

**NEUROPROTECTION AND NEUROENHANCEMENT IN A  
MODEL OF OPTIC NERVE NEURODEGENERATION (NON  
ARTERITIC ISCHEMIC OPTIC NEUROPATHY):  
STUDY OF MORPHO-FUNCTIONAL CHANGES RELATED TO  
TREATMENT WITH CITICOLINE ORAL SOLUTION**

## **GENERAL INFORMATION**

**Protocol NEU-03\_2016**

**Study title:** NEUROPROTECTION AND NEUROENHANCEMENT IN A MODEL OF OPTIC NERVE NEURODEGENERATION (NON ARTERITIC ISCHEMIC OPTIC NEUROPATHY): STUDY OF MORPHO-FUNCTIONAL CHANGES RELATED TO TREATMENT WITH CITICOLINE ORAL SOLUTION

**Version and date:** 2 - dated 20/12/2016

**Study type:** non profit, blinded, close labelled

**Product:** Neukron Ofta® mese, food supplement based on Citicoline oral solution

**Pharmaceutical form:** vials containing 10 ml of Citicoline oral solution

**Dosage:** 500 mg/die

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# 1. INTRODUCTION

## 1.1 Basic Information

The optic nerve is made up of approximately one million nerve fibres that originate from retinal ganglion cells (RGC).

The nerve fibres of the optic nerve can be the site of degeneration processes due to different etiopathogenic mechanisms such as an increase of intraocular pressure (in the case of Glaucoma), ischemic phenomena (in arteritic and non-arteritic ischemic optic neuropathy) infective-inflammatory processes (in Optical Retrobulbar Neuropathy in the case of Multiple Sclerosis).

In particular, non-arteritic Ischemic Optic Neuropathy (NAION) (i.e., not related to autoimmune diseases) is a disease that can have an acute onset with a chronic progression and can affect one eye (monocular) or both (binocular). The disease causes a severe impairment of the visual function understood as decreased visual acuity (VA), loss or reduction of contrast perception, visual field deficits of various kinds in relation to the selective involvement of the optic nerve fibres: arcuate scotoma, blind-central scotomas, upper or lower altitudinal deficit.

From a pathogenetic point of view NAION is most frequently caused by transient hypoperfusion of the head in the optic nerve at level of the posterior ciliary arteries that causes a compartmental syndrome inducing ischemia. In rare cases it can be traced to local embolic phenomena (Hayreh 2011, Kerr et al. 2009).

The neurodegeneration process of the nerve fibres of the optic nerve can be assessed both from a functional point of view using Pattern Electroretinogram recording (PERG) and Visual Evoked Potential (VEP) (Parisi et al. 2008a) and the morphological view by studying the thickness of nerve fibers using optical coherence tomography (OCT) (Kernstock et al. 2014, Akbari et al., 2016, Han et al. 2016).

The literature reports the possibility, in rare cases, of slight spontaneous improvement of the visual function (Hayreh and Zahoruk 1981). However, there is currently no evidence of substantial benefit from specific therapies.

Indeed, currently under debate are the use of intravitreal injections of triamcinolone and anti angiogenics, oral steroid therapy, and intravitreal injection of erythropoietin (Kernstock et al. 2014).

In the case of another neurodegenerative disease of the optic nerve, Glaucoma, a concept recently proposed is for "neuroenhancement", i.e., the possibility of increasing the impaired function of the RGC and optic nerve (Chang and Goldberg 2012). This concept is based on data

relating to the increase of the responses of the PERG (an objective method of function of RGC) and VEP evaluation (an objective method of assessing the functionality of the optic nerve) after treatment with Citicoline (Parisi et al. 1999). The increase in RGC functionality can produce, over time, protection mechanisms from the acceleration of the peculiar apoptotic processes of the glaucomatous disease with a consequent reduction in the number of RGC that undergo cell death (neuroprotection).

