UNIVERSITÄTSKLINIK FÜR KLINISCHE PHARMAKOLOGIE

MEDIZINISCHE UNIVERSITÄT WIEN

Allgemeines Krankenhaus der Stadt Wien

Medical University Vienna
Allgemeines Krankenhaus
Währinger Gürtel 18-20
A-1090 Vienna
Austria
Phone: + 43 1 40400 29810
Fax: + 43 1 40400 29980
E-mail: Klin-Pharmakologie@meduniwien.ac.at
http://www.meduniwien.ac.at/klpharm

Study protocol

A Phase II/III, Randomized, Double-Masked, Vehicle-Controlled, Efficacy, Safety and Tolerability Study of Chloroprocaine 3% Gel Eye Drops in Healthy Volunteers

Protocol No: CHL.3-02-2019

EudraCT No: 2020-000465-17

Version 2.0, 04-May-2020

Sponsor (AMG §§ 2a, 31, 32):
Sintetica S.A.
Via Penate 5,
6850 Mendrisio, Switzerland
Tel. +41 (0)91 640 42 50
Fax +41 (0)91 646 85 61

Sponsor's Representative:
Elisabetta Donati, PharmD, Corporate Director Scientific Affairs
Email: edonati@sintetica.com

Principal investigator (AMG §§ 2a, 35, 36):
Assoc. Prof. Priv. Doz. Dr. Gerhard Garhöfer
Department of Clinical Pharmacology
Währinger Gürtel 18-20
1090 Vienna
Austria
Tel: +43 1 40400 29810
Fax: +43 1 40400 29900

Clinical trial center:
Department of Clinical Pharmacology
Währinger Gürtel 18-20
1090 Vienna
Austria
Phone: +43 1 40400 29810
Fax: +43 1 40400 29900

CRO (AMG §§ 2a,33, 34):
Iris Pharma
Les Nertières
Allée Hector Pintus
06610 La Gaude
France

INVESTIGATOR SIGNATURE PAGE

A Phase II/III, Randomized, Double-Masked, Vehicle-Controlled, Efficacy, Safety and Tolerability Study of Chloroprocaine 3% Gel Eye Drops in Healthy Volunteers

I confirm that I have read and understood the protocol. I agree to meet all the obligations and restrictions outlined for me in the protocol. I will obtain written informed consent from all study participants prior to conducting any study procedures. All information regarding this protocol and the IMP will be treated as strictly confidential. I will conduct the study in all respects in accordance with the study protocol and the ethical principles of the current version of the declaration of Helsinki and GCP.

SPONSOR: Sintetica S.A.

Sponsor´s representative

04.05.2020 

_____ _____

Date signature

INVESTIGATOR CENTER

Principal Investigator:
Assoc. Prof. Priv. Doz. Dr. Gerhard Garhöfer

_____ _____

date signature

Study Synopsis

Title	A Phase II/III, Randomized, Double-Masked, Vehicle-Controlled, Efficacy, Safety and Tolerability Study of Chloroprocaine 3% Gel Eye Drops in Healthy Volunteers
Study phase	Phase II/III
Principal investigator	Assoc. Prof. Priv. Doz. Dr. Gerhard Garhöfer, Universitätsklinik für Klinische Pharmakologie, Allgemeines Krankenhaus Wien, Währinger Gürtel 18-20, A-1090 Wien, Tel: +43 1 40400 29810
Co-investigators	Assoc. Prof. PD Dr. Doreen Schmidl, Dr. Nikolaus Hommer, Dr. Martin Kallab, Dr. Andreas Schlatter, Universitätsklinik für Klinische Pharmakologie, Allgemeines Krankenhaus Wien, Währinger Gürtel 18-20, A-1090 Wien; Tel. 40400-29810
Data management Biostatistics	IDDI, 30 av Provinciale, 1340, Louvain-la-Neuve, Belgium Agilis, Patriarchou Ioakim 1 and Pireos 209, 177 78 Tavros, Greece
Background and aim	Safe and efficient topical anaesthesia is crucial for surgical procedures in ophthalmology, such as cataract surgery. Usually, eye drops based on oxybuprocaine, lidocaine or tetracaine as pharmacologically active agents are used to provide local anaesthesia of the eye. Chloroprocaine is a local anaesthetic that is indicated for the production of local anaesthesia by infiltration, peripheral nerve block and central blocks, including spinal and epidural blocks. One of its key advantages is the fast metabolism by ester hydrolysis leading to a very short plasma half-life of less than 30 seconds. Therefore, also high concentrations can be used with large volumes and minimal risk of toxicity. As such, chloroprocaine is the ideal anaesthetic for regional anaesthesia in patients undergoing short-duration surgeries, providing rapid onset of action, adequate potency, predictable duration, fast recovery, and a safe toxicological profile when compared with other local anaesthetics. In the present study, a Chloroprocaine 3% Gel formulation for topical anaesthesia of the eye will be evaluated in healthy subjects. In addition to the above-mentioned benefits, the gel formulation has several other advantages. Most importantly, it tends to stay on the eye for a longer duration than commonly used eye drops due to its viscosity and is not drained by the punctae as quickly as drops with low viscosity. Due to its high viscous formulation, systemic absorption through the nasolacrimal system is expected to be low, therefore reducing the potential for systemic toxicity. The study will be carried out in 2 parts. In part I, safety and tolerability will be assessed in three groups (12 subjects per group) for single and multiple instillations (1 drop, 3 drops and 3+3 drops). In each group, 9 subjects will be randomized to receive Chloroprocaine 3% Gel and 3 subjects will receive vehicle as control in the right eye. After part I is completed, an internal independent board will review safety endpoints of data collected from these first subjects and advise to go on with further enrollment. If no safety concerns arise, in part II efficacy, safety and tolerability will be assessed in 60 healthy subjects for the 3 drops dose regimen. 40 subjects will receive Chloroprocaine 3% Gel and 20 will receive vehicle (2:1 randomization) in the right eye.
Study objectives	Primary objective: To assess efficacy of Chloroprocaine 3% ophthalmic gel in healthy volunteers Secondary objectives: To assess safety and tolerability of Chloroprocaine 3% ophthalmic gel in healthy volunteers

Primary endpoint	Estimate the proportion of subjects experiencing full anaesthesia of the ocular surface 5 minutes after administration of Chloroprocaine 3% ophthalmic gel
Primary Hypothesis	Administration of Chloroprocaine 3% ophthalmic gel leads to topical anesthesia within 5 minutes in healthy subjects
Secondary endpoints:	• Estimate time to anesthesia and duration of anesthesia, as well as Cochet Bonnet assessments • Assess the occurrence of: - Ocular symptoms - Adverse events • Blood pressure and heart rate • Slit lamp examination • Corneal fluorescein staining • Intraocular pressure
Study design	Randomized, Double-Masked, Vehicle-Controlled, Single-Center Study
Study population	A total of 96 healthy male and female subjects will be included in the study. The study will be performed in 2 parts. Part I: Group 1 (12 subjects): Single instillation Group 2 (12 subjects): 3 drops Group 3 (12 subjects): 3+3 drops In each group, 9 subjects will be randomized to receive Chloroprocaine 3% ophthalmic gel and 3 subjects will receive vehicle in the right eye. On the first two study days of each group, one subject will be dosed per day. On the third study day up to two subjects can be dosed per day. On the following study day(s) up to 4 subjects can be dosed per day. Randomization will be performed in three blocks (3:1, Chloroprocaine vs. vehicle) for each group. After part I is completed, an internal independent board will review safety endpoints of data collected from these first subjects and advise to go on with further enrollment. Part II: 60 subjects will be included in Part II. 40 subjects will be randomized to receive 3 drops of Chloroprocaine 3% ophthalmic gel and 20 subjects will receive vehicle in the right eye (2:1 randomization, Chloroprocaine vs. vehicle).
Investigational medicinal product (IMP)	Chloroprocaine 3% ophthalmic Gel IMP: Chloroprocaine 3% ocular gel, Sintetica S.A., Switzerland Manufacturer: Unither Pharmaceuticals, France Pharmaceutical form: Gel Dose: 1 drop Frequency: 1, 3 and 3+3 times Administration route: Topical instillation
Control	Vehicle for Chloroprocaine 3% ophthalmic Gel IMP: Placebo, Vehicle for chloroprocaine 3% ocular gel, Sintetica S.A., Switzerland Manufacturer: Unither Pharmaceuticals, France Pharmaceutical form: Gel Dose: 1 drop Frequency: 1, 3 and 3+3 times Administration route: Topical instillation

