way, evidenced by the lack of statistically significant differences between groups in all ETDRS grid areas, except in the outer ring inferior area (-1.32±18.98 and 13.60±41.92; p=0.040).

The number of subjects that needed treatment for DME in the control group (4.44%) and in the study group (0%) is not significantly different (p=0.177).

The number of subjects that needed vitrectomy due to the occurrence of vitreous haemorrhage, tractional retinal detachment or other complications of DR in the study group and in the control group is not significantly different (2.50% and 11.11%, respectively; p=0.122).

The number of subjects that needed rescue treatment (DME treatment or vitrectomy) was significantly higher in the control group in comparison with the study group (2.50% and 15.56%, respectively; p=0.040).

Considering the combined endpoint, the number of subjects that had success with treatment in the study group is higher in comparison with the control group but this difference is not statistically significant (63.41% and 52.25%, respectively; p=0.299).

The mean number of PRP treatments is statistically higher in the control group at the loading phase and at follow-up (loading phase: 2.05±0.80 and 2.95±2.25, p=0.017; follow-up: 1.41±0.95 and 1.95±1.16, p=0.022).

The mean number of PRP spots is statistically higher in the control group only at the loading phase (loading phase: 1823.15±1105.60 and 2345.05±1201.76, p=0.041; follow-up: 1124.49±1043.45 and 1413.14±1243.52, p=0.252).

The mean area of PRP laser burns is higher in the control group, but this difference is not statistically significant (loading phase: 21.49 mm^2 and 27.16 mm^2, p=0.598; follow-up: 10.06 mm^2 and 11.10 mm^2, p=0.726).

Since this study allowed the inclusion of patients who had previous PRP treatments, a subgroup analysis exploring differences between treatment-naïve patients was performed, showing that the number of patients with NVT regression was significantly higher in PRP + ranibizumab group only in naïve patients (Naïve: 94.44% and 50%, respectively, p=0.004; No naïve: 91.30% and 80% , respectively, p=0.255).

In a multivariate analysis, the considered baseline factors: age, HbA1c and number of PRP treatments, did not show a significant association with BCVA difference from baseline to month-12 and there were no statistically significant differences between study group and control group (Group: p=0.264, Age: p=0.787, HbA_{1c}: p=0.450, number of study treatments: p=0.904).

Age, HbA_{1c} and number of PRP treatments did not affect the time to complete NV regression (Age: p=0.787, HbA_{1c}: p=0.450, number of study treatments: p=0.904).

Considering macular retinal thickness difference from baseline to month-12, age showed a statistically significant association in the inner ring inferior, nasal and temporal areas (IRI: p=0.045; IRN: p=0.047; IRT: p=0.039), HbA_{1c} showed also a statistically significant positive association in the outer ring superior (ORS: p=0.007).

In a logistic multivariate analysis, NV recurrence, NV recidivism and the need of vitrectomy were not affected by treatment group, age, HbA_{1c} and number of study treatments (NV recurrence - Group: p=0.108, Age: p=0.130, HbA_{1c}: p=0.535, number of study treatments: p=0.146; NV recidivism - Age: p=0.053, HbA_{1c}: p=0.994, number of study treatments: p=0.505; Need of vitrectomy - Group: p=0.452, Age: p=0.757, HbA_{1c}:

p=0.855, number of study treatments: p=0.193).

Safety: A total of 125 Adverse Events occurred in this study. 104 (83.20%) were not serious and 21 (16.80%) were considered as Serious Adverse Events.

The number of AEs in both groups is similar. Control Group (PRP) had 53.60% of all AEs (67) and Study Group (PRP + ranibizumab) had 46.40% (58).

Regarding AEs Severity, classified as Mild, Moderate and Severe, in this study 56.80% of all occurred AEs were considered as Mild, 31.20% as Moderate and 12.00% as Severe. Regarding AE Relationship with study drug, classified as Unrelated, Unlikely, Possible, Probable and Related, 88.80% of all AEs were categorized as Unrelated to study drug, 2.40% as Unlikely, 4.00% as Related, 1.60% as Probable and 3.20% as Possible. About the Action taken with study drug, classified as No action taken, Study drug temporarily interrupted and Study drug permanently discontinued, 96.80% of the Adverse Events had no action taken; the study drug was permanently discontinued in 1.60% of cases and temporarily interrupted in 1.60%. In regard to the Action Taken with AE, classified as Concomitant medication taken, Hospitalization, None and Other, 38.40% had concomitant medication taken; 31.20% had no action (None), 17.60% had Other actions and 12.80% led to Hospitalization. Lastly, in relation to AE Outcome, classified as Recovered, Recovered with sequels, Recovering, Not Recovered and Unknown, 56.00% of AEs were totally recovered, 3.20% were recovered with sequels, 14.40% were considered as Recovering, 17.60% were not recovered and 8.80% had an unknown outcome.

No deaths occurred and no unexpected adverse events were observed during the study.

No statistically significant differences were found for the laboratory values over time ($p \geq 0.056$) and vital signs ($p \geq 0.218$) between study group and control group at all visits. IOP was not significantly different between study group and control group at all visits except at month-4 ($p=0.013$).

Overall conclusions: PRP associated with ranibizumab was more effective than PRP alone in the regression of neovascularisation area in HR-PDR eyes during one year follow-up.

The combination of PRP associated with ranibizumab seems to be safe in this population.

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