Citicoline is a natural precursor of phosphatidylcholine, which constitutes the main component of neuronal and mitochondrial membranes. Regarding the physiology of RGC, phosphatidylcholine performs two very important roles: one is structural, as it represents the main phospholipid membrane, and one is functional in that, following the action of the phospholipase A2 enzyme, it is a source of fatty acids (AA and DAG), fundamental intracellular messengers (Secades and Lorenzo 2006).

Currently Citicoline is available in oral solution, the bioavailability of which is well known, about 98% (Agut et al., 1983, Roda et al. 1983). In this regard, the evidence bearing particular importance for Citicoline, administered as an oral solution at a dose of 500 mg/day in 4-month treatment cycles followed by 2 months of suspension, is its ability to slow the progression of visual field loss in glaucoma patients where there is a documented progressive loss of the visual field in the two years prior to starting treatment with Citicoline (Ottobelli et al. 2013)

A pilot study, conducted on patients suffering from NAION, demonstrated that after months of treatment with Citicoline administered in oral suspension, there was improvement in ganglion cell functionality, nerve conduction along the visual pathways and visual acuity (Parisi et al. 2008b).

## **1.2 Effects of Citicoline on the function of ganglion cells and the optic nerve in patients with non-arteritic ischemic optic neuropathy**

Our pilot study enrolled patients suffering with NAION in accordance with a set of inclusion/exclusion criteria which are listed comprehensively in the published work (Parisi et al. 2008b).

Patients with NAION were divided randomly into two groups matched by age:

- 1) NAION -T Group: 14 patients were treated with Citicoline. Citicoline was administered orally (Cebrolux - Tubilux, Italy, 1600 mg/day) for 60 days. followed by a suspension period of 4 months for a total of 6 months follow-up
- 2) NAION -NT Group: 12 patients were not treated and the natural evolution of the disease was observed during a follow-up of 6 months.

For this study, randomized for the first six months, it was decided in the sixth month of observation to continue the study as "open" and to continue to treat patients with NAION for a further period of 2 months, followed by 4 months of suspension.

For a more detailed description of the procedures followed and the electrophysiological techniques used, please refer to the original works. (Parisi et al. 2008a, Parisi et al. 2008b)

In analysing the VEP and PERG results, we considered the following parameters: P100 latency time and amplitude N75-P100 (VEP); latency time P50 and amplitude P50-N95 (PERG); difference between latency P100 and P50 (retinal cortical time, SCT).

During the study period the following was observed:

1. VEP: a decrease in the P100 latency time and an increase in amplitude N75-P100 after the two months of treatment; after the suspension period, we found a worsening in electrofunctional responses. At the end of the first follow-up (6 months) the VEP values were significantly different ( $P<0.01$ ) both compared to those of the baseline and compared to those of the control group (NAION -NT).
2. PERG: a decrease in the P50 latency time and an increase in amplitude P50-N95 after the two months of treatment; after the suspension period, we found a worsening in these electrofunctional responses. At the end of the first follow-up (6 months) the PERG values were significantly different ( $P<0.01$ ) both compared to those of the baseline and compared the control group (NAION -NT).
3. SCT: (electrofunctional index of post-retinal nerve conduction derived from the difference between the P100 latency time of the VEP and the P50 latency time of the PERG): a decrease after two months of treatment; after the suspension period we found a worsening of this electrofunctional index. At the end of the follow-up (6 months) the SCT values were significantly different ( $P<0.01$ ) both compared to the baseline and compared the control group (NAION -NT).
4. VA: increase after two months of treatment; after the suspension period, we found a worsening of these psychophysical responses. At the end of the follow-up (6 months) the VA values were significantly different ( $P<0.01$ ) both compared to the baseline and compared the control group (NAION -NT).

### **1.3 Citicoline mechanism of action**

From the data available in the literature, it is known that the exogenous administration of citicoline causes an increase in endogenous phosphatidylcholine synthesis and thus accelerates the repair of the previously damaged cell membranes (Secades 2001).