Topically administered medication for measurements	Fluoresceine-Oxybuprocaine SDU Faure (Omnivision, Neuhausen), dose: 1 drop per eye for measurement of intraocular pressure Minims-Fluorescein Sodium 2.0% (Chauvin Pharmaceuticals Ltd. UK); dose: 1 drop per eye for assessment of corneal staining
Study procedures	• Blood pressure and heart rate (automatic oscillometric device) • Ocular symptoms (100 mm visual analogue scale for burning, stinging, itching, foreign body sensation) • Far best corrected visual acuity using standard ETDRS charts • Slit lamp examination (eyelid swelling, eyelid redness, conjunctival chemosis, conjunctival hyperemia, cornea, anterior chamber flare) • Corneal fluorescein staining (according to the NEI scale) • Intraocular pressure (Goldmann applanation tonometry) • Funduscopy at the slit lamp • Pinching of the conjunctiva (0.3 mm forceps) • Cochet bonnet assessment of corneal sensitivity • Urine pregnancy test
Inclusion criteria	1. Signed and dated informed consent 2. Healthy male or female aged from 18 to 90 years 3. No clinically significant ocular or systemic disease 4. Ability to orally respond to pain 5. Ability to follow the visit schedule
Exclusion criteria	*Ophthalmic exclusion criteria* 1. Eye movement disorder (i.e. Nystagmus) 2. Dacryocystitis and all other pathologies of tears drainage system 3. History of Inflammatory ocular disease (Iritis, uveitis, herpetic keratitis) 4. Corneal, epithelial, stromal or endothelial, residual or evolutionary disease (including corneal ulceration and superficial punctuate keratitis) 5. History of ocular traumatism, infection or inflammation within the last 3 months 6. Best corrected visual acuity < 1/10 7. History of ophthalmic surgical complication (i.e. cystoid macular oedema) *Systemic/non ophthalmic exclusion criteria* 8. General history: 8.1 Deafness 8.2 Excessive anxiety 9. Any other medical or surgical history, disorder or disease such as acute or chronic severe organic disease: hepatic, endocrine neoplastic, haematological diseases, severe psychiatric illness, relevant cardiovascular abnormalities (such as unstable angina, uncontrolled hypertension: systolic blood pressure over 200 mm Hg, diastolic blood pressure over 100 mm Hg) and/or any complicating factor or structural abnormality judged by the investigator to be incompatible with the study 10. Allergic history: Known hypersensitivity to one of the components of the study medications or to test products *Specific non-inclusion criteria for women*: 11. Pregnancy, lactation 12. Women without an effective method of contraception (i.e. oral contraceptive, intra-uterine device, subcutaneous contraceptive implant) OR 13. Women not hysterectomised, not menopausal nor surgically sterilized *Exclusion criteria related to general conditions:* 14. Inability of subject to understand the study procedures and thus inability to give informed consent

	15. Non-compliant subject (e.g. not willing to attend the follow-up visits, way of life interfering with compliance) 16. Participation in another clinical study 17. Already included once in this study 18. Ward of court 19. Subject not covered by the Social Security *Exclusion criteria related to previous and concomitant medications (taken within 15 days prior screening visit)* 20. Use of systemic opioids and opioid drugs 21. Topical ocular treatment with anaesthetic action 22. Use of systemic analgesic drugs (except paracetamol, which will be allowed after Visit 2)
Planned Duration of the study	Approximately 6 months (first subject first visit to last subject last visit)
Benefit/risk assessment	Although there is no direct benefit for the subjects under study, the findings of this study may help to assess safety, tolerability and efficacy of ophthalmic Chloroprocaine 3% Gel. Chloroprocaine has been used for spinal, epidural and perineural anaesthesia for several years and is well tolerated. However, no data for the use in the human eye is currently available. Chloroprocaine 3% ophthalmic Gel is intended to be used for topical anaesthesia for patients undergoing eye surgery in the future. Due to its viscosity the gel tends to stay on the eye for a longer duration than eye drops and is not absorbed by the punctae as quickly as the tears and systemic absorption through the nasolacrimal system should be reduced, therefore reducing the potential for systemic toxicity. Data from the non-clinical development program reports good local tolerability of Chloroprocaine 3% ophthalmic gel on the ocular surface. Further, non-clinical data from a proof-of-concept study indicates a strong and long-lasting anaesthetic effect of Chloroporcaine 3% ophthalmic gel. However, at this stage of development, local toxicity of Chloroprocaine 3% Gel in human eyes cannot be ruled out but the risk is considered low based on the safety factor calculated using preclinical data. Considering the expected low systemic exposure, systemic side effects are unlikely to occur. The most common systemic side effect that was observed after intrathecal injection was systemic hypertonia. Although it is unlikely to occur after ophthalmic use, blood pressure and heart rate will be assessed after administration in the present study. Therefore, the risk/benefit ratio is acceptable.
Statistical analysis	Part I: In this part of the study, no formal statistical analysis (i.e. hypothesis testing) will take place. Safety will be assessed using the frequency of adverse events and results from the slit lamp evaluation. The safety board will decide whether the safety profile is acceptable to proceed in part II of the study. Part II: All parameters measured will be evaluated and presented using descriptive statistics, i.e. arithmetic mean, SD, minimum, median and maximum values for quantitative variables, and absolute and relative (%) frequencies for qualitative variables. In this part of the study, the list of quantitative variables includes time to anesthesia, duration of anesthesia, Cochet Bonnet assessment, blood pressure, heart rate, intraocular pressure, ocular symptoms, slit lamp examination and corneal fluorescein staining, while qualitative variables include the experience of full anesthesia of the ocular surface and the occurrence of adverse events. Moreover, results will be reported by treatment group. In terms of figures, quantitative variables will be presented by either histograms or box-plots, and

	qualitative variables will be presented by either bar-charts or pie-charts. Confidence intervals will be reported, where appropriate. If not stated elsewhere, these intervals will be two sided in each case and provide 95% confidence. The primary endpoint of the part II of the study is defined as the proportion of subjects gaining full anesthesia of the ocular surface 5 minutes after administration of the IMP for the 2 treatment arms (i.e. p1 for vehicle and p2 for Chloroprocaine arm). The null hypothesis of equal response rates between the 2 treatment arms (i.e. H_0: $p_1=p_2$) at the α=0.05 level will be rejected if: $$\left\lvert \frac{\widehat{p_1} - \widehat{p_2}}{\sqrt{\frac{\widehat{p_1}(1-\widehat{p_1})}{n_1} + \frac{\widehat{p_2}(1-\widehat{p_2})}{n_2}}} \right\rvert > Z_{\alpha/2}$$ In terms of statistical inference for the secondary endpoints, quantitative variables will be compared between the 2 groups using Mann-Whitney's test, in which the null hypothesis is that for each quantitative variable tested, its median value does not differ significantly between the 2 groups (i.e. H_0: $median_1=median_2$). Qualitative variables will be compared using Pearson's X^2 test, in which the null hypothesis for each pair of qualitative variables is that these two variables are not associated. To account for multiple comparisons, we will adjust the statistical significance level using Bonferroni corrections. In terms of adverse events, all AEs and SAEs will be reported in tabular form overall and separately for each treatment. Not available data will be evaluated as "missing values". A statistical analysis plan (SAP) will be provided before database closure. A p-value<0.05 will be considered statistically significant. The statistical analysis will be performed using RStudio v. 1.2.5033 and/or IBM SPSS Statistics v. 25 (or newer) for Windows.

Table of Contents

1. Introduction

1.1 Background

Safe and efficient topical local anaesthesia is crucial for surgical procedures in ophthalmology, such as cataract surgery, intravitreal injections, refractive surgery or others. The development of less invasive surgical techniques, which allow for most interventions to be performed as outpatient surgeries, has stimulated the development of new approaches in topical aesthesia. Currently, oxybuprocaine, lidocaine or tretracaine as pharmacologically active agents - mostly formulated as liquid eye drops - are used to provide local anesthesia of the eye.[1] However, it has been hypothesized that formulations with optimized physical and chemical properties such as high viscosity gel based formulations might be superior to eye drops in terms of efficacy and safety.

Background on Chloroprocaine

Chloroprocaine is a local anaesthetic that has been widely used for spinal, epidural and perineural anaesthesia.[2] Chloroprocaine, like other local anesthetics in this group, reduces signal conduction in neurons by altering the Na^+ channels in the neuronal cell membrane responsible for the action potential propagation. Pharmacologically speaking, one of the key advantages of Chloroprocaine is the fast onset (usually within minutes) and the fast metabolization by ester hydrolysis, which leads to a very short plasma half-life of less than 30 seconds. This leads to good controllability and also high concentrations can be used with minimal risk of toxicity. As such, Chloroprocaine is the ideal anesthetic for regional anesthesia in patients undergoing short-duration surgeries, providing rapid onset of action, adequate potency, predictable duration, fast recovery, and a safe toxicological profile when compared to other local anesthetics.

