SYNOPSIS FINAL CLINICAL STUDY REPORT

Name of Sponsor/Company: Promoter: "A. Meyer" University Children's Hospital, Florence (no profit)	Individual Study Table Referring to Part of the Dossier Volume: Page:	*(For National Authority Use Only)*
Name of Finished Product: Propranolol		
Name of Active Ingredient: Propranolol		

Title of Study:
Safety and Efficacy of propranolol eye drops in neonates with retinopathy of prematurity: a pilot study (DROP-PROP). EudraCT Number 2013-002062-39

Investigator: **Dr Luca Filippi (Terapia Intensiva Neonatale – AOU Meyer, Firenze)**

Study centre(s): 4

Publication (reference):

Studied period (years): first patient enrolled: **(28/08/2013)** last patient completed: **(14/11/2014)**	Phase of development: **Phase II**

Objectives:
Principal Objective: Oral propranolol reduces retinopathy of prematurity (ROP) progression.

Secondary Objective: to obtain low plasma concentrations of propranolol.

Methodology:
A multicenter open-label trial, planned according to the Simon optimal two-stage design, was performed to analyze safety and efficacy of propranolol 0.1% eye micro-drops in treating preterm newborns with gestational age <32 weeks, diagnosed with a stage 2 ROP without plus in zone II. To evaluate safety, hemodynamic and respiratory parameters were continuously monitorized, blood samples were collected weekly, for three weeks. Propranolol plasma levels were also monitored. The progression of the disease was evaluated by serial ophthalmologic examinations. In particular, the treatment was considered not-effective if we had observed at least 4 failures in the first 19 newborns enrolled (I stage), or at least 12 out of 55 newborns enrolled (II stage). Newborns were divided into two groups, of 23-25 and 26-32 weeks to evaluate the safety of propranolol treatment in newborns with different gestational ages. The study was stopped when it was observed a progression of the disease to stage 2 or 3 with plus in more than 3 patients the first 19 enrolled. In particular, it was observed a progression of the disease in 4 patients. At the moment of progression of the four patient, and when the study was stopped, the newborns enrolled were 23.

Number of patients (planned and analysed):
Planned: 55
Enrolled: 23
Treated: 23

Diagnosis and main criteria for inclusion:

Retinopathy of prematurity, preterm newborns with gestational age between >23 and <32 weeks, diagnosed with a stage 2 ROP without plus in zone II.
Test product, dose and mode of administration, batch number: propranolol (eye-drops) as ophthalmic solution (concentration 0.1%): three micro-drops of 6 µL propranolol solution (6 µg propranolol every micro-drop) in each eye, three times daily (every 8 hours).
Duration of treatment: The original duration of treatment was max 60 days; However, afterwards the protocol was amended and in case of rebound after 60 days of treatment, propranolol eye drops might be administer for further 30 days (max 90 days).
Reference therapy, dose and mode of administration, batch number:

ANNEX 1 cont.

Criteria for evaluation:

Primary Endpoint: to evaluate the number of newborns that will evolve in stage 2 or 3 ROP with plus disease, and to evaluate the values of plasma propranolol levels.

Secondary Endpoint: to evaluate the number of newborns that will evolve in stage 3 ROP without plus, in stage 4 and 5 ROP with partial or total retinal detachment; the number of newborns that will need to have a vitrectomy; number of collateral events to the treatment with propranolol.

Safety:

The safety endpoint is the demonstration that this treatment does not induce severe adverse effects (severe bradycardia, severe hypotension, severe bronchospasm) and that mean propranolol concentration are, at the steady state, less than 20 ng/mL

Statistical methods: The present study was planned according to the Simon optimal two-stage design for phase II clinical trials. A limited number of patients were enrolled in stage I: if the number of clinical failures had been more than a pre-determined value, the trial would be closed for failure to demonstrate efficacy. Otherwise, an additional group of patients would have been enrolled in stage II. If the cumulative number of clinical failures were greater than another pre-determined value, the trial would have ended by declaring not-effective the topical administration of propranolol at a concentration of 0.1%. Else, it would be concluded that the treatment would be effective enough for further investigations. In this study, clinical failure was defined as a progression of ROP to stage 2 or 3 with plus and/or a mean propranolol plasma concentration at the steady state greater than 20 ng/mL. The progression rate from stage 2 ROP to stage 2 or 3 plus previously reported in our units was around 38%. The treatment was considered effective if it halved the progression ratio. Hence, considering an alpha error of 0.05 and a power of 80%, the treatment was considered not-effective if we had observed at least 4 failures in the first 19 newborns enrolled (I stage), or at least 12 out of 55 newborns enrolled (II stage). Newborns were divided into two groups, of 23-25 and 26-32 weeks to evaluate the safety of propranolol treatment in newborns with different gestational ages. Biochemical data were grouped according with the data of samplings: at enrollment, after 7, 14 and 21 days. The t-test was used to assess possible differences in such data between these groups. The association between predictors and clinical failure was assessed using Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables. The null hypothesis was accepted with a p-value higher than 0.05. Statistical analyzes were performed with the Statistical Software Program.