The cell membrane dysfunction or degeneration induces the release of cytotoxic substances (lytic enzymes, excitotoxic amino acids, etc.) which induce an extension of the area of damage or activate the mechanisms that lead to apoptosis (for example, an increase in the concentration of intracellular ceramide, a compound produced by catabolism of sphingolipids, is a potent pro-apoptotic agent)

In particular, in the etiology of vascular diseases (for example in stroke) a loss of phospholipid components is found resulting from an alteration in their metabolism, which leads to irreversible damage to the cell membranes of neurons. Biochemically a loss of phosphatidylcholine is observed, which is degraded during the ischemic processes into fatty acids with the production of reactive oxygen species (ROS). Experimental studies suggest that the administration of citicoline induces a reactivation of the anabolism of the phospholipids with reduced degradation of phosphatidylcholine and thus reduced formation of ROS. In some animal models a reduction was also observed of the infarcted area volume after treatment with Citicoline.

Therefore the therapeutic approach with citicoline allows the maintaining and/or restoring of the integrity of the cell membranes of neurons subjected to ischemic insults, such as can occur in some of the many forms of glaucomatous syndrome.

### **1.4 Neuro-functional considerations on treatment with Citicoline systemically and safety and tolerability in humans**

The fundamental element for cell function and survival is the ability to maintain an internal environment different to the external one. This function is ensured by the cell membrane, a discriminating barrier that selects and regulates the passage of the substances inside the cell and controls the difference in electrical potential.

Also, most of the biochemical reactions occur close to the cell membrane and in its context surface receptors are located allowing a dialogue of the cell with the surrounding cells and the rest of the organism.

In fact, the cell membrane:

- is the site of receptors and synapses that govern communications with neighbouring cells and the rest of the organism,



- maintains and controls the difference in electrical potential, which is necessary to allow propagation of the nerve impulse,
- handles most enzymatic reactions,
- at the mitochondrial level, the inner membrane is the site of the main energy production processes.

A lesion of the cell membrane leads to three important events:

1. some ions normally concentrated in the extracellular environment, in particular  $\text{Ca}^{++}$ , and other potentially harmful molecules, can enter uncontrollably into the cytoplasm, altering the vital processes of the cell
2. the ability to communicate through surface receptors is lost;
3. the intracytoplasmic content, rich in lytic enzymes and toxic mediators, can flow outside of the cell resulting in an extension of the damage to the contiguous cells. Therefore, an alteration of the phospholipids turnover compromises the validity of the membrane protection systems. Furthermore, as a result of activating the particular lytic enzymes, the phospholipases, the catabolism of the membrane phospholipids is accelerated and, if the resynthesis mechanism is insufficient, toxic molecules accumulate, such as ceramide, which can activate the metabolic pathways that lead to programmed cell death (apoptosis). To maintain the perfect functionality of the cells, therefore, the cell membrane needs to stay structurally intact. This is absolutely necessary in the case of cells without replicative capacity like the RGC, the loss of which results in irreversible functional impairment.

The cell membrane is composed of a double layer of phospholipids. Phospholipids are continuously exposed to chemical and physical insults which damage the molecular structure: to protect itself from this, the cell possesses an enzyme system that catabolises the damaged phospholipids and reuses the constituent elements (phosphocholine, glycerol and fatty acids), in order to synthesise them again

An alteration of the phospholipids turnover compromises the validity of the membrane protection systems and puts the specific cell functionality at risk. In the case of peripheral neurons such as RGC, the peculiar length of their axonal endings makes these cells particularly sensitive to any alteration in the cell membrane protection systems. In fact, precisely by virtue of the enormous development of the membrane surfaces, due to the length of their axons, these cells are more susceptible than others to the harmful effects caused by a phospholipid synthesis slowdown.

The clinical conditions where a slowdown of the phospholipid metabolism occurs are those

that present when the neural tissue undergoes a type of traumatic, ischemic or degenerative process.

In glaucoma and in NAION a pathological situation is configured in which one or more of these 3 elements can contribute to the development of damage to the optic nerve. Both glaucoma and NAION are neurotrophicopathies where alterations of the retinal structures are observed (Quigley et al. 1995, Parisi et al. 2008a) and retinal structures (Yucel et al. 2000).