The current study aims to investigate the efficacy, safety and tolerability of a newly developed Chloroprocaine ophthalmic gel in humans. Based on theoretical considerations, one can hypothesize that a gel formulation could provide additional benefits compared to a drop solution. First, because of its high viscosity, gel formulations stay longer on the ocular surface and may therefore provide a longer anesthetic effect. Secondly, because of the gel nature of the formulation, the drainage of the drug through the nasolacrimal drainage system is reduced, which might limit absorption through the nasal mucosa and thus lead a low systemic exposure of the drug.

Regulatory status of Chloroprocaine

Chloroprocaine is marketed since 1955 in the USA and Canada for epidural aesthesia and peripheral blocks (Nesacaine® 1%, 2%, 3% injection). In the European Community, Chloroprocaine has received market authorization (Ampres® 10mg/ml Injektionslösung) for spinal anesthesia in adults where the planned surgical procedure should not exceed 40 minutes. An extension application for the existing marketing authorization (new strength of 20mg/ml, new route of administration: perineural anesthesia) is currently under review.

Non-clinical development

As Chloroprocaine is widely used for different routes of administration, the toxicological profile is well described.

Animal studies report a good safety profile and did not report any particular sign of systemic toxicity beside those already known for this class of local anesthetics. For more details on systemic toxicology please refer to the investigator's brochure.

Non-clinical safety and Toxicology on the eye

Local tolerability and safety and proof-of-concept of Chloroprocaine 3% eye drops has been investigated in a non-clinical development program. Data of a GLP ocular irritation study in rabbits reports good tolerability of the formation on the ocular surface (Study Number 14-01765-G2, Study on File). Further, a 7-day GLP local tolerance study including histopathological assessments in rabbits confirms good tolerance on the ocular surface. (Study number: S104CH20118, Study on file). A non-GLP efficacy study performed in a rabbit animal model supports the concept of Chloroprocaine 3% ophthalmic gel (Study A66C06315, S104CH20318, Studies on file), showing an anesthetic potency up to 60 minutes after instillation depending on the viscosity of the gel formulation. An in-depth description of the non-clinical development program is given in the investigator´s brochure.

1.2 Rationale of the study

Scientific advice on the planned clinical development plan has been obtained from the FDA in Sept. 2019. As part of this development plan and following the advice from the competent authority a combined phase II/III study will be performed as described here in this study protocol.

In the present study, the efficacy, safety and tolerability of Chloroprocaine 3% ophthalmic gel for topical anaesthesia of the eye will be evaluated in healthy subjects. Based on theoretical assumptions it is reasonable to expect that the formulation under study offers significant advantages compared to currently available formulations. Most importantly, because of its gel nature it stays longer on the ocular surface than commonly used eye drops due to its viscosity and is not drained by the punctae as quickly as drops with low viscosity.[3] Due to its high viscous formulation, systemic absorption through the nasolacrimal system is expected to be low, therefore reducing the potential for systemic toxicity.

The study will be carried out in 2 parts. In part I, safety and tolerability will be assessed in three groups (12 subjects per group) for single and multiple instillations (1 drop, 3 drops and 3+3 drops). In each group, 9 subjects will be randomized to receive Chloroprocaine 3% Gel and 3 subjects will receive vehicle as control in the right eye. After part I is completed, an internal independent board will review safety endpoints of data collected from these first subjects and advise to go on with further enrollment.

If no safety concerns arise, in part II efficacy, safety and tolerability will be assessed in 60 healthy subjects for the 3 drops dose regimen. 40 subjects will receive Chloroprocaine 3% Gel and 20 will receive vehicle (2:1 randomization) in the right eye.

Dosage rationale

The selected dose of Chloroprocaine gel is derived from animal data. In an ocular tolerance study (Study Number: S104CH20118) in New Zealand White Rabbits a 5 day 5 times daily administration

of Chloroprocaine ophthalmic gel was well tolerated. A transient absence of pupillary reflex and partial mydriasis were observed for eyes treated with Chloroprocaine gel 3% after each last daily administration. In summary, five daily instillations over 5 days of Chloroprocaine gel 3% in the right eyes of albino rabbits were both macroscopically and microscopically well tolerated.

A non-clinical dose finding study investigating Chloroprocaine 5% gel, Chloroprocaine 3% high viscosity gel and Chloroprocaine 3% low viscosity gel was performed in New Zealand White rabbits (Study number: A66C06315). Whereas Chloroprocaine 5% gel showed only limited tolerability on the ocular surface and was therefore not further continued, Chloroprocaine 3% showed good tolerability together with a long anesthetic potential (up to 60 minutes for the high viscosity gel, up to 45 minutes for the low viscosity gel). Based on this data, Chloroprocaine 3% high viscosity gel was chosen for further development. The performance of the selected formulation (Chloroprocaine 3% high viscosity gel, batch number 1708L08) was then confirmed in a further study in group of 6 albino rabbits (Study Number: S104CH20318), again showing a good anesthetic potential of Choroprocaine 3% gel.

1.3 Benefit/risk assessment

Although there is no direct benefit for the subjects under study, the findings of this study may help to assess safety, tolerability and efficacy of ophthalmic Chloroprocaine 3% Gel.

Chloroprocaine has been used for spinal, epidural and perineural anaesthesia for several years and is well tolerated. In particular, chloroprocaine solutions (i.e. Nesacaine® 1, 2, 3%) have been marketed in USA and Canada since 1955. Until now, no data for the use in the human eye is currently available. However, the local and systemic efficacy and safety of chloroprocaine is well-known from other routes of administration. Chloroprocaine 3% ophthalmic Gel is intended to be used for topical anaesthesia for patients undergoing eye surgery in the future. Due to its viscosity the gel tends to stay on the eye for a longer duration than eye drops is not absorbed by the punctae as quickly as the tears and systemic absorption through the nasolacrimal system should be reduced, therefore reducing the potential for systemic toxicity.

Data from the non-clinical development program reports good local tolerability of Chloroprocaine 3% ophthalmic gel on the ocular surface. Further, non-clinical data from a proof-of-concept study indicates a strong and long-lasting anesthetic effect of Chloroprocaine 3% ophthalmic gel.

However, at this stage of development, local toxicity of Chloroprocaine 3% Gel in human eyes cannot be ruled out but the risk is considered low based on the safety factor calculated using preclinical data. Considering the expected low systemic exposure, systemic side effects are unlikely to occur. The most common systemic side effect that was observed after intrathecal injection was systemic hypertonia. Although it is unlikely to occur after ophthalmic use, blood pressure and heart rate will be assessed after administration in the present study.

Therefore, the risk/benefit ratio is acceptable.

2. Study objectives

Primary objective

- To assess efficacy of Chloroprocaine 3% ophthalmic gel in healthy subjects

Secondary objective

- To assess safety and local tolerability of Chloroprocaine 3% ophthalmic gel

Primary endpoint

- Proportion of subjects gaining full anesthesia of the ocular surface 5 minutes after administration of Chloroprocaine 3% ophthalmic gel

Secondary Endpoints

- Safety and tolerability as assessed by the occurrence of ocular symptoms, adverse event occurrence and concomitant medication
- Time of anesthesia and duration of anesthesia, as well as Cochet Bonnet assessments
- Blood pressure and heart rate
- Slit lamp examination
- Corneal fluorescein staining
- Intraocular pressure

3. Investigational plan

3.1 Design

Randomized, Double-Masked, Vehicle-Controlled, Single-Center Study

3.2 Selection of study population

The participants will be recruited from the database of the Department of Clinical Pharmacology, Währinger Gürtel 18-20, A-1090 Wien, Austria.

3.2.1 Number of subjects

A total of 96 healthy male and female subjects will be included in the study. The study will be performed in 2 parts.

Part I:

- Group 1 (12 subjects): Single instillation
- Group 2 (12 subjects): 3 drops
- Group 3 (12 subjects): 3+3 drops

In each group, 9 subjects will be randomized to receive Chloroprocaine 3% Gel and 3 subjects will receive vehicle in the right eye. On the first two study days of each group, one subject will be dosed

per day. On the third study day up to two subjects can be dosed per day. On the following study day(s) up to 4 subjects can be dosed per day. Randomization will be performed in three blocks (3:1, Chloroprocaine vs. vehicle) for each group. After part I is completed, an internal independent board will review safety endpoints of data collected from these first subjects and advise to go on with further enrollment.

Part II:

60 subjects will be included in Part II. 40 subjects will be randomized to receive 3 drops of Chloroprocaine 3% Gel and 20 subjects will receive vehicle in the right eye (2:1 randomization, Chloroprocaine vs. vehicle).

