PRECLINICAL STUDY REPORT

A GLOBAL SERVICE PROVIDER OF PRECLINICAL AND CLINICAL RESEARCH IN OPHTHALMOLOGY

IRIS PHARMA Study Number: N50F25612

Non-GLP Study

ML7.

EVALUATION OF TOPICAL ADMINISTRATIONS OF ML7 IN A RAT MODEL OF SCOPOLAMINE-INDUCED DRY EYE.

IRIS PHARMA for Neuroptis Biotech

Study Director: Laurence FERAILLE, Ph.D.

Sponsor Representative: Djamel HAMANI, Ph.D.

Study Report version 2 – 41 pages

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1. SUMMARY

Purpose:

The aim of the study was to evaluate the therapeutic potential of topical applied ML7 formulation in a rat model of scopolamine-induced dry eye.

Method:

Dry eye symptoms were induced in albino Lewis female rats by systemic scopolamine diffusion (20 mg/day) over 21 days. Rats were randomized into 4 groups. One group was not induced and not treated (naïve group). The other groups were induced and received either three instillations of ML7, or three instillations of Placebo per day (TID) from the day of induction to the end of the study on Day 21. Oral Cyclosporine A treatment (25 mg/kg/day) from Day 0 to Day 21 was used as reference.

Tear production was measured using the phenol red thread test and corneal fluorescein staining was scored using the standardized National Eye Institute (NEI) grading system.

Results:

Scopolamine reduced tear production and increased corneal fluorescein staining after 7 days of induction and over a 3-week period.

3 rats were humanely euthanized, two in Placebo-treated group and one in Cyclosporine A-treated group because of edema and skin necrosis at the pump implantation sites.

Topical application of ML7 (TID) failed to improve tear production. The tear volumes were similar to those of the Placebo-treated group.

Topical application of ML7 (TID) reduced significantly corneal fluorescein staining after 7 days of treatment in comparison with the placebo treatment. After 21 days of treatment, the corneal staining was lower than in the vehicle group, however no statistical analysis could be performed at this time-point due to the number of animals left in the vehicle group (2 euthanized animals for ethical reason).

As expected, oral administration of Cyclosporine A improved tear production and reversed corneal staining by 3 weeks of treatment.

Conclusion:

Oral administration of Cyclosporine A improved tear production and reversed the corneal staining by 3 weeks of treatment and validated the assay.

Topical administration of ML7 was effective to reverse the corneal staining induced by the scopolamine on Day 7.

2. STATEMENT

ML7.

EVALUATION OF TOPICAL ADMINISTRATIONS OF ML7 IN A RAT MODEL OF SCOPOLAMINE-INDUCED DRY EYE.

MANAGEMENT AND STUDY DIRECTION

Director of Test Facility: **Pierre-Paul ELENA, Ph.D.**

_____ _____

Signature Date

I, the undersigned, declare that this study was performed according to the study plan and I assume the responsibility of the validity of reported data.

Study Director: **Laurence FERAILLE, Ph.D.**

_____ _____

Signature Date

3. GENERAL INFORMATION

3.1. Test facility

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3.3. General information

3.3.1. Study dates

Study plan version 1 (final version) signed on:	September 2nd, 2013
Start of experimental phase:	September 2nd, 2013
End of experimental phase:	September 23rd, 2013
Preliminary results:	September 26th, 2013
Report version 1:	January 14th, 2014
Report version 2:	January 08th, 2015

3.3.2. Confidentiality

All employees of Iris Pharma are contractually bound by a confidentiality clause.

4. INTRODUCTION

4.1. Background

Dry eye syndrome, along with cataract and AMD, the main eye pathology in the elderly population. About 15 to 25% of the population aged over 65 is treated with tear substitutes.

The causes of dry eye syndrome are varied and include both lacrimal hyposecretion or hypersecretion. The classification [**1**] differentiates dry eye by hyposecretion syndromes, such as Sjögren's syndrome and syndromes with dry tear film instability. This category comprises allergies, blepharitis, meibomis malfunctions, rosacea, and environmental factors [**2**]. In recent years, many discoveries have significantly changed the understanding of dry eye. Investigation of the lacrimal and meibomian gland functions in animal models and patients unveiled the important role played by inflammation of the ocular surface and lacrimal gland [**3**] as well as the involvement of hormonal factors [**4**] or the existence of anomalies of the tear and meibomian glands. On the other hand, malfunctions at the interconnections between nerves of the ocular surface, eyelids and the main lacrimal glands have been clearly implicated in the genesis of dry eye [**5**].