An improvement of the VA, the VEP and contrast sensitivity after treatment with Citicoline was also observed in partially sighted patients (Porciatti et al., 1998, Campos et al., 1995, Campos 1997). These studies suggested that the effects observed were attributable to the activity of dopamine-like effect of Citicoline (Gottlob et al. 1989, Gottlob and Stangler-Zuschrott 1990). This pharmacological property could explain, at least partly, the positive effects of PERG and PEV observed in patients with glaucoma (Parisi et al. 1999, Parisi 2005 Rejdak et al. 2003) and in patients with NAION (Parisi et al. 2008b). In fact, it is known that levodopa induces a shortening of the VEP latency in humans with a possible retinal contribution as the PERG also is improved by this substance (Secades 2001). The VEP variations found in patients with glaucoma and with NAION are attributable to a dysfunction of the innermost retinal layers (ganglion cells and their fibres), correlated to a delay in post-retina nerve conduction (Parisi 2001, Parisi et al. 2001, Parisi 1997, Parisi et al. 2008a), while the PERG alterations the can be attributed to a dysfunction of the innermost retinal layers, although the possibility of a functional alteration of the pre-ganglionic elements has also been suggested (Veagan et al. 1995, Parisi et al 2008a).

The improvements of the PERG can also be attributed to a dopaminergic-like action of Citicoline; in fact it has been observed that levodopa induces an increase in retinal function (Gottlob et al. 1989) and therefore a similar neuromodulating activity can be assumed to explain the effects of Citicoline.

In addition to the evaluation of retinal function, both in glaucoma patients and in those with NAION, postretinal nerve conduction was examined using SCT measurement (Celesia and Kaufmann, 1985). The reduction of the SCT, observed after treatment with Citicoline (Parisi et al. 2008b), can be attributed to an improvement of retinal function with consequent improvement of nerve conduction along the optic pathways and a related increase in bioelectric activity in those cells where cortical potential originates. And this may also explain the reduced latency and increased P100 amplitudes of the VEP after treatment with Citicoline.

Also the independent action of Citicoline on the postretinal nerve conduction or visual

cortex can be hypothesised, but this hypothesis has no clear, conclusive and appropriate experimental references in the literature.

Citicoline induces an improvement in retinal bioelectric activity: we are unable to demonstrate whether there are additional effects on retinal nerve fibres (for example an increase in the layer thickness of the retinal nerve fibre), since at this time only the retinal function has been studied and there have been no morphological assessments.

Therefore, the effects of Citicoline on the bioelectric responses of the visual cortex may derive from two sources of improvement: one at a retinal level (as indicated by the PERG with reduced latencies and increased amplitudes) and one at a postretinal level (as indicated by the shortening of the SCT). As has been recently observed, the perimetric indices (Mean Deviation of Humphrey perimetry) are significantly correlated with the parameters of PERG, VEP and SCT (Parisi 2001), thus it is likely that the sources previously mentioned as cortical improvement might also be suggested to explain the improvement in perimetric condition detected in patients with glaucoma after treatment with Citicoline (Pecori Giraldi et al. 1989).

In conclusion, our data suggest that Citicoline has a significant action in improving the retinal and cortical bioelectric responses in patients suffering from diseases of the optic nerve of glaucomous or ischemic nature.

As a further benefit of this therapy, it should be emphasised that no clinical trial in humans (Parisi et al. 1999 Porciatti et al., 1998, Campos et al., 1995, Campos 1997 Virno et al. 2000 Rejdak et al., 2003) has reported significant adverse reactions either ocular or systemic.

### **1.5 Rationale of the study**

The pilot study (Parisi et al 2008b) demonstrated the possibility of improving retinal functionality and nerve conduction along the optic pathways in patients suffering from NAION after treatment with Citicoline for oral suspension.

However, the limit of this study consisted in the fact that another dose of Citicoline (1600 mg/day) was used since the bioavailability (i.e. the presence of a percentage of active ingredient in the blood after a single administration) of this molecule administered in the formulation for oral suspension is certainly lower than that of intramuscular administration.