3.2.2 Inclusion criteria

1. Signed and dated informed consent
2. Healthy male or female aged from 18 to 90 years
3. No clinically significant ocular or systemic disease
4. Ability to orally respond to pain
5. Ability to follow the visit schedule

3.2.3 Exclusion criteria

Ophthalmic exclusion criteria

1. Eye movement disorder (i.e. Nystagmus)
2. Dacryocystitis and all other pathologies of tears drainage system
3. History of Inflammatory ocular disease (Iritis, uveitis, herpetic keratitis)
4. Corneal, epithelial, stromal or endothelial, residual or evolutionary disease (including corneal ulceration and superficial punctuate keratitis)
5. History of ocular traumatism, infection or inflammation within the last 3 months
6. Best corrected visual acuity < 1/10
7. History of ophthalmic surgical complication (i.e. cystoid macular oedema)

Systemic/non ophthalmic exclusion criteria

8. General history:
    8.1 Deafness
    8.2 Excessive anxiety
9. Any other medical or surgical history, disorder or disease such as acute or chronic severe organic disease: hepatic, endocrine neoplasic, haematological diseases, severe psychiatric illness, relevant cardiovascular abnormalities (such as unstable angina, uncontrolled hypertension: systolic blood pressure over 200 mmHg, diastolic blood pressure over 100 mmHg) and/or any complicating factor or structural abnormality judged by the investigator to be incompatible with the study
10. Allergic history: Known hypersensitivity to one of the components of the study medications or to test products

Specific non-inclusion criteria for women:

11. Pregnancy, lactation
12. Women without an effective method of contraception (i.e. oral contraceptive, intra-uterine device, subcutaneous contraceptive implant)
    OR
13. Women not hysterectomised, not menopaused nor surgically sterilized

Exclusion criteria related to general conditions:

14. Inability of subject to understand the study procedures and thus inability to give informed consent
15. Non-compliant subject (e.g. not willing to attend the follow-up visits, way of life interfering with compliance)
16. Participation in another clinical study
17. Already included once in this study
18. Ward of court
19. Subject not covered by the Social Security

Exclusion criteria related to previous and concomitant medications (taken within 15 days prior screening visit)

20. Use of systemic opioids and opioid drugs
21. Topical ocular treatment with anaesthetic action
22. Use of systemic analgesic drugs (except paracetamol, which will be allowed after Visit 2)

3.3 Medication

3.3.1 Investigational Medicinal Product (IMP)

IMP: Chloroprocaine 3% Gel

IMP	Chloroprocaine 3% ocular gel, Sintetica S.A., Switzerland
Manufacturer	Unither Pharmaceuticals, France
Pharmaceutical form	Gel
Dose	1 drop
Frequency	1, 3 and 3+3 times
Administration route	Topical instillation

Placebo: Vehicle for Chloroprocaine 3% Gel

IMP	Placebo, Vehicle for chloroprocaine 3% ocular gel, Sintetica S.A., Switzerland
Manufacturer	Unither Pharmaceuticals, France
Pharmaceutical form	Gel
Dose	1 drop
Frequency	1, 3 and 3+3 times
Administration route	Topical instillation

Dose:

Part I:

- Group 1 (12 subjects): Single instillation
- Group 2 (12 subjects): 3 drops (1 instillation every minute ± 15 seconds)
- Group 3 (12 subjects): 3+3 drops (1 instillation every minute ± 15 seconds for the first 3 drops, then wait for 5 minutes before new instillation every minute ± 15 seconds for the last 3 drops)

Part II:

- 3 drops (1 instillation every minute ± 15 seconds)

3.3.2 IMP handling and accountability

3.3.2.1 Handling and Storage

IMP and placebo will be stored at temperature between 15°C and 25°C and protected from direct light.

The investigator, the hospital pharmacist or other personnel allowed to store and dispense the IMP will be responsible for ensuring that the IMP used in the clinical investigation is securely maintained and in accordance with the applicable regulatory requirements.

3.3.2.2 Packaging and labelling

Packaging and labelling will be carried out by the Sponsor according to the randomization list.

All labels will be written in the local language. The content of the labelling is in accordance with the GMP specifications, according to the Annex 13 to the Good Manufacturing Practice and local requirements.

3.3.2.3 Administration

The IMP must be administered only to study subjects who have signed informed consent and in accordance with the protocol. A designated member of the study team (as set out in the delegation log) will be responsible for administration according to the randomization procedure. Only the right eye of each subject will be treated.

3.3.2.4 Accountability

The test and reference investigational products will be provided directly to the investigator by the sponsor.

After receipt of the investigational products supply, the pharmacist or the person identified as the recipient of the study drug supply will confirm receipt.

Each unit must be accounted in a specific form according to ICH GCP recommendation.

The investigational materials are to be handled by the investigator or by study team members at the site designated and are to be used in accordance with this protocol only. Under no circumstances

will the investigator allow the investigational product to be used other than as directed by this protocol.

The responsibilities of personnel designated to handle IMP will be specified in written prior to their undertaking responsibilities related to the handling of the IMP.

At the end of the study, used, unused and partially used supplies of Investigational Medicinal Products provided by the sponsor will either be destroyed on site (upon written authorization) or returned to the sponsor/manufacturer (upon written authorization), after assessment of drug accountability.

3.3.3 Topical medication for investigations

Fluorescein/Oxybuprocaine SDU Faure® (Oxybuprocainhydrochlorid with fluorescein, Omnivision AG, Austria, Neuhausen); dose: 1 drop per eye for measurement of intraocular pressure

Minims-Fluorescein Sodium 2,0% (Chauvin Pharmaceuticals Ltd. UK); dose: 1 drop per eye for assessment of corneal staining

3.3.4 Randomization and masking

A subject who has given informed consent and who is included will be assigned a specific randomization number on the first study day. Randomization numbers will be allocated in ascending order using the next available consecutive number. The randomization number should be recorded in the subject's source document and the Case Report Form (CRF).

For both study parts, treatment assignment will be implemented through an IRT (interactive response technology) system using an unstratified permutated block randomization.

The study will be carried out in a double-masked fashion, therefore the investigators and study site staff as well as the study participants will not be informed about the treatment dispensed. Drug accountability will also be performed only by designated personnel who is not involved in the study related procedures.

The study will be performed in 2 parts.

<u>Part I:</u>

- Group 1 (12 subjects): Single instillation
- Group 2 (12 subjects): 3 drops (1 instillation every minute ±15 seconds)
- Group 3 (12 subjects): 3+3 drops (1 instillation every minute ±15 seconds, 5 minutes between each 3 drops set)

In each group, 9 subjects will be randomized to receive Chloroprocaine 3% Gel and 3 subjects will receive vehicle in the right eye. On the first two study days of each group, one subject will be dosed per day. On the third study day up to two subjects can be dosed per day. On the following study day(s) up to 4 subjects can be dosed per day. Randomization will be performed in three blocks (3:1, Chloroprocaine vs. vehicle) for each group. After part I is completed, an internal independent board will review safety endpoints of data collected from these first subjects and advise to go on with further enrollment.

Part II:

60 subjects will be included in Part II. 40 subjects will be randomized to receive 3 drops (1 instillation every minute ±15 seconds) of Chloroprocaine 3% Gel and 20 subjects will receive vehicle in the right eye (2:1 randomization, Chloroprocaine vs. vehicle).

A randomized subject discontinued from the study without being treated will be replaced. This will be done according to the randomization list. Withdrawn subjects randomized and treated will not be replaced.

3.4 Study procedures

3.4.1 Prestudy Screening

Visit 1 (Day -30 to Day -7)

Time frame: 1.5 hours

The screening examination will be performed before the start of the experiments and is identical for Part I and Part II:

- Informed consent
- Pregnancy test in females of childbearing potential
- Demography
- Ocular medical and surgical history
- Systemic medical and surgical history
- Previous (last 15 days) and concomitant ocular and non-ocular treatments
- Check of inclusion/exclusion criteria
- Height and weight
- Blood pressure and heart rate.
- Ocular symptoms
- Best far corrected visual acuity in both eyes
- Slit lamp examination including corneal fluorescein staining in both eyes
- Measurement of intraocular pressure (IOP) by Goldmann applanation tonometry in both eyes
- Funduscopy in both eyes

3.4.2 Description of the study days

Visit 2 (Day 1)

Part I:

Time frame: 2,5 hours

The following examinations will be carried out in the following order:

- Adverse Events/concomitant medication

- Pregnancy test in females of childbearing potential
- Blood pressure and heart rate.
- Assessment of ocular symptoms
- Slit lamp examination (both eyes)
- Recheck of inclusion/exclusion criteria
- Randomization
- Instillation of the study medication in the right eye
- A break of at least 60 minutes ±10 minutes will be scheduled
- Blood pressure and heart rate
- Assessment of ocular symptoms
- Slit lamp examination (both eyes)
- Assessment of Adverse Events

Part II:

Time frame: 2,5 hours

The following examinations will be carried out in the following order:

- Adverse Events/concomitant medication
- Pregnancy test in females of childbearing potential
- Blood pressure and heart rate.
- Assessment of ocular symptoms
- Slit lamp examination (both eyes)
- Recheck of inclusion/exclusion criteria
- Randomization
- Instillation of the study medication in the right eye
- Assessment of conjunctival anaesthesia* (right eye only)
- Cochet Bonnet assessment** (right eye only)
- Assessment of ocular symptoms (at the end of the study day)
- Blood pressure and heart rate (at the end of the study day)
- Slit lamp examination (both eyes, at the end of the study day)
- Assessment of Adverse Events (throughout the study day)

*Subjects will be tested and then questioned about pain at 20 seconds, 40 seconds, and 1 minute and in cases where they do report pain at the 20 and 40 seconds time point but not at the 1 minute timepoint, additional testing will be performed at 80 seconds. The resting intervals will be extended to every 5 minutes once a subject reported no pain on two successive tests. These subjects will be considered to have achieved the primary endpoint of anesthesia by 5 minutes.