Substitution tears are the basis of the treatment of dry eye. But new treatments targetting immunological, inflammatory and hormonal causes are under development. Cyclosporine is a typical representative of this new generation of treatments.

4.2. Objective

The aim of the study was to evaluate the therapeutic potential of ML7 in a rat model of scopolamine-induced dry eye.

5. MATERIAL

Table 1: Material table – Test item

Material	***Test item***
Test article	ML7
Batch number	E753-2
Supplier	Octalia technologies
Characteristics	Solution
Preparation (concentration)	0.1%, ready to use
Number of administration per day	3
Volume needed per day (theoretical – practical)	60 µL - 100 µL
Storage conditions and stability (before/ after preparation, before/after opening)	At +2° to +8°C
Documentation	CA, MSDS, CS formulation protocol

Table 2: Material table – Control item

Material	***Control item 1***
Denomination	Placebo
Characteristics	Solution
Supplier	Octalia technologies
Batch number	E753-1
Number of administration per day	3
Volume needed per day (theoretical – practical)	60 µL - 100 µL
Preparation (concentration)	Ready to use
Storage conditions and stability (before/ after preparation, before/after opening)	At +2° to +8°C
Documentation	CA, MSDS, CS formulation protocol

Table 3: Material table – Reference item

Material	***Reference item***
Denomination	Cyclosporine A (Sandimmune®)
Characteristics	Solution
Supplier	Iris Pharma
Batch number	Not yet available
Number of administration per day	Once a day

Volume/amount needed per day (theoretical – practical)	6 mg, 600 µL per day
Preparation (concentration)	25 mg/mL
Storage conditions and stability (before/ after preparation, before/after opening)	Room temperature
Documentation	CA, MSDS, CS formulation protocol

Disposition of test item:

Following the completion of the experimental phase, empty containers of test item were discarded according to Iris Pharma standard operating procedures. The remaining of test item was sent back to Octalia.

Disposition of control item:

Following the completion of the experimental phase, empty containers of control item were discarded according to Iris Pharma standard operating procedures.

Disposition of reference item:

Following the completion of the experimental phase, empty containers of reference item were discarded according to Iris Pharma standard operating procedures.

6. ANIMALS AND HUSBANDRY

All standard operating procedures and protocols described in this study plan had been reviewed by Iris Pharma Internal Ethics Committee. All animals were treated according to the Directive 2010/63/UE European Convention for the Protection of Vertebrate Animals used for Experimental [**6**] and Other Scientific Purposes and to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research [**7**].

6.1. Animals

6.1.1. Animals

Species: Rat. This is the strain most commonly used in this model [**8**-**9**].
Strain: Lewis (albino).
Age: Approximately 6-7 weeks.
Weight: 150 - 175 g (on ordering).
Number/sex: 100 males (study 91; reserve 9).
Breeder: "ELEVAGE JANVIER" - FR-53941 LE GENEST-ST-ISLE.

6.1.2. Identification

All animals were identified by tag on tail following the inclusion examination.

6.1.3. Clinical examination and health status

Animals were held in observation for 4 weeks following their arrival. They were daily observed for signs of illness and particular attention was paid to their eyes.

6.2. Housing

6.2.1. Animal husbandry

Animals were housed by at least two and four at the most in standard cages, under identical environmental conditions. The temperature was held at 22 ± 2°C and the relative humidity at 55 ± 10%. Rooms were continuously ventilated (15 air volumes per hour). Temperature and relative humidity were continuously controlled and recorded. Animals were routinely exposed (in-cage) to 10-200 lx light in a 12-hour light and darkness cycle.

6.2.2. Food and water

Throughout the study, animals had free access to food and water. They were fed the standard dry pellet diet LASQCdiet® Rod16-H (Lasvendi GmbH, Soest Germany).

Tap water, regularly analyzed, was available *ad libitum* from plastic bottles.

7. DESIGN AND PROCEDURE

7.1. Study design

The **Table 4** below summarizes the study design:

Table 4: Study design

Group number	***Number of animals***	***Induction***	***Treatment***			***Clinical evaluation***		
			Name	**Dosing Regimen From D1 to D21**	**Route of administration**	**Body weight**	**PRT**	**Corneal staining**
1	10	Subcutaneous scopolamine infusion 20 mg/day	ML7	Three time a day	Instillation (10 µl) in both eyes	Baseline once a week	Baseline, D7, D14 and D21	
2	10		Vehicle					
3	10		Reference (CsA)	Once daily	Per os 1 mL/kg			
4	5	No	-	-	-			

The **Table 5** below summarizes the schedule:

Table 5: Schedule table

Day	Design
Baseline	Phenol red thread (PRT) test and corneal staining
D0	Placement of scopolamine pump
D1- 6	Administration
D7	Administration + PRT and corneal staining
D8-13	Administration
D14	Administration + PRT and corneal staining
D15-20	Administration
D21	PRT and corneal staining + sacrifice

The animals were allocated to coded treatment groups (**Table 6**). The investigators were kept masked to the treatment groups throughout the study.

