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ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ

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Θέμα: «An observational, open-label, multi-center prospective study for assessing the patients' adherence to IKERVIS® treatment in routine daily clinical practice»

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Λάρισα, Σεπτέμβριος 2020

PROTOCOL OF A NON-INTERVENTIONAL CLINICAL STUDY

Protocol title:	**An observational, open-label, multi-center prospective study for assessing the patients' adherence to IKERVIS® treatment in routine daily clinical practice.**
Study code:	**IKERVIS**
Protocol no:	CRO-Sponsor-Ikervis
Date:	20 Sept 2020
Version:	1
Authors:	Samos Zacharias

Contract Research Organization (CRO):	CRO
Sponsor:	SPONSOR

STATEMENT OF COMPLIANCE

This document is a clinical research protocol. The study will be conducted in compliance with the protocol and in accordance with the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP), with the ethical principles laid down in the Declaration of Helsinki and with the Greek National Organization of Medicines (EOF) regulations.

All personnel involved in the conduct of this study have completed human subjects' protection training.

Study Protocol Synopsis

Title:	An observational, open-label, multi-center prospective study for assessing the patients' adherence to IKERVIS® treatment in routine daily clinical practice.
Phase:	Phase IV
Number of study sites:	10
Primary Objective:	To investigate patients' adherence to IKERVIS® (Ciclosporin 1 mg/ml Eye Drops) in adult patients with dry eye disease, when administered according to standard clinical practice.
Secondary Objective:	To identify potential factors that may be associated with IKERVIS® non-adherence in daily clinical practice.
Study Design:	Multicenter, prospective, non-interventional, one arm, open label cohort study of patients with dry eye disease receiving IKERVIS®. The total duration of the study is 36 weeks (12 weeks recruitment period and 24 weeks follow-up period).
Target population:	Male and female patients with dry eye disease, aged ≥18 years, receiving Ciclosporin 1 mg/ml Eye Drops.
Inclusion criteria:	• Adult patient (≥18 years old) with dry eye disease • Patient receiving IKERVIS®. • Patients willing and able to provide written informed consent personally or by legal proxy. • Patients willing to comply with all study procedures and to be available for the duration of the study.

Exclusion criteria:	• Patient with uncontrolled dry eye disease. • Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test • Patients in treatment for dry eye disease with more than one therapy (tear supplements excluded). • Patient with hypersensitivity to the active substance or to any of the excipients of this medicinal product. • Current enrollment in other clinical trial.
Data collection plan	At baseline, at 8 weeks and end of follow-up period (24 weeks).
Primary endpoint:	Proportion of patients reporting high adherence to IKERVIS® at 24 weeks defined as a Morisky Medication Adherence Scale (MMAS-4) score equal to 0.
Secondary endpoints:	• Proportion of patients with a Medication Persistence Ratio (MPR), defined as the number of days without receiving medication after study enrolment divided by the number of days participating in the study, less than 0.20. • Proportion of patients encountering difficulties with medication supply due to coexisting medical conditions. • Proportion of patients encountering difficulties with medication supply due to lack of insurance coverage. • Proportion of patients encountering difficulties with medication supply due to inability to access or difficulty accessing pharmacy.

	• Proportion of patients with IKERVIS® discontinuation during the follow-up period.
Safety endpoints	• Visual acuity(VA) • Intraocular pressure (IOP) • Schirmer's test • Tear Break Up Time (TBUT) • Cornea Fluorescein Staining(CFS) score on the modified Oxford scale • Ocular Surface Disease Index(OSDI) • Ocular/systemic adverse events (AEs)
Sample size:	The sample size calculation was conducted with the precision-based sample size calculation. In order to calculate a 95% confidence interval for the proportion of patients reporting high adherence to IKERVIS® at 24 weeks, which is expected to be 80% with a margin error (Δ) (Baudouin et al, 2017) no more than 15 %, the required sample size is at least 266 patients. A total sample size of at least 320 patients (20% additional) will allow compensation for incomplete datasets.
Statistical methods:	Demographic and clinical/prognostic data of the patients at baseline will be described with numerical and graphical summary statistics. The statistical significance of the proportion of patients reporting high adherence to IKERVIS® will be assessed using a binomial test and the respective 95% confidence interval (CI). For the secondary and safety endpoints, categorical data will be analyzed using a binomial

	test with the respective 95% CI, and continuous data will be analyzed using the paired t-test or the Wilcoxon signed rank test, depending on the nature of the data. The association between categorical variables will be tested using a chi-squared test and/or logistic regression. A result will be considered significant when P≤0.05. The statistical analysis will be performed using SPSS v.21

STUDY CONTACT DETAILS

Principal Investigator:

CRO:

Sponsor:

PROTOCOL SPONSOR APPROVAL

An observational, open-label, multi-center prospective study for assessing the patients' adherence to IKERVIS® treatment in routine daily clinical practice

Study code: IKERVIS

Signature: .....

SPONSOR
Date: .....
(DD-mmm-YYYY)

PROTOCOL AUTHORS APPROVAL

Signature: .....