SUMMARY – CONCLUSIONS

Twenty-three newborns were enrolled. Since the fourth of the first 19 newborns enrolled in the first stage of the study showed a progression to stage 2 or 3 with plus, the second stage was prematurely discontinued. Even though the objective to complete the second stage was not reached, the percentage of ROP progression (26%) was similar to that obtained previously with oral propranolol administration. However, no adverse effects were observed and propranolol plasma levels were significantly lower than those measured after oral administration.

EFFICACY RESULTS:

The study was discontinued when a total of 23 newborns were enrolled, when the fourth of the first 19 newborns presented a progression of ROP from stage 2 to the threshold for the ophthalmologic treatment (stage 2 or 3 with plus). Therefore, the objective to complete the second stage was not reached. Univariate logistic regression analysis was first performed to evaluate the influence of the known risk factors for ROP progression **(Table 1)**. The multivariate analysis was not performed because of a small number of enrolled subjects. The ophthalmologic outcomes, assessed by RetCam images, are summarized in **(Table 2)**. One newborn was treated for the development of ROP stage 2 plus after only 14 days of eye micro-drops administration. Five newborns developed ROP stage 3 plus. Two of these newborns were treated for a few days (13 and 19 days), because ROP progressed rapidly; the other 3 newborns were treated for a longer time (38, 44, and 65 days). Therefore, overall, 6 of 23 newborns (26.1 %; CI 95% 44.0 to 8.1%) showed ROP progression to stage 2 or 3 plus. Five of them belonged to the group with GA 23-25 weeks. Three of them received treatment with laser photocoagulation, but in two of them (in three eyes) a progression to stage 4 ROP was recorded, vitrectomy was performed in two eyes of two newborns, and

one eye progressed to stage 5 despite a rescue treatment with bevacizumab. The other three newborns were treated with bilateral intravitreal bevacizumab as the first choice, and no one of these newborns progressed to a more advanced stage. Interestingly, all these newborns showed a high incidence of comorbidities including more than one surgical operation. Two twins (patient number 14 and 15) who did not progress to stage plus showed a stable ROP at stage 2 for all the 60 days of treatment. However, after 4-5 days of treatment discontinuation, a rebound of the disease with a progression to stage 3 was observed in both twins. The treatment with propranolol eye micro-drops was resumed for other fifteen days, and ROP regressed. Therefore, for the following 8 patients, the limit of the treatment was extended to 90 days.

Table 1
Univariate analysis of predictors of clinical failure

		Clinical Failure (stage 2 or 3 plus)		p-value
Predictors		Yes*	No	
Sex	Male	6 (46.1)	7 (53.9)	0.017
	Female	0 (0)	10 (100)	
Gestational age	23-25 weeks	5 (41.7)	7 (58.3)	0.095
	26-32 weeks	1 (9.1)	10 (90.9)	
Weight at enrolment	< 750 g	2 (25.0)	6 (75.0)	0.666
	≥ 750 g	4 (26.7)	11 (73.3)	
Transfusion	Yes	6 (28.6)	15 (71.4)	0.538
	No	0 (0)	2 (100)	
Bronchopulmonary dysplasia	Yes	6 (33.3)	12 (66.7)	0.184
	No	0 (0)	5 (100)	
Candida sepsis	Yes	1 (33.3)	2 (66.7)	0.616
	No	5 (25.0)	15 (75)	
Necrotizing Enterocolitis	Yes	3 (37.5)	5 (62.5)	0.334
	No	3 (20.0)	12 (80.0)	
O_2 days exposure	mean±SD	72.5±50.2	39.9±24.6	0.172

* Number (row percent)