Table 6: Animal allocation

Group number	Animal ID	Treatment
1	1, 2, 3, 4, 5, 6, 7, 8, 9, 10	Placebo (code A)
2	11, 12, 13, 14, 15, 16, 17, 18, 19, 20	ML7 (code B)
3	21, 22, 23, 24, 25, 26, 27, 28, 29, 30	Cyclosporin A
4	31, 32, 33, 34, 35	Naïve

7.2. Experimental procedure

7.2.1. Animal selection and randomization

Thirty-five (35) animals were selected based on good health and homogeneous body weight. Animals with no visible sign of ocular defect were randomly assigned to the study groups, using a function in Excel® software based on the mean of the corneal fluorescein staining scores from both eyes at baseline.

7.2.2. General clinical signs

7.2.2.1. Body weight

Body weights were recorded during the pre-test period (baseline) and once a week.

7.2.2.2. General appearance

General clinical signs and appearance of all animals were observed daily.

7.2.3. Induction of dry eye

Scopolamine was continuously and systemically delivered to the animals by an osmotic pump (2 ML4 Alzet; Charles River Laboratoires) filled with scopolamine and implanted subcutaneously on Day 0.
Pumps delivered 20 mg/day of a scopolamine solution over 21 days.
The pump was filled as mentioned in the procedure provided by Alzet®. Briefly, the pump was placed in sterile 0.9% NaCl overnight before implantation. Then the pump was filled with the scopolamine solution (280 mg/mL in 0.9% NaCl) using a syringe and a blunt-tipped filling tube.
Once the animal was anesthetized, the skin was shaved and scrubbed with betadine® over the implantation site.
An incision was made on the back of the animal and a hemostat inserted into the incision, and by opening and closing the jaws of the hemostat, the subcutaneous tissue was spread to create a pocket for the pump. The filled pump was inserted into the pocket, delivery portal first. The wound was closed with wound clips.

7.2.4. Ocular clinical signs

7.2.4.1. Measurement of aqueous tear production

Tear production was measured with the phenol red thread test (Zone-Quick, FCI-Ophthalmics) on both eyes. The threads were placed in the lateral cantus of the lateral conjunctival fornix for 30 seconds. The thread was wet by the tear and turned red, indicating the aqueous tear production. This data was expressed in millimeters.

7.2.4.2. Fluorescein staining

At the different time points, eyes were examined by slit-lamp observation using blue light after 0.5% fluorescein eye drop instillation (0.5 µL). Punctuate staining was recorded with standardized National Eye Institute (NEI) grading system giving a 0-3 score to each of the 5 areas in which the corneas were divided. For each cornea, the scores from each area were added, leading to a maximum score of 15 (see section **14.1** page **14**). If at least two area were not evaluable, the score was not calculated then «nd» (not determined) was noted.

7.2.5. In life phase termination

Animals were euthanized on Day 21 by systemic injection of overdosed pentobarbital. This method is one of the recommended methods for euthanasia by the European authorities [**6**]. At the end of the study, each pump was withdrawn and their scopolamine levels were visually compared.

8. DATA PROCESSING

Results were expressed in the form of data tables using Microsoft Excel® software.

9. STATISTICAL ANALYSIS

The statistical analyses were performed using the software GraphPad Prism 6.0. A two-way ANOVA analysis was performed on the individual scores at both time points. The induced effect was assessed using the Sidak test for two comparisons: naïve group versus placebo-treated and induced group.

The drug effect was assessed using the Dunnett's test for multiple comparisons between treated-groups and placebo-treated groups. The comparison was performed only for the 7-day time-point, since the number of animals in the placebo-treated group was too small for the other time-points.

The p value had to be lower than 0.05 for the difference to be significant.

10. RESULTS

10.1. Study plan amendments and deviations

No study plan amendment was required within the study period and two study plan deviation were reported:

- the first instillation on September 19th was missed.

- the lacrimation production on D21 was not performed because of Phenol red thread supplying.

These study deviations were not considered to have an impact on the integrity of the results.

10.2. General behavior and mortality

General behavior and appearance were normal for all animals. Chromodacryoorrhea (red tears) was observed in some animals during the first week after the surgery. This phenomenon is usually observed during acute stress and involves muscarinic mechanisms.