Zacharias Samos

Date: .....
(DD-mmm-YYYY)

INVESTIGATOR'S AGREEMENT

I have read the foregoing protocol, entitled "An observational, open-label, multi-center prospective study for assessing the patients' adherence to IKERVIS® treatment in routine daily clinical practice" and agree to conduct the study as detailed herein and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

Principal Investigator's Name Signature

Date

Investigational Site

LIST OF ABBREVIATIONS AND KEY TERMS

Abbreviation	Full term
AE	Adverse Event
ADR	Adverse Drug Reaction
CRF	Case Report Form
CRO	Contract Research Organization
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonization
SAE	Serious Adverse Event
SmPC	Summary of Product Characteristics
VA	Visual Acuity
logMAR	Logarithm of the Minimum Angle of Resolution
IOP	Intraocular pressure
DED	Dry eye disease
TBUT	Tear Break Up Time
CFS	Cornea Fluorescein Staining
OSDI	Ocular Surface Disease Index

Table of Contents

1. Background

1.1 Introduction

Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homoeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities have aetiological roles' (Nelson et al, 2017).

Types of dry eye disease and its prevalence

As is well known, there are two major categories of DED, aqueous-deficient (equating with reduced lacrimal gland function) and evaporative (equating very largely with meibomian gland dysfunction). Evaporative dry eye is far commoner than aqueous-deficient dry eye, and many patients have both forms concurrently (Lemp et al, 2012). The global prevalence of DED is not constant. It is highest in South East Asia, and lowest in Europe and the USA. Prevalence increases with age, with signs showing a greater increase per decade than symptoms. Women have a higher prevalence than men. Although most workers have found that tear parameters change with age, with volume, Schirmer score and break-up time reducing, and osmolarity increasing (Mathers et al, 1996, Ozdemir et al, 2010) others have found no significant age differences, except that osmolarity increases with age in females. Tomlinson's group suggested that in DED there are pathological processes at work, particularly meibomian gland dysfunction, and that dry eye is hence an acquired disease rather than an age-related change.

There is a huge literature on DED Management. Last year a Cochrane Systematic Review found that most over-the-counter artificial tear preparations are similarly effective (Pucker, 2016). Some success has been achieved with antioxidants, and viscosity enhancers such as drops containing HPMC or hyaluronic acid are much used. Hypo-osmolar drops have been available for years and there is interest in newer

osmoprotectants, including trehalose, a non-reducing sugar whose molecules cluster in aqueous solution.

Autologous serum, prepared from the patient's own blood, is very similar to natural tears, being of the correct pH and containing nutrients, vitamins, fibronectin, epithelial, and nerve growth factors, but there are legal hurdles involved in its preparation and it is very expensive, a single batch costing £1300 in 2008 (Macnellan et al, 2008). Steroids have been used for many years in DED where there is significant inflammation, and the usual caveats of steroid usage apply: short periods of use and the 'less-penetrating' drugs are to be preferred. However, a recently published multi-centre European RCT of ciclosporin 0.1% emulsion showed improvement of symptoms and signs in patients with moderate to severe DED (Baoudin et al, 2017).

1.2 Name and description of study product.

The current preparation of IKERVIS® 1mg/ml contains 1 mg of ciclosporin. Ciclosporin (also known as ciclosporin A) is a cyclic polypeptide immunomodulator with immunosuppressant properties. It has been shown to prolong survival of allogeneic transplants in animals and significantly improved graft survival in all types of solid organ transplantation in man.

Ciclosporin has also been shown to have an anti-inflammatory effect. Studies in animals suggest that ciclosporin inhibits the development of cell-mediated reactions. Ciclosporin has been shown to inhibit the production and/or release of pro-inflammatory cytokines, including interleukin 2 (IL-2) or T-cell growth factor (TCGF). It is also known to up-regulate the release of anti-inflammatory cytokines. Ciclosporin appears to block the resting lymphocytes in the G0 or G1 phase of the cell cycle. All available evidence suggests that ciclosporin acts specifically and reversibly on

lymphocytes and does not depress haematopoiesis or has any effect on the function of phagocytic cells.
In patients with dry eye disease, a condition that may be considered to have an inflammatory immunological mechanism, following ocular administration, ciclosporin is passively absorbed into T-lymphocyte infiltrates in the cornea and conjunctiva and inactivates calcineurin phosphatase. Ciclosporin-induced inactivation of calcineurin inhibits the dephosphorylation of the transcription factor NF-AT and prevents NF-AT translocation into the nucleus, thus blocking the release of pro-inflammatory cytokines such as IL-2.

Clinical efficacy and safety

The efficacy and safety of IKERVIS were evaluated in two randomised, double-masked, vehicle-controlled clinical studies in adult patients with dry eye disease (keratoconjunctivitis sicca) who met the International Dry Eye Workshop (DEWS) criteria.
In the 12 month, double-masked, vehicle controlled, pivotal clinical trial (SANSIKA study), 246 Dry Eye Disease (DED) patients with severe keratitis (defined as a corneal fluorescein staining (CFS) score of 4 on the modified Oxford scale) were randomised to one drop of IKERVIS or vehicle daily at bedtime for 6 months. Patients randomised to the vehicle group were switched to IKERVIS after 6 months. The primary endpoint was the proportion of patients achieving by month 6 at least a two-grade improvement in keratitis (CFS) and a 30% improvement in symptoms, measured with the Ocular Surface Disease Index (OSDI). The proportion of responders in the IKERVIS group was 28.6%, compared to 23.1% in the vehicle group. The difference was not statistically significant (p=0.326).