Alternatively, if the subject experiences pain at 20, 40, and 60 seconds, pinching will be suspended to the 5-minute time point. If the subject experiences pain at this testing interval (5 minutes), no more testing will be performed and the subject will be considered not to have gained anesthesia.

**Cochet Bonnet assessment will either be performed at the 1 minute or 80 seconds time point only. Thereafter, Cochet Bonnet assessment will always be performed immediately after conjunctival pinching.

Visit 3 (Day 2)

Time frame: 10 minutes

A phone visit will be performed in Part I and II and subjects will be asked about:

- Changes in concomitant medication
- Adverse Events

Visit 4 (Day 8 ± 1)

Time frame: 1 hour

The following examinations will be carried out (Part I and Part II):

- Adverse Events/concomitant medication
- Blood pressure and heart rate
- Assessment of ocular symptoms
- Best far corrected visual acuity in both eyes
- Slit lamp examination including corneal fluorescein staining in both eyes
- Measurement of intraocular pressure (IOP) in both eyes
- Funduscopy in both eyes

Visit 5 (Day 29 ± 3)

Time frame: 10 minutes

A phone visit will be performed in Part I and II and subjects will be asked about:

- Changes in concomitant medication
- Adverse Events

3.4.3 Withdrawal and replacement of subjects

Subjects must be withdrawn under the following circumstances:

- at their own request,
- if the investigator feels it would not be in the best interests of the subject to continue,
- if the subject violates the conditions laid out in the consent form/information sheet or disregards instructions by the study personnel on purpose.

In all cases, the reasons why subjects are withdrawn must be recorded in detail in the case report form and in the source data sheet. A final examination is to be done for each withdrawn subject.

Only randomized subjects not treated will be replaced.

3.4.4 Study stopping rules

Any subject can withdraw its consent to participate in the study without any reason. A final examination should be performed to ensure safety of the patient.

The study may be prematurely terminated for a subject if SAEs or other significant side effects occur or if any other medical or ethical reasons do not justify continuation of the study, according to Investigator judgement.

At the end of part 1, the Independent Board will review safety data of the subjects included in part 1 and advise to go on with further enrollment with part 2.

The sponsor or the investigator has the right to close this study at any time. As far as possible, this should occur after mutual consultation. As required by the Clinical Trials legislation, the IEC/IRB/CA must be informed.

Should the study be discontinued prematurely, all study materials (completed, partially completed and empty case record forms) will be retained.

3.4.5 Protocol deviations

The Investigator should not implement any deviation from the protocol without prior review and agreement by the Ethics Committee and the competent authorities, except when necessary to eliminate an immediate hazard to study subjects. Any deviation from the protocol will be documented in writing (note to file or protocol deviation) by the principal investigator.

For purposes of this protocol, major protocol deviations are defined as:

- Subject entered into the study even though he/she did not satisfy entry criteria
- Subject who developed withdrawal criteria during the study and was not withdrawn

In case of a major protocol deviation, subjects may be excluded from the analysis.

3.4.5.1 Withdrawal criteria

It will be documented whether or not each subject completed the clinical study. If, for a subject, study treatment or observations are discontinued, the type of discontinuation and the primary reason for discontinuation will be recorded.

Patients who will present any kind of complications preventing primary endpoint assessment to be performed, will be withdrawn from the efficacy analysis. However, these patients will be followed for safety parameters assessments.

Moreover, if, in the opinion of the investigator, there is any other situation or condition which puts the subject at significant risk, patients should be withdrawn from the study too.

Any patient may voluntarily discontinue the study at any time he or she chooses (Declaration of Helsinki), without prejudice. The investigator may elect to discontinue a patient for reasons related to the study product (e.g., AE). Such details should be recorded on the patient source data and CRF and reported separately. A final examination is to be done for each withdrawn subject.

3.4.5.2 Discontinuation procedures

For any subject discontinuing from interventions and findings, the investigator will:

- ask the subject to undergo, as far as possible, a final medical visit to examine the subject's health conditions
- arrange for alternative medical care of the withdrawn subject, if necessary
- report in the source data and CRF date and time of the investigational product administration, and date and primary reason of study discontinuation
- record in the source data and CRF any follow-up, if the subject is withdrawn for an AE

Discontinued subjects will not be replaced.

3.5 Variables and schedule of observations

3.5.1 Outcome variables

Primary endpoint:

- Efficacy of Chloroprocaine 3% gel eye drops, which is defined as the proportion of subjects gaining full anesthesia of the ocular surface 5 minutes after administration of the IMP

Secondary endpoints:

- Assess the time to anesthesia and duration of anesthesia, as well as Cochet Bonnet assessments
- Assess the occurrence of:
  - Ocular symptoms
  - Adverse event occurrence
- Blood pressure and heart rate
- Slit lamp examination
- Corneal fluorescein staining
- Intraocular pressure

3.6 Equipment used for assessing the clinical investigation variables

All used equipment should be monitored and calibrated if necessary and maintained according to the instructions of use.

4. Methods of evaluation

4.1 Measurement techniques

4.1.1 Noninvasive measurement of systemic hemodynamics

Systolic, diastolic and mean blood pressures (SBP, DBP, MAP) are measured on the upper arm by an automated oscillometric device. Pulse rate is automatically recorded from a finger pulse-oxymetric device.

4.1.2 Ocular symptoms

Scores will be determined using a 100 mm VAS on which 0 means “no symptoms” and 100 means “worst possible discomfort”. The following symptoms will be assessed using VAS: burning, stinging, itching, foreign body sensation.

4.1.3 Far best corrected visual acuity

Far best-corrected visual acuity will be measured using the standard ETDRS acuity charts.

4.1.4 Slit lamp examination

Slit lamp biomicroscopy will be performed for the following parameters: Conjunctival redness, anterior chamber flare, conjunctival chemosis, eyelid swelling and will be graded on a 4 point scale: (0) none, (1) mild, (2) moderate, (3) severe.

4.1.5 Corneal fluorescein staining

Minims-Fluorescein Sodium 2.0% eye drops will be used to detect corneal epithelial defects using slit lamp biomicroscopy. As grading scale for corneal damage, the NEI/Industry Workshop guidelines will be used [4]. The cornea will be divided into five sectors (central, superior, inferior, nasal and temporal), each of which is scored on a scale of 0–3, whereas 0 means no staining and 3 means maximum staining, with a maximal score of 15.

4.1.6 Intraocular pressure

Intraocular pressure will be measured with a slit-lamp mounted Goldmann applanation tonometer. Before each measurement one drop of oxybuprocainhydrochloride combined with sodium fluorescein will be used for local anesthesia of the cornea.

4.1.7 Funduscopy

Indirect ophthalmoscopy will be performed at the slit lamp using a +90 diopters Volk lens.[5]

4.1.8 Assessment of conjunctival anaesthesia

Conjunctival anaesthesia will be assessed by pinching of the conjunctiva with 0.3-mm forceps. Subjects will be tested every 20 seconds during the first minute following last instillation of the study product. Subjects will be asked to immediately report if they feel pain.

The testing intervals will be extended to every 5 minutes once a subject reported no pain on two successive tests.

Assessment will be done as follows: Subjects will be tested at 20 seconds, 40 seconds, 60 seconds and 80 seconds if needed (if a subject reports pain at 20 and 40 seconds, but no pain at 60 seconds, a forth test should be performed at 80 seconds).

These subjects with no pain on two successive tests will be considered to have achieved the primary endpoint of anesthesia by 5 minutes. Testing will be then repeated every 5 minutes to assess duration of anaesthesia.

Alternatively, if the subject experiences pain at 20, 40, and 60 seconds, pinching will be suspended to the 5-minute time point. If the subject experiences pain at this testing interval (5 minutes), no more testing will be performed and the subject will be considered not to have gained anesthesia.[6]

To assess the duration of anesthesia, the testing will be concluded when the study subject reported 'pain' on two successive tests with 5 minutes in between.