Three rats were humanely euthanized for ethical reason (skin necrosis on the scare):

- rat #4 (placebo group) on D15;
- rat #5 (placebo group) on D11;
- rats #27 (cyclosporine A -treated group) on D15.

10.3. Animal body weight

The animal body weights are reported in **Table 17** to **Table 20** page **24** to **25**.

Table 7: Animal body weight (in g)

Treatment	Baseline		Day 7		Day 14		Day of sacrifice (Day 21)		Body weight gain (%, Day 21 vs Baseline)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Naïve	184	6	208	8	212	6	207	4	12	3
Placebo	183	9	197	7	204	5	201	9	9	4
ML7	184	8	189	7	199	7	196	8	6	4
Cyclosporine A	183	7	181	4	189	10	200	6	10	4

SD= Standard Deviation

Body weights were all within a normal range at baseline: mean group values were in a range of 183 to 184 g.

On Day 21, all surgery animals had a lesser body weight gain in comparison with the naïve group. This might be related to the scopolamine treatment. The ML7 treated group had a lesser body weight gain in comparison with the placebo or Cysclosporin A -treated group.

10.4. Aqueous tear production

The level of tear production for each group was measured with the phenol-red thread test.

Results are summarized in **Table 8** page **15**. Individual data are reported in **Table 9** to **Table 12** page **16** to **19**.

Figure 1: Tear production measurement

Seven days after induction, the Placebo treated rats showed a reduction in tear production compared with that of the naïve rats. This reduction in tear production continued over the 2-weeks period. Oral treatment with Cyclosporine A improved tear production compared with the vehicle group on Day 14. On Day 14, the mean PRT in Cyclosporine A –treated group was greater than the placebo –treated group (15.1 ± 7.5 vs. 11.2 ± 5.9) and similar to the Naïve group (15.1 ± 7.5 vs. 16.0 ± 7.3). Topical administration of ML7 was similar to the placebo administration.

10.5. Corneal fluorescein staining

The corneal fluorescein staining was measured using the National Eye Institute grading scheme.

Results are summarized in **Table 8** page **15**. Individual data are reported in **Table 13** to **Table 16** page **20** to **23**. Statistical analyses are reported in **Table 21** to **Table 22** page **26** to **27**.

Figure 2: Corneal fluorescein staining

At baseline, rats demonstrated only minimal corneal punctate staining. Scopolamine significantly increased the fluorescein staining. The score was significantly higher in the Placebo-treated dry eye animal group than in the naïve control animals on Day 7 (9.4 ± 1.1 versus 4.0 ± 1.0, $p < 0.001$), on Day 14 (8.6 ± 2.2 versus, 4.4 ± 1.0, $p < 0.001$) and Day 21 (6.0 ± 2.3 versus 2.4 ± 1.8, $p < 0.001$).

Oral Cyclosporine A demonstrated a reduction in corneal staining scores after 21 days of treatment. The difference was statistically significant when compared with the Placebo treatment group on Day 7 ($p<0.0001$).

ML7 applied topically produced a statistically significant difference in corneal staining score on Day 7 when compared with the placebo-treated group ($p<0.0001$). On Days 21, the corneal staining was lower than the Placebo-treated group (5.2 ± 2.2 vs. 6.0 ± 2.3).

11. CONCLUSIONS

In the present study, and under our experimental conditions, the effects of ML7 topically applied TID, were evaluated in a rat model of scopolamine-induced dry eye. Under these conditions it can be stated that:

- ML7 did not improve the reduction of tear production induced by the scopolamine treatment.
- ML7 reduced the staining score after 7 days of treatment. After 21 days of treatment, the corneal staining was lower than in the vehicle group, however no statistical analysis could be performed at this time-point due to the number of animals left in the vehicle group (2 euthanized animals for ethical reason).

As expected, oral administration of Cyclosporine A improved tear production and reversed the corneal staining by 3 weeks of treatment and validated the assay.

12. ARCHIVING OF DATA

All source documents, raw data, study plan and report (all paper data) will be available for 5 years at IRIS PHARMA following the Study Director's approval of study report, upon which they will be either discarded or sent back to the Sponsor or kept for another 5 years at the Sponsor's request (at an additional cost).

13. REFERENCES

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scopolamine-induced dry eye model. **Graefes Arch Clin Exp Ophthalmol**. 2008 JUN; 246(6): 857-67.