The severity of keratitis, assessed using CFS, improved significantly from baseline at month 6 with IKERVIS compared to vehicle (mean change from baseline was -1.764 with IKERVIS vs. -1.418 with vehicle, p=0.037). The proportion of IKERVIS-treated patients with a 3-grade improvement in CFS score at month 6 (from 4 to 1) was 28.8%, compared to 9.6% of vehicle-treated subjects, but this was a post-hoc analysis, which limits the robustness of this outcome. The beneficial effect on keratitis was maintained in the open phase of the study, from month 6 and up to month 12.

The mean change from baseline in the 100-point OSDI score was -13.6 with IKERVIS and -14.1 with vehicle at month 6 (p=0.858). In addition, no improvement was observed for IKERVIS compared to vehicle at month 6 for other secondary endpoints, including ocular discomfort score, Schirmer test, use of concomitant artificial tears, investigator's global evaluation of efficacy, tear break-up time, lissamine green staining, quality of life score, and tear osmolarity.

A reduction in the ocular surface inflammation assessed with Human Leukocyte Antigen-DR (HLA-DR) expression (an exploratory endpoint), was observed at month 6 in favour of IKERVIS (p=0.021).

In the 6 month, double-masked, vehicle controlled, supportive clinical trial (SICCANOVE study), 492 DED patients with moderate to severe keratitis (defined as a CFS score of 2 to 4) were also randomized to IKERVIS or vehicle daily at bedtime for 6 months. The co-primary endpoints were the change in CFS score, and the change in global score of ocular discomfort unrelated to study medication instillation, both measured at month 6. A small but statistically significant difference in CFS improvement was observed between the treatment groups at month 6 in favor of IKERVIS (mean change from baseline in CFS -1.05 with IKERVIS and -0.82 with vehicle, p=0.009).

The mean change from baseline in ocular discomfort score (assessed using a Visual Analogic Scale) was -12.82 with IKERVIS and -11.21 with vehicle (p=0.808).
In both studies, no significant improvement of symptoms was observed for IKERVIS compared to vehicle after 6 months of treatment, whether using a visual analogue scale or the OSDI.
In both studies one third of the patients in average had Sjögren's syndrome; as for the overall population, a statistically significant improvement in CFS in favor of IKERVIS was observed in this subgroup of patients.
At completion of the SANSIKA study (12-month study), patients were asked to enter the Post SANSIKA study. This study was an open-label, non-randomized, one-arm, 24-month study extension of the Sansika Study. In Post SANSIKA study patients alternatively received IKERVIS treatment or no treatment depending on CFS score (patients received IKERVIS when there was a worsening of keratitis).
This study was designed to monitor the long-term efficacy and relapse rates in patients who have previously received IKERVIS.
The primary objective of the study was to assess the duration of the improvement following IKERVIS treatment discontinuation once the patient was improved with respect to the baseline of the SANSIKA study (i.e. at least 2 grade improvement on the modified Oxford scale).
67 patients were enrolled (37.9% of the 177 patients having ended Sansika). After the 24-month period, 61.3% of 62 patients included in the primary efficacy population did not experience a relapse based on CFS scores. Percentage of patients who experienced a severe keratitis recurrence was 35% and 48% in patients treated 12 months and 6 months with IKERVIS respectively in the SANSIKA study.
Based on the first quartile (the median could not be estimated due to the small number of relapses), time to relapse (back to CFS grade 4) was ≤224 days and ≤175 days in patients previously treated 12 months and 6 months with IKERVIS, respectively.

Patients spent more time on CFS grade 2 (Median 12.7 weeks/year) and grade 1 (Median 6.6 weeks/year) than CFS grade 3 (Median 2.4 weeks/year), CFS grades 4 and 5 (Median time 0 week/year).

Assessment of DED symptoms by VAS showed a worsening of patient's discomfort from the time treatment was first stopped to the time it was restarted except pain which remained relatively low and stable. The median global VAS score increased from the time treatment was first stopped (23.3%) to the time treatment was restarted (45.1%).

Blood concentrations of IKERVIS were measured using a specific high-pressure liquid chromatography-mass spectrometry assay. In 374 patients from the two efficacy studies, plasma concentrations of ciclosporin were measured before administration and after 6 months (SICCANOVE study and SANSIKA study) and 12 months of treatment (SANSIKA study). After 6 months of ocular instillation of IKERVIS once per day, 327 patients had values below the lower limit of detection (0.050 ng/mL) and 35 patients were below the lower limit of quantification (0.100 ng/mL). Measurable values not exceeding 0.206 ng/mL were measured in eight patients, values considered to be negligible. Three patients had values above the upper limit of quantification (5 ng/mL) however they were already taking oral ciclosporin at a stable dose, which was allowed by the studies' protocol. After 12 months of treatment, values were below the low limit of detection for 56 patients and below the low limit of quantification in 19 patients. Seven patients had measurable values (from 0.105 to 1.27 ng/mL), all considered to be negligible values. Two patients had values above the upper limit of quantification, however they were also on oral ciclosporin at a stable dose since their inclusion in the study.