Table 2
Ophthalmologic outcome

		All newborns	Group 23-25 weeks	Group 26-32 weeks
		n=23	n=12	n=11
Progression to stage 2 ROP with plus, *n* (%)	newborns	1/23 (4.3)	0/12 (0)	1/11 (9.1)
	eyes	2/46 (4.3)	0/24 (0)	2/22 (9.1)
Progression to stage 3 ROP without plus, *n* (%)	newborns	10/23 (43.5)	6/12 (50)	4/11 (36.4)
	eyes	20/46 (43.5)	12/24 (50)	8/22 (36.4)
Progression to stage 3 ROP with plus, *n* (%)	newborns	5/23 (21.7)	5/12 (41.7)	0/11 0)
	eyes	9/46 (19.6)	9/24 (37.5)	0/22 (0)
Clinical failures (stage 2 or 3 plus), *n* (%)	newborns	6/23 (26.1)	5/12 (41.7)	1/11 (9.1)
	eyes	11/46 (23.9)	9/24 (37.5)	2/22 (9.1)
Treatment with laser photocoagulation, *n* (%)	newborns	3/23 (13.0)	2/12 (16.7)	1/11 (9.1)
	eyes	5/46 (10.9)	3/24 (12.5)	2/22 (9.1)
Treatment with bevacizumab, *n* (%)	newborns	4/23 (17.4)	3/12 (25.0)	1/11 (9.1)
	eyes	8/46 (17.4)	6/24 (25.0)	2/22 (9.1)
Progression to stage 4 ROP, *n* (%)	newborns	2/23 (8.7)	1/12 (8.3)	1/11 (9.1)
	eyes	3/46 (6.5)	1/24 (4.2)	2/22 (9.1)
Vitrectomy, *n* (%)	newborns	2/23 (8.7)	1/12 (8.3)	1/11 (9.1)
	eyes	2/46 (4.3)	1/24 (4.2)	1/22 (4.5)
Progression to stage 5 ROP, *n* (%)	newborns	1/23 (4.3)	0/12 (0)	1/11 (9.1)
	eyes	1/46 (2.2)	0/24 (0)	1/22 (4.5)

SAFETY RESULTS:
During the study, no severe adverse events usually related to propranolol (i.e. bradycardia, bronchospasm, apnea, severe hypotension) or severe local signs due to propranolol eye micro-drops were observed. Hemodynamic and respiratory parameters, continuously monitored during the treatment period, did not present relevant abnormalities, and no significant differences were observed between newborns of different gestational ages. Electrocardiographic and cardiac ultrasound resulted normal in all newborns. Finally, no significant abnormalities were recorded for the variables of blood gas analysis, complete blood count, blood glucose, serum electrolytes, serum total protein, C-reactive protein, renal and liver function tests. In particular, no substantial differences were appreciated between values at the enrollment and values after 7, 14 or 21 days. As shown in **Figure 1**, plasma propranolol during the first 3 days of treatment and on the 10th day (steady state) was consistently below the cut-off value of 20 ng/mL, being approximately 10 times lower than that reported after oral administration of 1 mg/kg/day of propranolol. None of the enrolled newborns showed propranolol values higher than 20 ng/mL in any blood samplings, and, therefore, no clinical failure was attributable to excessively high levels of plasma propranolol.

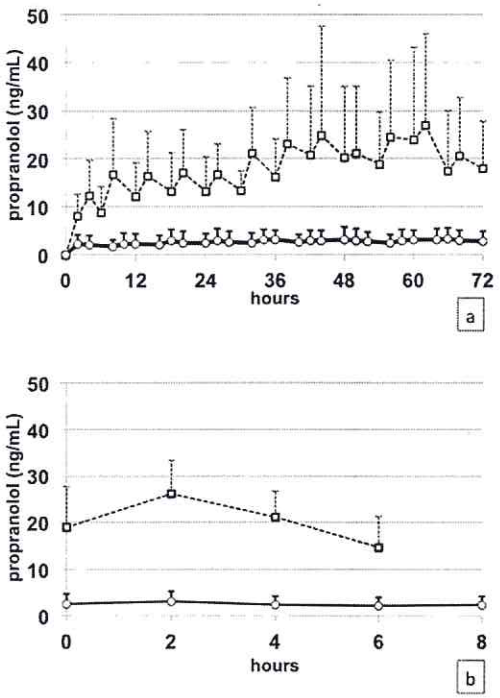

Figure 1

CONCLUSION:
The present pilot study inquired for the first time the safety and the efficacy of a new topical delivery system for the administration of propranolol in newborns with ROP. Propranolol 0.1% eye micro-drops seem to be well tolerated even in high-risk patients, as preterm newborns with a high prevalence of comorbidities, but not sufficiently effective. However, the number of newborns evaluated was small and further prospective studies are needed to reach meaningful conclusions.
In this trial a very low dosage of propranolol was employed and newborns were enrolled late, with an advanced stage of the disease. The reassuring profile of safety and tolerability of propranolol eye micro-drops, together with the very low plasmatic levels, encourages further experimentation by administering a higher dose of propranolol to newborns in an earlier stage of the disease.

Date of the report:
November 23rd, 2015