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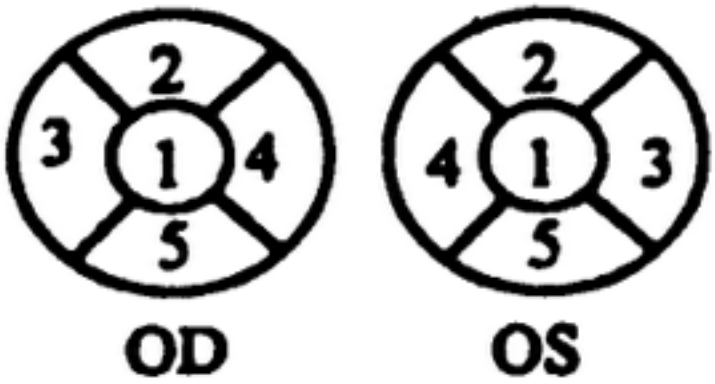
14. APPENDICES

14.1. Corneal staining

The scale is based on the NEI grading system. The cornea is divided into five areas (see below). The amount of staining in each area is graded from 0 to 3 according to the punctuate fluorescein staining. The total maximum score is 15.

0:	no staining
1:	mild punctuate staining
2:	moderate punctuate staining
3:	severe punctuate staining



14.2. Table 8: Summarized data

Table 8: Evaluation of dry eye symptoms in albino rats

Treatment group	Time-point	Lacrimation (mm)		Corneal staining (0-15)	
		Mean	*SD*	**Mean**	*SD*
3 instillations of Placebo/day	Baseline	**16.15**	*7.08*	**2.15**	*1.18*
	D7	**10.45**	*4.26*	**9.35**	*1.14*
	D14	**11.22**	*5.93*	**8.61**	*2.17*
	D21	**-**	-	**6.00**	*2.31*
3 instillations of ML7/day	Baseline	**16.85**	*5.98*	**2.20**	*1.06*
	D7	**7.65**	*2.28*	**8.00**	*1.49*
	D14	**11.00**	*7.23*	**8.55**	*1.70*
	D21	**-**	-	**5.20**	*2.19*
Cyslosporin A per os 1x/day	Baseline	**16.80**	*5.31*	**2.25**	*1.12*
	D7	**13.65**	*5.02*	**4.45**	*1.00*
	D14	**15.11**	*7.49*	**4.11**	*1.49*
	D21	**-**	-	**2.44**	*1.54*
Naïve	Baseline	**15.80**	*2.82*	**2.00**	*0.82*
	D7	**20.50**	*4.55*	**4.00**	*1.05*
	D14	**16.00**	*7.29*	**4.40**	*0.97*
	D21	**-**	-	**2.40**	*1.78*

14.3. Tables 9-16: Individual data

14.3.1. Tables 9-12: Tear production evaluation

Table 9: Tear production evaluation with three instillations/day (Placebo)

Rat n°	Time-point	PRT Test (mm)						Mean	SD
		Right eye	Mean	SD	Left eye	Mean	SD		
1	Baseline	25	17.7	7.1	15	14.6	7.1	16.2	7.1
2		6			11				
3		17			6				
4		22			18				
5		7			8				
6		15			12				
7		28			32				
8		20			16				
9		20			15				
10		17			13				
1	D7	7	9.1	4.5	12	11.8	3.8	10.5	4.3
2		19			15				
3		3			5				
4		5			15				
5		10			10				
6		8			14				
7		11			15				
8		11			15				
9		6			11				
10		11			6				
1	D14	12	11.9	6.2	16	10.6	5.9	11.2	5.9
2		16			16				
3		25			8				
4		12			13				
5		dead			dead				
6		9			9				

7	**12**	**20**
8	**5**	**5**
9	**12**	**3**
10	**4**	**5**

Table 10: Tear production evaluation with three instillations/day (ML7)

Rat n°	Time-point	PRT Test (mm)						Mean	*SD*
		Right eye	**Mean**	*SD*	Left eye	**Mean**	*SD*		
11	**Baseline**	**20**	**16.6**	***4.6***	**26**	**17.1**	***7.4***	**16.9**	*6.0*
12		**18**			**23**				
13		**19**			**25**				
14		**20**			**7**				
15		**14**			**15**				
16		**25**			**26**				
17		**12**			**11**				
18		**10**			**9**				
19		**13**			**17**				
20		**15**			**12**				
11	**D7**	**4**	**7.6**	***2.3***	**9**	**7.7**	***2.4***	**7.7**	*2.3*
12		**7**			**9**				
13		**11**			**7**				
14		**9**			**9**				
15		**9**			**7**				
16		**9**			**9**				
17		**10**			**5**				
18		**5**			**4**				
19		**6**			**6**				
20		**6**			**12**				
11	**D14**	**6**	**11.7**	***7.5***	**9**	**10.3**	***7.2***	**11.0**	*7.2*
12		**11**			**12**				
13		**11**			**8**				
14		**26**			**13**				
15		**5**			**5**				
16		**25**			**28**				
17		**7**			**12**				
18		**8**			**2**				