Currently Citicoline is however available in oral solution, the bioavailability of which is well known, about 98% (Agut et al., 1983, Roda et al. 1983), comparable to that of intramuscular administration. In this regard, the evidence bearing particular importance for Citicoline,

administered as an oral solution at a dose of 500 mg/day in 4-month treatment cycles followed by 2 months of suspension, is its ability to slow the progression of visual field loss in glaucoma patients where there is a documented progressive loss of the visual field in the two years prior to starting treatment with Citicoline (Ottobelli et al. 2013).

It will be particularly interesting to evaluate the changes in the function (via recordings of PERG) and morphology (via the OCT evaluations) of retinal and nerve conduction along the optic pathway (using VEP recordings) in patients with NAION who are treated with Citicoline oral solution whose bioavailability is well known.

## **2. OBJECTIVES AND AIMS OF THE STUDY**

### **2.1 Primary objective**

Evaluation of potential neuroprotective action of Citicoline for maintenance and stabilisation of visual acuity in patients with Non-arteritic Ischemic Optic Neuropathy (NAION).

### **2.2 Secondary objectives**

1) to study the neurodegenerative processes of the optic nerve in patients suffering from NAION using the correlations between the function of RGC and optic nerve (using the PERG and VEP recording) and the structural modifications of the nerve fibres of the optic nerve (using the OCT evaluation);

2) to determine in patients with NAION the potential for neuroenhancement linked to Citicoline administration in oral solution. This will be obtained using the evaluations of the functional condition (PERG/VEP) found after treatment with Citicoline compared to the condition at the time of enrolment. Correlations will also be evaluated between stabilisation and/or function improvement of RGC and the optic nerve with changes in the visual field.

3) to study in patients with NAION the potential for neuroprotection linked to Citicoline administration oral solution. This will be evaluated by comparing, at the end of a 9-month follow-up period, the changes of the nerve fibre structure and the optic nerve (via the OCT assessment) found in the group of patients with NAION treated with Citicoline oral solution compared to a group of patients with untreated NAION where the natural progression of neurodegenerative processes will be observed.

### **3. PARTICIPATING SITES**

IRCCS - Fondazione Bietti, Roma

### **4. STUDY POPULATION**

A number of patients (from a minimum of 16 patients to a maximum of 32) will be enrolled, within a maximum time frame of 24 months, who have a diagnosis of unilateral or bilateral NAION aged 45 to 80 years such as to provide the study 32 eyes affected by this condition with the following

#### **4.1 Inclusion criteria.**

- 1) Acute visual reduction episode from NAION occurring for more than 6 months
- 2) Typical defects of the visual field evidenced with the Goldmann perimetry or with Humphrey perimetry 30-2
- 3) Visual acuity not less than 1/10
- 4) Having suspended any potential neuroprotective therapies (e.g., Coenzyme Q10) for at least 6 months.

#### **4.2 Exclusion criteria**

- Ocular surgery in the 3 months preceding the study, including surgery for cataracts in the previous three months.
- Cataract or maculopathy
- Known hypersensitivity to the study product
- Positive history for diseases of the optic nerve (retrobulbar optic neuropathy, glaucoma) or systemic diseases which could preclude the enrolment in the study according to the investigators' judgement
- Pregnant or nursing women, or women of potential childbearing age not using adequate contraception.
- Diabetes, SLE, rheumatoid arthritis, mixed connective tissue disease

## **5. TRIAL DESIGN**

### **5.1 Trial Plan**

A prospective, single-center, randomized, blinded close-label study providing for the enrolment of a minimum of 16 to a maximum of 32 patients (see sample size in the section "Statistics") affected by NAION.

Considering that according to the inclusion/exclusion criteria it is very difficult to recruit patients suffering from NAION with the specific characteristics that enable them to be enrolled in this study, it is likely that with the presence of the binocular NAION, both eyes of the patients will be considered as recruited. Therefore, in the event of all patients having monocular NAION, enrolment must include 32 patients and this number is lower if we need to enrol patients with NAION in both eyes.