Non-clinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, repeated dose toxicity, phototoxicity and photoallergy, genotoxicity, carcinogenic potential, toxicity to reproduction and development.

Effects in non-clinical studies were observed only with systemic administration or at exposures considered sufficiently in excess of the maximum human exposure indicating little relevance to clinical use.
No significant changes have been observed in the other secondary endpoints (TBUT, lissamine green staining and Schirmer test, NEI-VFQ and EQ-5D) over the course of the extension study (SmPC , 2020).

2. Study objectives

2.1 Primary objective

The primary objective of the current study is to investigate patients' adherence to IKERVIS® treatment in adult patients with dry eye disease.

2.2 Secondary objective

The secondary objective comprises the following: To identify potential factors that may be associated with IKERVIS® non-adherence in daily clinical practice.

2.3 Study design

Multicenter, open – label, one – arm, prospective, non-interventional cohort study of patients with dry eye disease receiving IKERVIS®. The total duration of the study is 36 weeks (12 weeks' recruitment period and 24 weeks' follow-up period). The recruitment period may be extended until the total number of patients is reached.

3. Study population

3.1 Target population

The target population of the current clinical study is male and female patients with dry eye disease, aged ≥18 years, receiving Ikervis according to standard clinical practice.

3.2 Inclusion criteria

To be eligible to participate in this study, a patient must meet all the following eligibility criteria at baseline:

•Adult patient (≥18 years old) with dry eye disease.

•Patient receiving IKERVIS®.

•Patients willing and able to provide written informed consent personally or by legal proxy.

•Patients willing to comply with all study procedures and to be available for the duration of the study

3.3 Exclusion criteria

A patient will be excluded from the study if any of the following exclusion criteria apply:

•Current enrolment in other clinical trial.

•Patient with uncontrolled dry eye disease.

•Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test

•Patients in treatment for ocular diseases (including DED) with more than one therapy (tear supplements excluded).

•Patient with hypersensitivity to the active substance or to any of the excipients of this medicinal product.

4. Study endpoints

4.1 Primary endpoints

The primary endpoints of the study is the Proportion of patients reporting high adherence to IKERVIS® at 24 weeks defined as a Morisky Medication Adherence Scale (MMAS-4) score equal to 0.

4.2 Secondary endpoints

The secondary endpoints of the study comprise the following:

- Proportion of patients with a Medication Persistence Ratio (MPR), defined as the number of days without receiving medication after study enrolment divided by the number of days participating in the study less than 0.20.
- Proportion of patients encountering difficulties with medication supply due to coexisting medical conditions.
- Proportion of patients encountering difficulties with medication supply due to lack of insurance coverage.
- Proportion of patients encountering difficulties with medication supply due to inability to access or difficulty accessing pharmacy.
- Proportion of patients with IKERVIS® discontinuation during the follow-up period.

4.3 Safety endpoints

- Mean change in Visual acuity(VA)

- Mean change in Intraocular pressure (IOP)
- Mean change in Schirmer's test
- Mean change in Tear Break Up Time (TBUT)
- Mean change in Cornea Fluorescein Staining(CFS) score on the modified Oxford scale
- Mean change in Ocular Surface Disease Index(OSDI)
- Ocular/systemic adverse events (AEs),

5. Total study duration

Total study duration is three (3) months recruitment period and 6 months' follow-up period for each patient.

6. Data collection plan and study variables

Data pertinent to the present study will be collected on three visits and recorded on the Case Report Form (CRF). A schedule of patients' visits and assessments are presented in attached Table.

6.1 Baseline visit (week 0)

The Baseline visit will include the screening phase during which inclusion and exclusion criteria are checked, the patient is informed about the study, and informed consent form is signed. Should the patient be eligible for participation in the study and upon signature of the informed consent form; the following data will be recorded: demographic data, medical history (type of dry eye disease, date of diagnosis, previous and current treatment DED), IOP, VA, Schirmer's test. CFS, TBUT measurements, comorbidities and concomitant medications.

6.2 Interim Visit 1 (week 8)

During the visit at week 8(±7 days), the following will take place: administration of the MMAS-4 questionnaire, calculation of the Medication Persistence Ratio and the following data will be recorded: description of potential factors associated with non-adherence, treatment discontinuations and adverse events. IOP, VA, Schirmer's test. CFS, TBUT measurements, comorbidities and concomitant medications will be recorded as well.

6.3 Final visit (week 24)

During the final visit at week 24 (±14 days), the following will take place: administration of the MMAS-4 questionnaire, calculation of the Medication Persistence Ratio and the following data will be recorded: description of potential factors associated with non-adherence, treatment discontinuations and adverse events. IOP, VA, Schirmer's test. CFS, TBUT measurements, comorbidities and concomitant medications will be recorded as well

6.4 MMAS-4

The Morisky Medication Adherence Scale (MMAS) is a generic self-reported, medication-taking behavior scale, validated for hypertension but used for a wide variety of medical conditions. The original version of the scale (MMAS-4) consists of four items with a scoring scheme of "Yes" = 0 and "No" = 1. The items are summed to give a range of scores from low adherence (3-4) to high adherence (0) (Morisky et al, 1986, Med Care).