19		**8**			**4**				
20		**10**			**10**				

Table 11: Tear production evaluation with cyclosporin (per os) 1x/day

Rat n°	Time-point	PRT Test (mm)						Mean	SD
		Right eye	**Mean**	*SD*	Left eye	**Mean**	*SD*		
21	**Baseline**	**17**	**14.8**	***5.2***	**20**	**18.8**	***4.8***	**16.8**	*5.3*
22		**11**			**17**				
23		**20**			**25**				
24		**15**			**23**				
25		**8**			**15**				
26		**10**			**18**				
27		**13**			**10**				
28		**23**			**25**				
29		**10**			**20**				
30		**21**			**15**				
21	**D7**	**16**	**13.5**	***4.5***	**13**	**13.8**	***5.7***	**13.7**	*5.0*
22		**13**			**13**				
23		**16**			**26**				
24		**18**			**16**				
25		**14**			**15**				
26		**14**			**19**				
27		**18**			**9**				
28		**5**			**6**				
29		**15**			**12**				
30		**6**			**9**				
21	**D14**	**16**	**17.8**	***7.4***	**18**	**12.4**	***6.9***	**15.1**	*7.5*
22		**28**			**8**				
23		**28**			**20**				
24		**15**			**9**				
25		**5**			**7**				
26		**21**			**11**				
27		**dead**			**dead**				
28		**12**			**10**				
29		**20**			**25**				


30
15
4

Table 12: Tear production evaluation with no induction (Naïve)

Rat n°	Time-point	PRT Test (mm)						Mean	SD
		Right eye	**Mean**	*SD*	Left eye	**Mean**	*SD*		
31	**Baseline**	**13**	**15.4**	***2.9***	**12**	**16.2**	***3.0***	**15.8**	*2.8*
32		**20**			**18**				
33		**16**			**15**				
34		**13**			**20**				
35		**15**			**16**				
31	**D7**	**20**	**23.8**	***3.0***	**21**	**17.2**	***3.2***	**20.5**	*4.6*
32		**28**			**15**				
33		**25**			**18**				
34		**24**			**19**				
35		**22**			**13**				
31	**D14**	**5**	**17.2**	***7.9***	**5**	**14.8**	***7.4***	**16.0**	*7.3*
32		**18**			**18**				
33		**24**			**13**				
34		**15**			**25**				
35		**24**			**13**				

14.3.2.Tables 13-16: Corneal staining

Table 13: Corneal staining evaluation with three instillations/day (Placebo)

Rat n°	Time-point	Corneal staining (0-15)						Mean	SD
		Right eye	Mean	SD	Left eye	Mean	SD		
1	Baseline	1	2.7	1.3	2	1.6	0.7	2.2	1.2
2		2			1				
3		1			1				
4		2			1				
5		2			2				
6		3			2				
7		4			1				
8		3			3				
9		4			2				
10		5			1				
1	D7	11	9.7	1.1	10	9.0	1.2	9.4	1.1
2		10			9				
3		8			7				
4		10			9				
5		10			11				
6		11			10				
7		9			9				
8		10			8				
9		8			9				
10		10			8				
1	D14	13	8.7	2.3	9	8.6	2.2	8.6	2.2
2		8			9				
3		9			11				
4		9			8				
5		dead			dead				
6		8			8				
7		7			6				
8		11			12				

9		**5**			**9**				
10		**8**			**5**				
1		**8**			**6**				
2		**7**			**3**				
3		**4**			**6**				
4		**dead**			**dead**				
5		**dead**			**dead**				
6	**D21**	**8**	**6.4**	***1.8***	**12**	**5.6**	***2.8***	**6.0**	*2.3*
7		**4**			**4**				
8		7			4				
9		**8**			**6**				
10		**5**			**4**				

Table 14: Corneal staining evaluation with three instillations (ML7)

Rat n°	Time-point	Corneal staining (0-15)						Mean	SD
		Right eye	Mean	SD	Left eye	Mean	SD		
11	Baseline	1	2.5	1.1	1	1.9	1.0	2.2	1.1
12		2			0				
13		2			2				
14		3			1				
15		3			2				
16		2			3				
17		1			3				
18		4			2				
19		3			2				
20		4			3				
11	D7	7	8.1	1.7	8	7.9	1.4	8.0	1.5
12		10			9				
13		8			8				
14		8			9				
15		11			9				
16		7			7				
17		10			10				
18		7			6				
19		6			6				
20		7			7				
11	D14	8	9.3	1.3	8	7.8	1.8	8.6	1.7
12		10			9				
13		11			6				
14		11			10				
15		10			9				
16		9			9				
17		7			8				
18		9			7				
19		8			4				