4.1.9 Cochet-Bonnet assessment

For the assessment of corneal sensitivity the Luneau Cochet-Bonnet aesthesiometer (Western Ophthalmics Corporation©) will be used.

The Cochet-Bonnet aesthesiometer contains a thin, retractable, nylon monofilament that extends up to 6 cm in length. Variable pressure can be applied to the cornea by adjusting the monofilament length. The monofilament length ranges from 6 to 0.5 cm. As the monofilament length is decreased the pressure increases from 11 mm/g to 200 mm/g.

Only one assessment will be performed immediately after the last conjunctival anaesthesia assessment (either after 60 or if applicable after 80 seconds).

4.1.10 Urine Pregnancy test

Pregnancy test will be performed in females of childbearing potential at visit 1 and visit 2.

A negative urine pregnancy test result is required at visit 1 and visit 2 for the patient to be involved in the study.

4.2 Adverse events

4.2.1 Adverse events recorded throughout the study

A brief summary of AE definition, classification and management is reported below.

4.2.2 Definitions

- **Adverse event (AE)**

  Any untoward medical occurrence in a clinical trial subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

- **Adverse Drug Reaction (ADR)**

  Any noxious and unintended response to a medicinal product (i.e. a causal relationship between a medicinal product and an AE is at least reasonably possible in the investigator's or sponsor's opinion, the relationship cannot be ruled out) resulting not only from the authorized use of a medicinal product at normal doses, but also from medication errors and uses outside the terms of the marketing authorization, including the misuse and abuse of the medicinal product.

- **Pre-treatment AE (PTAE)**

  Any AE occurring before the first dose of a medicinal product and not worsening after the first dose. The following medical occurrences and clinical investigations are the only clinically significant events which, according to the investigator judgement, can be defined and recorded as PTAEs:

  - trauma (fractures, sprains, strains, falls, domestic accidents, car accidents, etc.) occurred after the signature of the informed consent and before the first medicinal product administration
  - new measurements (vital signs, ECG, laboratory parameters, etc.), performed after the signature of the informed consent and before the first medicinal product administration, which show a clinically significant worsening in comparison with a previous (baseline) measurement performed after the signature of the informed consent
  - any disease diagnosed after the anamnesis recorded at visit 1 and before the first medicinal product administration
  - physical and mental status changes (pre-syncope, anxiety, dizziness, fainting, etc.) occurred after the signature of the informed consent and before the first medicinal product administration

- **Treatment-emergent AE (TEAE)**

  Any AE occurring or worsening after the first dose of a medicinal product

- **Serious Adverse Event (SAE)**

  Any untoward medical occurrence that at any dose:

  - results in death
  - is life-threatening

- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event that may jeopardize the subject's health status or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are cancer, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse

➢ **Unexpected ADR**:

An ADR the nature or severity of which is not consistent with the Reference Safety Information (RSI)

➢ **Reference Safety Information (RSI)**:

In order to assess whether an adverse reaction is expected, the Investigator's Brochure (IB) for the test formulation will be used.

➢ **Suspected Unexpected Serious Adverse Reaction (SUSAR)**

An ADR that is both unexpected (not consistent with the RSI) and also meets the definition of a SAE.

4.2.3 AEs monitoring window

- Start of monitoring: from immediately after the signature of the informed consent
- End of monitoring: last follow-up visit/Early Termination Visit (ETV)

An AE occurring after the last follow-up visit/ETV and coming to knowledge of the investigator (e.g. by spontaneous reporting by study subjects) must be recorded only if it is an ADR, according to the investigator's judgment.

4.2.4 AEs recording

All AEs derived by spontaneous, unsolicited reports of the subjects, by observation and by routine open questioning should be collected and reported.

The following minimal information will be recorded for an AE in the source documents and later transcribed into the CRF:

1. Adverse Event: progressive number of the adverse event or progressive number of follow-up of the adverse event
2. Description: verbatim description of the adverse event

   Start Date/Time: start date/time of the adverse event or

Follow-up Date/Time: follow-up date/time of the adverse event

3. End Date/Time: end date/time of the adverse event
4. Affected Body Area: anatomical location relevant for the event; in case of an ophthalmological AE, then it needs to be recorded whether or not it is the study eye
5. Whether the adverse event start before or after the first intake of the study drug or whether the adverse event has worsened or not after the first intake of the study drug
6. Last Study Drug Administration Date/Time Before Onset: if the adverse event started after the first administration of the study drug, the date/time of last administration of the study drug before the onset of the adverse event or

   Last Study Drug Administration Date/Time Before Worsening: In case of treatment emergent adverse event, the date/time of the last administration of the study drug(s) before the worsening of the adverse event.
7. Investigator's opinion about the reasonable possibility of a causal relationship with the study drug.
8. Investigator's opinion about other causal relationship (e.g. non study drug, concomitant therapy, study device, etc.).
9. Severity: the severity or intensity of the event

   1 Mild

   2 Moderate

   3 Severe

10. Pattern: Used to indicate the pattern of the event over time

    1 Single Event

    2 Continuous

    3 Intermittent
11. Serious Adverse Event
12. Action Taken with Study Drug: describes changes to the study drug as a result of the event. It is specifically for actions taken with the study drug

    1 Dose Not Changed

    2 Dose Increased

    3 Dose Reduced

    4 Drug Interrupted (i.e. temporary stop)

    5 Drug Withdrawn (i.e. definitive stop)

    6 Not Applicable (e.g. drug administration not started yet or completed)

    7 Unknown
13. Concomitant Therapy: if a concomitant therapy is given, it must be reported in the specific CRF forms
14. Study Discontinuation: if the adverse event causes the subject to be discontinued from the study
15. Other Action Taken: other actions taken as a result of the event that are unrelated to dose adjustments of study drug
16. Outcome: Outcome of the event

    1 Recovered/Resolved

    2 Recovered/Resolved with Sequelae

    3 Recovering/Resolving

    4 Not Recovered/Not Resolved

5 Fatal

6 Unknown

4.2.5 SAEs reporting

The pharmacovigilance will be under the control of the Sponsor, Sintetica SA.

The investigator must report to the sponsor any SAE within 24 h of becoming aware of the event. The Investigator, within the same timeframe, should also inform the study monitor and the CRO. The investigator shall notify the competent Ethics Committee (EC) within 7 days of any SAE with lethal outcome occurred during a study, when required. If the Investigator is initially unable to obtain all the necessary details for completing the form, he/she should in any case transmit all the available information. The Investigator should provide an appropriate follow-up of SAEs to all concerned parties.

Seriousness and causality must be assessed by the investigator. Expectedness is usually assessed by the sponsor.

If the investigator is unable to assess the causality it is recommended to adopt a conservative approach and treat the event as a suspected adverse reaction until follow-up information is available.

The sponsor may also make an assessment of causality, independent of that of the investigator. The most conservative approach should be taken when it comes to regulatory reporting. Under no circumstances should the sponsor downgrade the investigator's opinion or put the investigator under pressure to change his/her assessment. In case of disagreement, both the opinion of the investigator and the sponsor should be provided on the report.

The sponsor will evaluate the SAE expectedness on the basis of the RSI.

4.2.6 SUSARs management

The clock for initial expedited reporting starts as soon as the information containing the minimum reporting criteria has been received by the sponsor (day 0).

For fatal and life-threatening SUSARs the EC and Competent Authority (CA) should be informed as soon as possible and in any case within 7 days.

If the initial report is incomplete, e.g. not all the information/assessments were available, a complete report should be sent within an additional 8 days.

SUSARs which are not fatal and not life-threatening are to be reported within 15 days.

The minimum information to be reported includes:

- Valid EudraCT number (where applicable)
- Sponsor study number
- One identifiable coded subject
- One identifiable reporter
- One SUSAR

- One suspect IMP (including active substance name, code)
- A causality assessment (a reasonable possibility of a causal relationship with the study drug can be excluded only if there is information supporting this decision, otherwise it cannot be excluded).

4.2.7 Other events qualified for expedited reporting

Other safety issues also qualify for expedited reporting when they might materially alter the current benefit-risk assessment of a medicinal product or would be sufficient to consider changes in the medicinal product administration or in the overall conduct of the trial, for instance:

- single case reports of an expected serious adverse reaction with an unexpected outcome (e.g.: a fatal outcome)
- an increase in the rate of occurrence of an expected serious adverse reaction, which is judged to be clinically important.
- post-study SUSARs that occur after the subject has completed a clinical trial and are reported to the investigator by the subject.
- new events relating to the conduct of the trial or the development of the medicinal product likely to affect the safety of the subjects, such as:
  - a SAE which could be associated with the trial procedures and which could modify the conduct of the trial
  - a significant hazard to the subject population such as lack of efficacy of a medicinal product used for the treatment of a life-threatening disease
  - a major safety finding from a newly completed animal study (such as carcinogenicity) or from other clinical trials.