6.5 MPR

The Medication Persistence Ratio (MPR) was defined as the number of days without receiving medication after study enrolment divided by the number of days participating in the study. High adherence was defined as a value less than 0.20.

6.6 Visual acuity(VA)

The visual acuity test is a routine part of an eye examination or general physical examination. The term visual acuity refers to an angular measurement relating distance to the minimal object size resolvable at that distance. Visual acuity test is used to determine the smallest letters a person can read on a standardized chart (Snellen chart) or a card held 14 - 20 feet away. Most of the lights should be turned off during the visual acuity test.

Visual acuity may be initially assessed using the patient's current distance glasses or most recent refraction obtained from prior examination. Refraction should then be performed on both eyes to determine the best corrected visual acuity. The patient should be asked to read slowly and not to proceed until they have given a definite answer. If the patient is not sure of the letter, they should be encouraged to guess. Examiners should never point to the chart or to specific letters/numbers. Instead, a sheet of paper may be used to guide the patient to the proper location on the chart. This test is done at each eye, one at a time.

If visual acuity is so poor that the patient cannot read from 20 feet, then he/she could approach to the chart and repeat the process. Special modification on the result should be taken into consideration by the examiner. If visual acuity still remains very poor, then count fingers or hand motion visual acuity test is performed. All room illumination should be turned on. Eccentric fixation if present should be encouraged. In count fingers vision it is important to record the distance between the patient and the examiner's hand.

Visual acuity may be expressed in different ways as shown in the following Table 2:

Table 2: Visual Acuity Scales

Foot	Metre	Decimal	LogMAR
20/200	6/60	0.10	1.00
20/160	6/48	0.125	0.90
20/125	6/38	0.16	0.80
20/100	6/30	0.20	0.70
20/80	6/24	0.25	0.60
20/63	6/19	0.32	0.50
20/50	6/15	0.40	0.40
20/40	6/12	0.50	0.30
20/32	6/9.5	0.63	0.20
20/25	6/7.5	0.80	0.10
20/20	6/6	1.00	0.00
20/16	6/4.8	1.25	-0.10
20/12.5	6/3.8	1.60	-0.20
20/10	6/3	2.00	-0.30

The top number refers to the distance the patient stands from the chart. This is usually 20 feet. The bottom number indicates the distance at which a person with normal eyesight could read the same line the patient correctly read. For example, 20/20 is considered normal. 20/40 indicates that the line the patient correctly read at 20 feet away can be read by a person with normal vision from 40 feet away. Even if the patient

misses one or two letters on the smallest line he is still considered to have vision equal to that line. Table 2 also lists the corresponding values, e.g. for logMAR. Visual acuity score will be recorded on the CRF.

6.7 Intraocular pressure (IOP)

Intraocular pressure (IOP) is the fluid pressure inside the eye. This pressure depends on the balance between the rate of production of aqueous humor and its resistance to drainage,

Intraocular pressure can be measured using the current gold standard instrument for measuring IOP, which is the Goldmann Applanation Tonometer. It provides the most accurate, reliable, and reproducible measurements. It measures the force necessary to flatten an area of cornea of 3.06 mm diameter, which equals multiplied by 10 with IOP. Because the instrument needs to come into contact with the eye, the test requires previous instillation of a topical anesthetic and fluorescein.

The investigation requires that the patient is sitting in the required exploration position before the slit lamp. The patient's eyelid is kept separated by the examiners non-dominant hand. Subsequently, the clinical trial subject is advised to bend forward, look ahead and breathe through the nose normally.

A special disinfected prism is mounted on the tonometer head and then placed against the cornea. The examiner then uses a cobalt blue filter to view two green semi circles. The force applied to the tonometer head is then adjusted using a dial connected to a variable tension spring until the inner edges of the green semicircles in the viewfinder meet. When an area of 3.06 mm has been flattened, the opposing forces of corneal rigidity and the capillary attraction of the tear film meniscus are counterbalanced allowing the pressure in the eye to be determined from the force applied.

6.8 Schirmer's test

Schirmer's test determines whether the eye produces enough tears to keep it moist. This test is used when a person experiences very dry eyes or excessive watering of the eyes. It poses no risk to the subject A negative (more than 10 mm of moisture on the filter paper in 5 minutes) test result is normal. Both eyes normally secrete the same amount of tears. The test works by the principle of capillary action, which allows the water in tears to travel along the length of a paper test strip in an identical fashion as a horizontal capillary tube. The rate of travel along the test strip is proportional to the rate of tear production. The patient is instructed to look upward, and the patient's eyelid is pulled down. The bent end of the test strip is placed in the eye such that it rests between the palpebral conjunctiva of the lower eyelid and the bulbar conjunctiva of the eye. Schirmer's test uses paper strips inserted into the eye for several minutes to measure the production of tears. Both eyes are tested at the same time. Most often, this test consists of placing a small strip of filter paper inside the lower eyelid (inferior fornix). The eyes are closed for 5 minutes. The paper is then removed and the amount of moisture is measured. After five minutes, the patient is asked to open both eyes and look upward so the test strips may be removed. The Schirmer test score is determined by the length of the moistened area of the strips (using the scale packaged with the strips) The use of the anesthetic ensures that only basal tear secretion is being measured.