20		**10**			**8**				
11		**7**			**4**				
12		**5**			**6**				
13		**8**			**2**				
14		**4**			**4**				
15		**8**			**6**				
16	**D21**	**8**	**6.1**	***1.9***	**5**	**4.3**	***2.2***	**5.2**	*2.2*
17		**7**			**8**				
18		**4**			**5**				
19		**3**			**1**				
20		**7**			**2**				

Table 15: Corneal staining evaluation with cyclosporin (per os) 1x/day

Rat n°	Time-point	Corneal staining (0-15)						Mean	SD
		Right eye	Mean	SD	Left eye	Mean	SD		
21	Baseline	3	2.6	1.1	0	1.9	1.1	2.3	1.1
22		1			1				
23		2			1				
24		3			1				
25		1			3				
26		3			2				
27		4			3				
28		3			2				
29		2			3				
30		4			3				
21	D7	5	4.5	1.0	5	4.4	1.1	4.5	1.0
22		4			4				
23		3			3				
24		6			6				
25		5			6				
26		5			4				
27		3			4				
28		4			3				
29		5			4				
30		5			5				
21	D14	4	4.7	1.2	4	3.6	1.6	4.1	1.5
22		7			3				
23		5			5				
24		6			5				
25		4			1				
26		4			2				
27		dead			dead				
28		3			2				
29		5			5				

30		**4**			**5**				
21	**D21**	**4**	**2.6**	***1.4***	**1**	**2.3**	***1.7***	**2.4**	*1.5*
22		**1**			0				
23		**5**			**2**				
24		**2**			**2**				
25		**4**			**2**				
26		**2**			**5**				
27		**dead**			**dead**				
28		**1**			**3**				
29		**2**			**1**				
30		**2**			**5**				

Table 16: Corneal staining evaluation with no induction (Naïve)

Rat n°	Time-point	Corneal staining (0-15)						Mean	SD
		Right eye	Mean	SD	Left eye	Mean	SD		
31	Baseline	3	2.6	0.5	2	1.4	0.5	2.0	0.8
32		3			2				
33		2			1				
34		3			1				
35		2			1				
31	D7	5	4.4	0.9	4	3.6	1.1	4.0	1.1
32		5			3				
33		4			4				
34		3			5				
35		5			2				
31	D14	5	4.8	0.4	5	4.0	1.2	4.4	1.0
32		5			4				
33		4			2				
34		5			5				
35		5			4				
31	D21	1	2.8	2.2	1	2.0	1.4	2.4	1.8
32		2			4				
33		1			1				
34		6			3				
35		4			1				

14.4. Tables 17-19: Individual data animal body weight

Table 17: Body weight without treatment

Rat n°	Body Weights (g)											
	D0	Mean	*SD*	D7	Mean	*SD*	D14	Mean	*SD*	D21	Mean	*SD*
31	192			216			218			209		
32	182			212			218			211		
33	184	184.4	*6.1*	208	208.0	*8.4*	210	211.6	*6.2*	207	206.6	4.2
34	176			194			204			200		
35	188			210			208			206		

Table 18: Body weight with three instillations (Placebo)

Rat n°	Body Weights (g)											
	D0	Mean	*SD*	D7	Mean	*SD*	D14	Mean	*SD*	D21	Mean	*SD*
1	178			192			202			187		
2	188			194			204			201		
3	184			194			206			210		
4	192			202			202			na		
5	170	183.2	*8.8*	184	196.8	*6.7*	na	204.2	*5.1*	na	200.5	*9.2*
6	178			194			206			203		
7	198			206			208			202		
8	186			198			200			208		
9	172			198			196			186		
10	186			206			214			207		

Table 19: Body weight with three instillations (ML7)

Rat n°	Body Weights (g)											
	Baseline	Mean	*SD*	D7	Mean	*SD*	D14	Mean	*SD*	D21	Mean	*SD*
11	176			184			192			195		

12	**182**			**180**			**194**			**188**	
13	**193**			**194**			**196**			**192**	
14	**176**			**182**			**192**			**190**	
15	**180**	**184.1**	***8.1***	**188**	**189.4**	***6.9***	**200**	**199.2**	***7.2***	**192**	**195.7**	***8.0***
16	**194**			**200**			**212**			**210**	
17	**182**			**184**			**196**			**190**	
18	**174**			**190**			**196**			**197**	
19	**188**			**194**			**204**			**193**	
20	**196**			**198**			**210**			**210**	