Patients selected according to the inclusion/exclusion criteria after signing informed consent will be randomized into two groups:

- a) In one group of patients with NAION no type of treatment will be performed (16 eyes, Control Group)
- b) in another group of patients with NAION, Citicoline will be administered in oral solution (500 mg/day) for 6 months followed by three months of suspension (16 eyes, Treated Group)

The division into two groups will enable the identification of a specific neuroprotective action of Citicoline. Randomization will be done by dividing patients with similar characteristics including age, refraction and visual impairment (perimeter defect and visual acuity) into two groups. Patients will be assigned to each group by an investigator not involved in the functional and structural tests.

## 5.2 Schematic diagram of the trial plan

The schematic diagram of the study is shown below.

|  | Baseline |           |           |
|--|----------|-----------|-----------|
| Visit  | T0       | T1        | T2        |
| Months   |          | 6         | 9         |
| Time window  |          | ± 10 days | ± 10 days |
| Informed consent   | ✓        |           |           |
| Demographic data   | ✓        |           |           |
| Medical records  | ✓        |           |           |
| Visual Field   | ✓        | ✓         | ✓         |
| PERG   | ✓        | ✓         | ✓         |
| PEV  | ✓        | ✓         | ✓         |
| OCT  | ✓        | ✓         | ✓         |
| Concomitant diseases   | ✓        | ✓         | ✓         |
| Previous treatments  | ✓        |           |           |
| Concomitant treatments   | ✓        | ✓         | ✓         |
| Complete ophthalmological examination:<br>visual acuity<br>anterior and posterior<br>biomicroscopy<br>ophthalmoscopy<br>intraocular pressure | ✓        | ✓         | ✓         |
| Inclusion/exclusion criteria   | ✓        |           | ✓         |
| Randomization  | ✓        |           |           |
| Compliance   | ✓        | ✓         | ✓         |
| Delivery of food supplement  | ✓        |           |           |
| Incidents  | ✓        | ✓         | ✓         |



### **5.3 Trial term and visits**

The study will have a total duration of 9 months, during which period the patients will have 3 visits:

- baseline visit T0
- visit at 6 months T1
- visit at 9 months T2

### **5.4 Scheduled visits**

The visits will be made at baseline, at 6 and 9 months of follow-up, not knowing to which group the patient belongs. The key will be opened only at the end of the second treatment period in order to assess the initial effects (after 9 months).

After screening, the patients enrolled will make a baseline visit during which they will be randomized into one of two groups. The visits will take place 6 and 9 months after the baseline visit.

At the baseline and each visit a complete ophthalmic examination will be performed, including the following assessments: corrected visual acuity, biomicroscopy, indirect ophthalmoscopy, measurement of intraocular pressure with Goldmann applanation tonometer, field of vision testing, recording of PERG and VEP, imaging of the optic nerve and retinal nerve fibre layer with OCT.

### **5.5 Outcomes**

The visual function will be determined by [Visual] acuity assessment, the function of retinal ganglion cells RGC will be assessed by electroretinogram (PERG) and the optic nerve function will be evaluated using the Visual Evoked Potential (VEP).

The primary objective is to evaluate the stabilisation and/or improvement in visual acuity.

Secondary objectives include stabilising and/or improving RGC functionality and optic nerve function assessed with the recording of PERG and VEP. Correlations will also be evaluated between stabilisation and/or function improvement of RGC and the optic nerve with changes in the visual field and the morphology of the optic nerve head (evaluated by OCT).

### **5.5.1. Psychophysical Evaluations**

#### **a) Visual Acuity (VA)**

The visual acuity (VA) examination for distance will be made with optotype projection of letters at 4 meters and the use of trial lenses in mesopic environment. Backlit ETDRS (Early Treatment Diabetic Retinopathy Study) Cards will be used for measuring the best visual acuity for distance. The expressed value acquired, LogMAR (logarithm of the minimum angle of resolution), will be converted to a decimal Snellen fraction according to the equivalence tables.