6.9 Tear Break Up Time (TBUT)

Tear breakup time (TBUT) is a clinical test used to assess dry eye disease. To measure TBUT, fluorescein is instilled into the patient's tear film and the patient is asked not to blink while the tear film is observed under a broad beam of cobalt blue illumination. The TBUT is recorded as the number of seconds that elapse between the last blink and the appearance of the first dry spot in the tear film, as seen in this

progression of these slit lamps photos over time. A TBUT under 10 seconds is considered abnormal.

6.10 Cornea Fluorescein Staining(CFS) score on the modified Oxford scale

Staining is represented by punctate dots on a series of panels (A-E). Staining ranges from 0-5 for each panel and 0-15 for the total exposed inter-palpebral conjunctiva and cornea. The dots are ordered on a log scale.

Conduct of Test:The dye is instilled. Slit-lamp is set (eg.16 magnifications with x10 oculars with Haag-Streit). The upper eyelid is lifted slightly to grade the whole corneal surface. In order to grade the temporal zone, the subject looks nasally; to grade the nasal zone the subject looks temporally.The upper and lower conjunctiva can also be graded.

6.11 Ocular Surface Disease Index (OSDI

The Ocular Surface Disease Index (OSDI), developed by the Outcomes Research Group at Allergan Inc is a 12-item questionnaire designed to provide a rapid assessment of the symptoms of ocular irritation consistent with dry eye disease and their impact on vision-related functioning (Walt et al, 1997). The 12 items of the OSDI questionnaire were graded on a scale of 0 to 4, where 0 indicates none of the time; 1, some of the time; 2, half of the time; 3, most of the time; and 4, all of the time. The total OSDI score was then calculated on the basis of the following formula: OSDI=[(sum of scores for all questions answered) ×

100]/[(total number of questions answered) × 4].Thus, the OSDI is scored on a scale of 0 to 100, with higher scores representing greater disability.

7. Treatment and concomitant medication

Since this is an observational, non-interventional study, the treatment schedule is defined according to investigator's criteria. Treatment with IKERVIS® should be according to the indication for treatment as defined in the Summary of Product characteristics (SmPC) and according to standard clinical practice. During this non interventional study, any changes and/or additions to the concomitant medications are allowed according to investigator's criteria. All concomitant medications will be recorded on the CRF.

8. Treatment discontinuation

A patient should be withdrawn from the study if:

- according to the investigator's criteria treatment with IKERVIS® should be discontinued
- the patient withdraws his/her consent for participation in the study
- any of the exclusion criteria becomes applicable.

The reason of patient's withdrawal should be recorded on the CRF.

9. Safety assessments

9.1 Definition of an adverse event

An AE is any untoward medical occurrence in a patient administered a pharmaceutical product, regardless of whether it has a causal relationship with this treatment. In this study, any AE occurring after the study patient has signed the informed consent form should be recorded and reported as an AE. An AE can,

therefore, be any unfavourable and unintended physical sign, symptom, or laboratory parameter that develops or worsens in severity during the course of the study, or significant worsening of the disease under study or of any concurrent disease, whether or not considered related to the study drug. A new condition or the worsening of a pre-existing condition will be considered an AE. Stable chronic conditions (such as arthritis) that are present before study entry and do not worsen during the study will not be considered AEs. Accordingly, an AE can include any of the following:

- Intercurrent illnesses
- Physical injuries
- Events possibly related to concomitant medication
- Significant worsening (change in nature, severity, or frequency) of the disease under study or other pre-existing conditions. (Note: A condition recorded as pre-existing that is intermittently symptomatic [e.g., headache] and which occurs during the study should be recorded as an AE.)
- Drug interactions
- Events occurring during diagnostic procedures or during any washout phase of the study
- Laboratory or diagnostic test abnormalities that result in the withdrawal of the patient from the study, are associated with clinical signs and symptoms or a serious adverse event (SAE), or require medical treatment or further diagnostic workup, or are considered by the physician to be clinically significant.

9.2 Definition of an Adverse Drug Reaction

An adverse drug reaction is defined as a response to a medicinal product which is noxious and unintended. Response in this context means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility. Adverse reactions may arise from use of the product within or outside the terms of the

marketing authorisation or from occupational exposure. Conditions of use outside the marketing authorisation include off-label use, overdose, misuse, abuse and medication errors.

9.3 Definition of a Serious Adverse Event

A SAE is an AE occurring at any dose that results in any of the following outcomes or actions:

•Death (other than disease progression in this study)

•A life threatening AE (i.e., the patient was at immediate risk of death from the event as it occurred); does not include an event that, had it occurred in a more severe form, might have caused death

•Inpatient hospitalization or prolongation of existing hospitalization means that hospital inpatient admission and/or prolongation of hospital stay were required for treatment of an AE, or that they occurred as a consequence of the event. Hospitalizations scheduled for an elective procedure or for treatment of a preexisting condition that has not worsened during participation in the study will not be considered SAEs.