4.2.8 SAEs: contacts

The Pharmacovigilance can be contacted using the phone and fax numbers stated in this protocol.

SAEs must be reported on SAE reporting forms and faxed/emailed within 24 H to sponsor - contact details below:

Corporate Drug Safety Unit
Fax: +41(0)91.646.85.61 - Phone: +41(0)91.640.42.50
Email: corporate_drug_safety@sintetica.com

4.3 Data handling procedures

Designated site staff must enter the information required by the protocol onto the CRFs. A subject ID list will be kept in the source data of the Department of Clinical Pharmacology.

Data management will be performed by IDDI under the responsibility of the sponsor. The sponsor will provide the investigator site with a web base electronic data capture (EDC) system that is fully validated and conforms to 21 CFR Part 11 requirements. Investigator site staff will not be given access to EDC system until they have been trained on the EDC system.

4.4 Statistical methods

All parameters measured will be evaluated and presented using descriptive statistics, i.e. arithmetic mean, SD, minimum, median and maximum values for quantitative variables, and absolute and relative (%) frequencies for qualitative variables. Results will be reported by treatment group. Confidence intervals will be reported, where appropriate. If not stated elsewhere, these intervals will be two sided in each case and provide 95% confidence.

Not available data will be evaluated as “missing values”. A statistical analysis plan (SAP) will be provided before database closure. The statistical analysis will be performed using RStudio v. 1.2.5033 and/or IBM SPSS Statistics v. 25 (or newer) for Windows.

4.4.1 Determination of sample size

Part I:

The rationale for the sample size and the randomization scheme was based on similar studies investigating eye drops in healthy subjects in a dose-escalating manner. For example, in the Tanihara et al study (2013)[7] there were 10 subjects per group (50 subjects in total), and the randomization scheme was 4:1. Considering the exploratory nature of part I of the study and the sample size used in similar studies, it deemed appropriate to use 12 subjects per group and 36 subjects in total. No power calculations have been performed for this part of the study.

Part II:

60 healthy female or male subjects will be included in Part II: 40 subjects will be treated with chloroprocaine and 20 subjects will be treated with placebo. We chose the 2:1 allocation rate (with the experimental treatment having more patients than the vehicle arm) since the experimental treatment is a new product and we wanted to be able to estimate its success rate with more precision.

Sample size per group was calculated for comparing two independent proportions (experimental and placebo success rate) with a two-sided alpha=0.05 and a power of 0.95. This is based on the assumption, that the experimental success rate is 80% and the vehicle success rate is 30%, taking a dropout rate of 20% into account. For sample size estimation, RStudio (Version 1.2.5001) was used implementing formulas described in Chow et al (2008).[8]

4.4.2 Analysis sets

The following analysis sets will be considered:

Safety set: All patients enrolled in the study, for which there is evidence that they used study medication and for whom any follow-up information is available.

Full Analysis Set (FAS): All patients enrolled in the study for which any follow-up efficacy

information is available.

Per Protocol Set (PPS): All patients of the FAS who did not show any major protocol violation.

The exclusion of patients from the analysis sets will be discussed during a blind review meeting that will be held before database lock.

The statistical analysis of the onset data and of efficacy will be performed on the basis of the FAS and the PPS. The primary population for the assessment of efficacy will be the FAS, while the statistical analysis on the PPS population will be considered as sensitivity analysis. The analysis of safety will be performed on the basis of the safety set. Patients preventing primary endpoint assessment to be performed will be withdrawn from the efficacy analysis. Such patients' characteristics will be tabulated and compared with the characteristics of the rest of the patients to investigate potential between group differences.

4.4.3 Handling of missing data

Not available data will be evaluated as “missing values”. All missing data will not be replaced.

4.4.4 Statistical analysis plan

Part I:

In this part of the study, no formal statistical analysis (i.e. hypothesis testing) will take place. Safety will be assessed using the frequency of adverse events and results from the slit lamp evaluation. The safety board will decide whether the safety profile is acceptable to proceed in part II of the study.

Part II:

All parameters measured will be evaluated and presented using descriptive statistics, i.e. arithmetic mean, SD, minimum, median and maximum values for quantitative variables, and absolute and relative (%) frequencies for qualitative variables. In this part of the study, the list of quantitative variables includes time to anesthesia, duration of anesthesia, Cochet Bonnet assessment, blood pressure, heart rate, intraocular pressure, ocular symptoms, slit lamp examination and corneal fluorescein staining, while qualitative variables include the experience of full anesthesia of the ocular surface and the occurrence of adverse events. Moreover, results will be reported by treatment group. In terms of figures, quantitative variables will be presented by either histograms or box-plots, and qualitative variables will be presented by either bar-charts or pie-charts. Confidence intervals will be reported, where appropriate. If not stated elsewhere, these intervals will be two sided in each case and provide 95% confidence.

The primary endpoint of the part II of the study is defined as the proportion of subjects gaining full anesthesia of the ocular surface 5 minutes after administration of the IMP for the 2 treatment arms (i.e. p1 for vehicle and p2 for Chloroprocaine arm). The null hypothesis of equal response rates between the 2 treatment arms (i.e. H_0: $p_1=p_2$) at the $\alpha=0.05$ level will be rejected if:

$$\left| \frac{\widehat{p_1} - \widehat{p_2}}{\sqrt{\frac{\widehat{p_1}(1-\widehat{p_1})}{n_1} + \frac{\widehat{p_2}(1-\widehat{p_2})}{n_2}}} \right| > Z_{\alpha/2}$$

In terms of statistical inference for the secondary endpoints, quantitative variables will be compared between the 2 groups using Mann-Whitney's test, in which the null hypothesis is that for each quantitative variable tested, its median value does not differ significantly between the 2 groups (i.e. H_0: $median_1$=$median_2$). Qualitative variables will be compared using Pearson's X^2 test, in which the null hypothesis for each pair of qualitative variables is that these two variables are not associated. To account for multiple comparisons, we will adjust the statistical significance level using Bonferroni corrections.

In terms of adverse events, all AEs and SAEs will be reported in tabular form overall and separately for each treatment. Not available data will be evaluated as "missing values". A statistical analysis plan (SAP) will be provided before database closure. A p-value<0.05 will be considered statistically significant. The statistical analysis will be performed using RStudio v. 1.2.5033 and/or IBM SPSS Statistics v. 25 (or newer) for Windows.

4.4.5 Safety and tolerability evaluation

AEs

Adverse events (AEs) are described analytically in 4.2 and will be coded by System Organ Class (SOC) and Preferred Term (PT), using the Medical Dictionary for Regulatory Activities (MedDRA).

AEs will be classified as pre-treatment AEs (PTAEs) and treatment-emergent AEs (TEAEs), according to the period of occurrence, as follows:

- PTAEs: all AEs occurring before the first dose of IMP and not worsening after the first dose of IMP
- TEAEs: all AEs occurring or worsening after the first dose of IMP

Individual PTAEs and TEAEs will be listed in subject data listings. No summary table will be provided for PTAEs. TEAEs will be summarized by treatment and overall. The number and percentage of subjects with any TEAE and the number of TEAEs will be tabulated by SOC and PT, seriousness, relationship to treatment and severity.

Physical examination

Significant findings/illnesses, reported after the start of the study and that meet the definition of an AE (see section 4.2.1), will be recorded in the subject source documents. Date of the physical examination and overall investigator's interpretation (as normal, abnormal not clinically significant [NCS] or abnormal clinically significant [CS]) will be reported in the CRF.

Vital signs

Values of vital signs will be listed and summarized by descriptive statistics.

5. Ethical and legal aspects

The study will be performed in accordance with the guidelines of the Declaration of Helsinki (1964), including current revisions. The study will be also conducted in accordance with the protocol and ICH-GCP and the applicable regulatory requirement(s).

5.1 Informed consent of subjects

In obtaining and documenting informed consent, the Investigator must comply with the applicable regulatory requirement(s) and adhere to the ICH guideline for GCP and the requirements in the Declaration of Helsinki.

Prior to any trial-related activity, the Investigator must give the subject oral and written information about the trial expressed in the appropriate local language and form that the person giving consent can read and/ or understand. The measures taken to safeguard the subject´s privacy and the protection of personnel data should be described. This includes information on how the identity of the subject and any recorded data will be coded, stored and protected. Information should be given about the person(s) who will have access to the code list data, where the list will be kept and for how long and who will be responsible for keeping and destroying it.

A voluntary, signed and dated Subject Information Sheet/Informed Consent Form (SIS/ICF) will be obtained from the subject prior to any trial-related activity. The subject will be given a copy of the signed SIS/ICF.

5.2 Approval of the study/Protocol Amendment/Study Termination

5.2.1 Approval of the study

Before the start of the study, the study protocol will be submitted to the Ethics Committee (EC) of the Medical University of Vienna and the competent authorities (BASG). Without any approval, the study shall not begin. If any additional requirements are emposed by the EC or competent authorities, they shall be followed.