The visual acuity examination for near vision will be performed in the presence of good lighting with trial lenses added to the lens in use by the patient or to the lens measured for distance (best optical correction). The examination is performed with the aid of optical tables for near vision and measured according to the maximum quantity of vision in terms of character for near vision (I character for near vision indicates maximum acuity, X character indicates worst acuity for near vision).

#### **b) Visual Field (VF)**

A static perimetry will be performed for the visual field (VF) measurements (Humphrey 30-2 SITA Standard program, with losses of fixation, rate of false positives and false negatives that must be <20%). The main indexes of the Humphrey perimetry are: Mean Deviation (MD) and Pattern Standard Deviation (PSD). The MD establishes the average of the defect obtained at all the points tested, considering the increasing dispersion of sensitivity values compared to the data obtained in normal persons based on the eccentricity and thus able to represent a severity index of the global damage. PSD indicates the homogeneity of the distribution of the visual field defect and therefore provides information on local damage.

### **5.5.2. Electrophysiological Evaluations**

#### **a) Pattern electroretinogram (PERG)**

The PERG shows the bioelectric activity of the innermost layers of the retina (ganglion cells, their fibres). PERG response is characterised by a series of waves with three successive peaks, with polarity negative, positive and negative respectively. In normal people, these peaks have the following implicit times: 35, 50 and 95 msec (N35, P50 and N95). An increase in the N35, P50 and N95 times and a decrease in amplitude N35-P50 and P50-N95 was found in glaucoma patients. These abnormalities in PERG are significantly related to visual field defects and to a reduction in the thickness of the layer of nerve fibres.

Also, PERG has shown a high clinical capacity (sensitivity: 100%, specificity: 100%) to detect malfunctions of the ganglion cells in glaucoma patients (Parisi et al. 2006).

#### **b) Visual evoked potentials (VEP)**

VEPs reflect the bioelectric activity of the visual cortex in response to visual stimulation and thus evaluate the function of the visual pathway as a whole. VEP response is characterised by a series of waves with three successive peaks, with polarity negative, positive and negative respectively. In normal people, these peaks have the following times: 75, 100 and 145 msec (N75, P100 and N145). An increase in the N75, P100 and N145 times and a decrease in amplitude N75-P100 and P100-N145 was found in glaucoma patients. These VEP abnormalities are significantly correlated with visual field defects. Also, VEP has shown a high clinical capacity (sensitivity: 100%, specificity: 100%) to detect a delay in the neural conduction along the visual pathways in patients with glaucoma (Parisi et al. 2006). Comparing VEP and PERG times allows for the obtaining a neural conduction index in the postretinal visual pathways. In fact, the difference between the VEP P100 and P50 times is known as "Retino-cortical Time (RCT)".

#### **5.5.3. Morphological Evaluation**

##### **Optical coherence tomography (OCT))**

OCT is a non invasive imaging technique that allows for the obtaining of high-resolution images in section of the retina and its individual layers and the optic nerve, using light radiation as an energy source. Recently a new generation of OCT has been developed and introduced on the market, a high-definition Spectral Domain OCT (HD-OCT), which in comparison to the previous version allows a faster capture rate of the image and a higher spatial resolution.

#### **5.6 Efficiency parameters**

- For the VA examination, the visual acuity values will be recorded for distance and near vision corrected with the best optical correction.
- For the visual field examination, the following parameters will be recorded: mean deviation (MD), pattern standard deviation (PSD), number of points, with  $p < 5\%$  and  $< 1\%$  in the pattern deviation map and the total deviation map.
- Each PERG examination will record the following parameters: latency P50 and amplitude P50-N95.

- The following parameters will be recorded for each VEP test: latency N75 and P100, amplitude N75-P100 and P100-N145
- For each OCT imaging session the following parameters will be recorded: the average thickness of the retinal nerve fibre layer and the thickness divided according to sectors.
- At baseline and at each control visit (6 and 9 months) a complete eye examination will be performed including the following assessments: corrected visual acuity, biomicroscopy, indirect ophthalmoscopy, measurement of intraocular pressure with Goldmann applanation tonometer, field of vision testing, recording of PERG and VEP, imaging of the optic nerve and retinal nerve fibre layer with OCT.