•Persistent or significant disability or incapacity (refers to a substantial disruption of one's ability to conduct normal life functions)

•A congenital anomaly/birth defect

•An important medical event that may not result in death, be life threatening, or require hospitalization, but may jeopardize the patient and may require medical intervention to prevent one of the outcomes listed in this definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or the development of drug dependency or drug abuse. Note: Any suspected

transmission of an infectious agent via a medicinal product is considered an important medical event.
An AE that does not meet any of the criteria for seriousness listed above will be regarded as a non-serious AE.

9.4 Recording and Reporting Adverse Events

Serious and non-serious Adverse Events that occur during the study period which are recorded in the patient's medical records or source documentation must be transcribed onto the CRF, regardless of the severity of the event. The clinical course of each AE will be monitored at suitable intervals until resolved or stabilized or returned to baseline, or until the patient is referred to the care of a health care professional, or until a determination of a cause unrelated to the study drug or study procedure is made. The onset and end dates, duration (in case of AE duration of less than 24 hours), action taken regarding study drug, treatment administered, and outcome for each AE must be recorded on the source documentation and transcribed onto the eCRF. The relationship of each AE to study drug treatment and study procedures, and the severity and seriousness of each AE, as judged by the physician, must be recorded as well.

To satisfy regulatory requirements, all serious adverse events regardless of judged relationship to treatment with IKERVIS®, that occur during the study period, must be reported to the sponsor by the physician. The event must be reported within 24 hours of when the physician learns about it or, if the event occurs on a weekend or national holiday, on the next working day. Contact details of the sponsor are provided below:

"sponsor"

Completing the Adverse Events/Adverse Drug Reaction Form and reporting the event must not be delayed, even if not all the information is available. The physician does not need to actively monitor patients for adverse events once the study has ended. Serious adverse events occurring to a patient after the treatment of that patient has ended should be reported to the sponsor if the physician becomes aware of them.

9.5 Relationship of an Adverse Event to the Study Drug

The relationship of an AE to the study drug is characterized as follows:

No reasonable possibility (not related)

This category applies to adverse events which, after careful consideration, are clearly due to extraneous causes (disease, environment, etc) or to adverse events, which, after careful medical consideration at the time they are evaluated, are judged to be unrelated to the study drug. The relationship of an adverse event may be considered “no reasonable possibility” if it is clearly due to extraneous causes or if at least 2 of the following apply:

•It does not follow a reasonable temporal sequence from the administration of the test drug.

•It could readily have been produced by the patient’s clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

•It does not follow a known pattern of response to the test drug.

It does not reappear or worsen when the drug is re-administered.

Reasonable possibility (related)

This category applies to adverse events for which, after careful medical consideration at the time they are evaluated, a connection with the test drug administration cannot be ruled out with certainty nor felt with a high degree of certainty to be related to the study drug. The relationship of an adverse event may be considered “reasonable possibility” if at least 2 of the following apply:

•It follows a reasonable temporal sequence from administration of the drug.

•It cannot be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

•It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists.

It follows a known pattern of response to the test drug.

9.6 Pregnancy

All pregnancies that occur during the study, or within 14 days of completion of the study, are to be reported immediately to the individual identified in the clinical study personnel contact information section of this protocol, and the physician must provide the LSO with the pregnancy form. The process for reporting a pregnancy is the same as that for reporting an SAE. All patients who become pregnant will be monitored to the completion or termination of the pregnancy. If the pregnancy continues to term, the outcome (health of the infant up to 8 weeks of age), including spontaneous or voluntary termination, details of birth, and presence or absence of any birth defect, congenital abnormalities, or maternal and new-born complications, will be reported to the sponsor. Any complication of pregnancy will be reported as an AE or SAE, as appropriate. For pregnancies of partners of men participating in the study, the Sponsor's Pharmacovigilance (PhV) Department will determine the procedure to appropriately follow up after notification as described above. All partners who become pregnant and provide appropriate consent to Sponsor's PhV will be monitored to the completion or termination of the pregnancy.

If the pregnancy does not continue to term, 1 of the following actions will be taken:

- For a spontaneous abortion, report an SAE.
- For an elective abortion due to developmental anomalies, report as an SAE.
- For an elective abortion **not** due to developmental anomalies, report on the pregnancy form.

10. Sample size

In order to calculate a 95% confidence interval for the proportion of patients reporting high adherence to IKERVIS® at 24 weeks, which is expected to be 80% with a margin error (Δ) (Baudouin et al, 2017) no more than 15 %, the required sample size is at least 266 patients. A total sample size of at least 320 patients (20% additional) will allow compensation for incomplete datasets.

11. Statistical methods

Demographic and clinical/prognostic data of the patients at baseline will be described with numerical and graphical summary statistics. The statistical significance of the proportion of patients reporting high adherence to IKERVIS® will be assessed using a binomial test and the respective 95% confidence interval (CI). For the secondary endpoints, categorical data will be analyzed using a binomial test with the respective 95% CI, and continuous data will be analyzed using the paired t-test or the Wilcoxon signed rank test, depending on the nature of the data. The association between categorical variables will be tested using a chi-squared test and/or logistic regression. A result will be considered significant when $P \leq 0.05$. The statistical analysis will be performed using SPSS v.21.