5.2.2 Protocol Amendment

Full compliance with this protocol is expected.

If any modifications become necessary or desirable, these will be documented in writing, major changes require the approval of all investigators, the sponsor, the ethics committee and the competent authorities.

5.2.3 Study Termination

As required by the legislation the sponsor must submit to the IEC/IRB/Competent Authorities:

- Information on suspected unexpected serious adverse reactions,
- Periodic safety reports, when applicable,
- Periodic reports on the progress of the study, when applicable,

- New information that may affect adversely the safety of the patients or the conduct of the trial
- Serious breaches inherent to the study protocol, as per regulatory requirements.

The study will be considered terminated at the date of the last visit of the last subject or upon completion of any follow-up procedure described in protocol.).

Information about the end of the study will be transmitted to EC/CA according to local regulation.

5.3 Insurance

All subjects participating in this clinical study will be insured in accordance with § 32 of the Austrian Medicines Act, 93.

5.4 Confidentiality

All subject names will be kept confidential in the investigator's files. Subjects will be identified throughout documentation and evaluation by the number(s) assigned to them at the beginning of the study. The subjects will be told that all study findings will be stored and handled in strictest confidentiality.

The investigator will maintain a personal patient identification list (patient numbers with the corresponding patient names) to enable records to be identified.

Patients will be informed that representatives of the sponsor or the sponsor's representative, IEC/IRB, or competent authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

Each participating site will maintain appropriate medical and research records for this trial in compliance with ICH E6(R2) GCP Sections 1.51, 1.52 and 4.9 and any other regulatory and institutional requirements for the protection of patient's confidentiality.

5.5 Patients compensation

Patient compensation is proposed due to the constraints required by the study protocol and will be submitted to the IRB/IEC for approval.

5.6 Study Duration

The study will be closed when all patients have completed the last study visit.

6. Data quality assurance

6.1 Monitoring

Monitoring visits will be performed by a study monitor designated by the sponsor. The study monitor will be dully trained to the study and will be responsible for ensuring and verifying that each study site conducts the study according to the protocol, standard operating procedures, other written instructions/agreements, ICH-GCP, and applicable regulatory guidelines/requirements. The

investigator will permit the study monitor to visit the study site at appropriate intervals to observe the progress of the study, review study records/documentation, and ensure that informed consent has been obtained.

6.2 Documentation of study findings

All findings collected during the study will be entered in the electronic CRFs provided by the CRO and data management activities will be performed by the CRO designated by the sponsor.

eCRFs will be completed based on the source data within 3 working days of the patient visit.

The medical records upon which the CRF is based will be kept for at least 15 years.

6.3 Auditing and inspections

The investigator will permit study-related monitoring, audits, review of the Ethics Committee, and regulatory inspection(s) to evaluate study conduct and compliance with the protocol, standard operating procedures, other written instructions/agreements, ICH GCP, and applicable regulatory guidelines/requirements. For this purpose, the investigator will provide direct access to source data documents. This will be carried out giving due consideration to data protection and medical confidentiality.

7. Publication policy

The sponsor agrees that the study results (including negative and inconclusive as well as positive results) can be made publicly available by the investigator publishing in peer reviewed journals, presenting results at scientific congresses and posting information and results on internet-based public registers and databases.

Study results will be communicated in full to the competent Health Authorities by the submission of a complete clinical study report. The sponsor will be in charge of the Clinical Study Report writing

As the sponsor agrees that the study results can be published by the investigator(s), the investigator agrees to submit any manuscript (abstract, publication, paper, etc.) to the sponsor before any public disclosure.

This will be done in order to ensure that clinical study results are reported in an objective, accurate and balanced manner. The sponsor reviews the proposed manuscripts, before submission, within a reasonable period of time (30-90 days in relation with the complexity of the work).

The investigator(s) will also be provided by the sponsor with the clinical study report and the results of any additional analysis, tables, figures, etc. undertaken for the purposes of the article, in order to take responsibility for the content of the publication(s).

On an exceptional basis, the sponsor may temporarily delay registration of certain data elements (e.g. compound, name, outcome, measures, etc.) to seek necessary intellectual property protection. This is because early disclosure of such data could, in some circumstances, prevent or negatively impact patentability.

8. References

1. Pham DT, Castello R. [Topical anaesthesia in cataract surgery]. *Klin Monbl Augenheilkd* 2010;227:605-610.
2. Goldblum E, Atchabahian A. The use of 2-chloroprocaine for spinal anaesthesia. *Acta Anaesthesiol Scand* 2013;57:545-552.
3. Moisseiev E, Loberman D, Zunz E, Kesler A, Loewenstein A, Mandelblum J. Pupil dilation using drops vs gel: a comparative study. *Eye (Lond)* 2015;29:815-819.
4. Lemp MA. Report of the National Eye Institute/Industry workshop on Clinical Trials in Dry Eyes. *CLAO J* 1995;21:221-232.
5. Gellrich M-M. The fundus slit lamp. *SpringerPlus* 2015;4:56-56.
6. Busbee BG, Alam A, Reichel E. Lidocaine hydrochloride gel for ocular anesthesia: results of a prospective, randomized study. *Ophthalmic Surg Lasers Imaging* 2008;39:386-390.
7. Tanihara H, Inoue T, Yamamoto T, Kuwayama Y, Abe H and Araie M for the K-115 Clinical Study Group. Phase 1 clinical trials of a selective Rho kinase inhibitor. JAMA Ophthalmol 2013; 131(10):1288-95.
8. Chow S-C, Shao J, Wang H. *Sample size calculations in clinical research*: Chapman and Hall/CRC; 2008.

9. Appendix: Schedule of assessments

Schedule of assessments: Part I:

	Visit 1 (Day -30 to -7)	**Visit 2** (Day 1)	**Visit 3** (phone) (Day 2)	**Visit 4** (Day 8 ± 1)	**Visit 5** (phone) (Day 29 ± 3)
Informed consent	X				
Urine Pregnancy test	X	X			
Demography	X				
Ocular medical and surgical history	X				
Systemic medical and surgical history	X				
Ocular and systemic concomitant medication	X	X	X	X	X
Check of inclusion/exclusion criteria	X	X			
Height and Weight	X				
Blood pressure and heart rate	X	X[1)]		X	
Ocular symptoms	X	X[1)]		X	
Best corrected visual acuity (ETDRS)	X			X	
Slit lamp examination	X	X[1)]		X	
Corneal fluorescein staining	X			X	
Intraocular pressure	X			X	
Funduscopy	X			X	
Assessment of Adverse Events		X[2)]	X	X	X
Randomization		X			
Instillation of the IMP in the right eye		X			

1) Before IMP instillation and at the end of the study day (at least 60 minutes ±10 minutes after instillation)

2) Throughout the study day

Schedule of assessments: Part II:

	Visit 1 (Day -30 to -7)	**Visit 2** (Day 1)	**Visit 3** (phone) (Day 2)	**Visit 4** (Day 8 ± 1)	**Visit 5** (phone) (Day 29 ± 3)
Informed consent	X				
Urine Pregnancy test	X	X			
Demography	X				
Ocular medical and surgical history	X				
Systemic medical and surgical history	X				
Ocular and systemic concomitant medication	X	X	X	X	X
Check of inclusion/exclusion criteria	X	X			
Height and Weight	X				
Blood pressure and heart rate	X	X[1)]		X	
Ocular symptoms	X	X[1)]		X	
Best corrected visual acuity (ETDRS)	X			X	
Slit lamp examination	X	X[1)]		X	
Corneal fluorescein staining	X			X	
Intraocular pressure	X			X	
Funduscopy	X			X	
Assessment of Adverse Events		X[3)]	X	X	X
Randomization		X			
Instillation of the IMP in the right eye		X			
Assessment of conjunctival anaesthesia[4)]		X[2)]			
Cochet Bonnet assessment[4)]		X[5)]			

1) Before IMP instillation and at the end of the study day
2) Subjects will be tested and then questioned about pain at 20 seconds, 40 seconds, and 1 minute. The resting (pinching and cornea touching) intervals will be extended to every 5 minutes once a subject reports no pain on two successive tests.
   These subjects will be considered to have achieved the primary endpoint of anesthesia by 5 minutes.
   Alternatively, if the subject experiences pain at 20, 40, and 60 seconds, pinching will be suspended to the 5-minute time point. If the subject experiences pain at this testing interval (5 minutes), no more testing will be performed and the subject will be considered not to have gained anesthesia.
3) Throughout the study day
4) Examinations will only be performed in the right eye
5) Cochet Bonnet assessment will be performed starting from 60 (or 80) seconds after instillation, immediately after assessment time points for conjunctival anesthesia