Table 20: Body weight with with cyclosporin (per os)

Rat n°	Body Weights (g)											
	D0	Mean	*SD*	D7	Mean	*SD*	D14	Mean	*SD*	D21	Mean	*SD*
21	172	183.4	*7.2*	180	180.8	*4.2*	172	188.9	*9.6*	191	200.0	*5.5*
22	182			186			192			193		
23	190			178			174			199		
24	176			178			196			204		
25	186			174			190			204		
26	196			186			196			201		
27	188			176			na			na		
28	180			182			190			197		
29	186			184			190			203		
30	178			184			200			208		

14.5. Tables 21-22: Statistical analyses

Table 21: Corneal staining Sidak's test (Naïve vs. Placebo groups)

Table Analyzed Two-way ANOVA, CS

Two-way ANOVA Ordinary
Alpha 0.05

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	110.2	3	36.72	F (3, 106) = 14.98	P < 0.0001
times	392.6	3	130.9	F (3, 106) = 53.38	P < 0.0001
treatment	302.0	1	302.0	F (1, 106) = 123.2	P < 0.0001
Residual	259.8	106	2.451		

Number of missing values 27

Sidak's multiple comparisons test

Nalve - Placebo	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Baseline	0.1500	-1.386 to 1.686	No	ns	0.9986
Day 7	5.700	4.164 to 7.236	Yes	****	< 0.0001
Day 14	4.211	2.646 to 5.776	Yes	****	< 0.0001
Day 21	3.600	2.001 to 5.199	Yes	****	< 0.0001

Table 22: Corneal staining Dunnett's test (ML7, Placebo and Cyclosporine A -treated groups)

Table Analyzed One way ANOVA, CS, Day 7

ANOVA summary	
F	92.96
P value	< 0.0001
P value summary	****
Are differences among means statistically significant? (P < 0.05)	Yes
R square	0.8086

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	385.2	3	128.4	F (3, 66) = 92.96	P < 0.0001
Residual (within columns)	91.15	66	1.381		
Total	476.3	69			

Dunnett's multiple comparisons test

	Mean Diff.	95% CI of diff.	Significant?	Summary	Adjusted P Value
Placebo vs. ML7	**1.700**	**0.8064 to 2.594**	**Yes**	****	**< 0.0001**
Placebo vs. CsA (per os)	**5.250**	**4.356 to 6.144**	**Yes**	****	**< 0.0001**
Placebo vs. Naïve	**5.700**	**4.606 to 6.794**	**Yes**	****	**< 0.0001**

14.6. Certificate of analysis of scopolamine

14.7. Certificate of analysis of ML7

octalia technologies

CERTIFICATE OF CONFORMITY FOR FORMULATIONS ML7 / NEUROPTIS

Number: CC024 Version: 01

Approved: _____ Date : May 16th, 2013

Batch number : TREATMENT B Description: Solution

Manufacturing date : July 31st, 2013 Packaging size : 2 mL in 3 mL amber glass vial

Study number : N50F25612

pH : 6.9

Osmolality : 293 mOsm/kg

Assay : Conforms

Name and signature : _____ Date : August 01st, 2013

14.8. Certificate of analysis of placebo

octalia technologies

CERTIFICATE OF CONFORMITY FOR FORMULATIONS ML7 / NEUROPTIS

Number: CC024 Version: 01

Approved: _____ Date : May 16th, 2013

Batch number : TREATMENT A Description: Solution

Manufacturing date : July 31st, 2013 Packaging size : 8 mL in 8 mL amber glass vial

Study number : N50F25612

pH : 6.9

Osmolality : 293 mOsm/kg

Assay : Conform

Name and signature : _____ Date : August 08th, 2013

14.9. Coding instructions

octalia technologies

Approved by:
Date: Aug 8th, 2013 Signature:

CODING AND LABELLING INSTRUCTIONS

Study : N50F25612

CRO : Iris Pharma **Fill volume: 2 mL**

Coding list

Coding	Batch #	ID	Number of vials
A	E753-1	Placebo	21 + 3
B	E753-2	0.1%w/w ML7 solution	21 + 4

Labels for formulation A

N50F25612 study
Treatment A
Per: 31/09/13
Storage: 4°C
Octalia Technologies

Labels for formulation B

N50F25612 study
Treatment B
Per: 31/09/13
Storage: 4°C
Octalia Technologies

Labels for sample box

octalia technologies

N50F25612 study
Treatment A (21 + 3 vials at 2 ml)
Per: 31/09/13

N50F25612 study
Treatment B (21 + 4 vials at 2 ml)
Per: 31/09/13

Storage: 4°C