12. Insurance coverage

Since this is a non-interventional clinical study, an insurance coverage is not applicable.

13. Regulatory Authority approval

The protocol will be submitted for approval to the Scientific Committee of each participating clinical site. Approval by the Greek Regulatory Agency (Hellenic Organization for Medicines, EOF) is not required due to the nature of the study (phase IV).

14. Data collection – Quality control

Data will be entered at the study sites into a central database using an electronic CRF (eCRF) with a delegated CRO as an administrator.

The investigator should keep accurate files to record study conduction. Study documents will be kept by the investigator on site as confidential documents. All applicable data will be transferred to the patient's Case Report Form (CRF). The Case Report Form is essential for the study conduction and should be completed for all patients entering the study by the investigator. The Sponsor has the rights of the completed original CRFs and therefore data should not be released in any format without the prior written approval of the Sponsor. Monitoring visits by authorized personnel may take place at the site to ensure conduction of the study in accordance to the protocol and ICH GCP Guidelines. The study monitor will be given access by the investigator or sub-investigator to the patient files and any relevant documents to ensure source data verification and will be assisted in resolving any issues arisen during the monitoring visits.

The investigational site may also get quality control audits by the sponsor company or the Regulatory Authorities. The investigator will ensure access of any study related documents to the authorized personnel of the sponsor company or the Regulatory Authorities during the quality control audits. For the smooth conduction of the study, it is important that investigational staff will be available during the monitoring visits or the quality control audits.

15. Protocol Amendments and violations

No changes from the final approved (signed) protocol will be initiated without the prior written approval or favorable opinion of a written amendment by the scientific committees as applicable to local regulations.

Any significant deviation from the protocol will be considered a protocol violation. Protocol violations include non-adherence on the part of the patient, the physician, or the sponsor to protocol-specific inclusion/exclusion criteria. Protocol violations will be identified and recorded by investigational site personnel on the CRF. If investigational site personnel learn that a patient who did not meet protocol eligibility criteria was entered into a study, they must immediately inform the sponsor of the protocol violation. If such patient has already completed the study or has withdrawn early, no action will be taken but the violation will be recorded.

16. Ethics

16.1 Informed Consent

This study is non-interventional, and by definition no additional procedures will be performed on the patient in addition to the normal clinical practice of the treating physician. Informed consent should be obtained from patients for their clinical data to be recorded anonymously. They will also be informed of their right to withdraw their consent at any time. The patient's willingness to participate in the study will be documented in writing in a consent form, which will be signed and personally dated by the patient. The physician will keep the original consent forms, and copies will be given to the patients. Written information about the study in a language understood by the patient will be given to all patients.

16.2 Health Authorities and Independent Ethics Committees/Institutional Review Boards

This study will be conducted in full accordance with the all relevant European and national guidelines and regulations for conducting studies with human subjects. Specifically, the study will comply with the Helsinki Declaration and the guidelines for Good Epidemiological Practice (GEP), Good Pharmacoepidemiology Practices and Good Pharmacovigilance Practices (GVP).

16.3 Confidentiality Regarding Study Patients

The physician must assure that the privacy of the patients, including their identity and all personal medical information, will be maintained at all times. In CRFs and other documents or image material submitted to the sponsor, patients will be identified not by their names, but by an identification code (e.g., identification number).

Personal medical information may be reviewed for the purpose of patient safety and/or verifying data in the source and transcribed onto the CRF. This review may be conducted by the study monitor, properly authorized persons on behalf of the sponsor, the quality assurance unit, and/or regulatory authorities. Personal medical information will always be treated as confidential.

17. Study Termination

The sponsor may terminate the study, or a site’s participation at any time. The end of study will be when the last subject completes the last scheduled visit.

18. References

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Summary of Product Characteristics, last updated March 2020

Walt et al, 1997 Evaluating the functional impact of dry eye: the Ocular Surface Disease Index [abstract]. Drug Inf J.;311436

Table. Flow Chart of Study Assessments for patients

Assessments	**Baseline visit**	**Interim visit 1**	**End of observation Final visit**
	Day 0	8 weeks after patient enrollment (± 7 days)	24 weeks after patient enrollment (± 14 days)
Informed consent[1]	X		
Demographic data	X		
Medical history	X		
Concomitant medication	X	X	X
Study drug administration	X	X	X
MMAS-4 assessment	-	X	X
MPR assessment	-	X	X
Assessment of difficulties in medication supply due to coexisting medical conditions	X	X	X
Assessment of difficulties in medication supply due to lack of insurance coverage	X	X	X
Assessment of difficulties in medication supply due to inability to access or difficulty accessing pharmacy	X	X	X
Treatment discontinuation	-	X	X
Adverse Event Reporting	-	X	X

Assessments	**Baseline**	**Interim visit**	**End of observation visit**
	Day 0	8 weeks after patient enrollment (± 7 days)	24 weeks after patient enrollment (± 14 days)
IOP	x	x	x
TBUT	x	x	x
CFS	x	x	x
Schirmer's Test	x	x	x
OSDI	x	x	x

NOTES: The signed and dated informed consent should be in file before recording any study-related data in the study case report form (CRF).