## **6. ASSIGNMENT AND MASKING**

Patients will be selected from a large cohort of known NAION patients who have been attending our facility for at least 12 months (approximately 125 patients). Only patients who meet the inclusion criteria will be selected. Patients will perform a baseline screening visit during which the conditions corresponding to the inclusion criteria will be evaluated and the study proposed. Only after signing the informed consent, will a single investigator ("recruiting doctor"), not involved in the clinical and instrumental assessments, decide whether to assign the selected patient to the Citicoline-treated or non-Citicoline-treated group.

The assignment to either group will take place in sequential order of presentation of the patient (with a 1:1 probability of being assigned to one or the other group) in order to constitute two homogeneous groups in terms of number.

The aforementioned assignment will be known only to the patient and the "recruiting doctor" who will not be involved in the clinical and instrumental assessments envisaged by the research and will therefore be the only investigating doctor to know the group to which the selected patient belongs; this feature of the study will be used to ensure "objectivity" of the clinical and instrumental assessments and to prevent them from being influenced in any way by the knowledge that the patient examined has or has not been treated with Citicoline. In this regard, it should be noted that the patient, by signing the informed consent, promises not to provide any type of information regarding the group to which he/she belongs and/or any treatment administered to other staff of the Institute involved in the clinical and instrumental assessments apart from the "recruiting doctor".

As a result, throughout the duration of the study (both at baseline and at the 6 and 9 month visits),

all study assessments (AV, CV, VEP, PERG, OCT) will be conducted by investigators who do not know if the patient being examined belongs to the Citicoline-treated or non-Citicoline-treated group.

## **7. TREATMENT OF SUBJECTS**

The active form of the food supplement in the study will be manufactured according to Good Manufacturing Principles, GMP and labelled according to the applicable regulatory provisions.

### **7.1 Dosage, posology and route of administration**

During the period of randomized treatment, patients will take the CDP-choline food supplement (Cytidine Diphosphocholine or Citicoline); water; fructose; methylparahydroxybenzoate; potassium sorbate; citric acid. This supplement contains Citicoline in the amount of 500 mg per 10 ml of solution. The supplement is available in this dosage in solution only and is administered in 10 ml vials under the trade name Neukron Oftamese. The treatment dose is 1 vial per day to be taken preferably in the morning for 6 months followed by a period of suspension of 3 months.

### **7.2 Composition of the treatment in use**

The food supplement (Neukron Ofta mese) of the study is produced by Omikron Italia Srl.

Each vial of oral supplement will contain:

10 ml oral solution for use containing:

-CDP-choline (Cytidine Diphosphocholine or Citicoline) 500 mg containing an equivalent of 102 mg of Choline

-Excipients: Osmotised water, Fructose, Potassium sorbate, Citric acid, Methylparahydroxybenzoate

The vials used for the study will be identified by batch number and expiry date.

### **7.3 Packing of integrator supplement**

The samples of Neukron Ofta@nese required for the conducting of the study will be provided free of charge by Omikron Italy.

### **7.4 Preservation method for integrator supplement**

The Investigator must store the samples of food supplement at appropriate temperature and humidity conditions as indicated on the label, in the Institution's internal Pharmacy or in his/her department in a locked room, accessible only to persons authorised to withdraw the samples.

### **7.5 Drug accountability**

The Investigator and the Pharmacy will be responsible for receiving, storing and consigning the food supplement to patients; the Investigator will keep an inventory of the amounts stored and of those returned by individual patients.

### **7.6 Preservation of materials**

The vials of food supplement will be sent to the Pharmacy of the Study Centre and must be stored at the Pharmacy of the Study Centre in a locked area, with access limited to the personnel involved only, out of reach of children, at appropriate temperature and humidity conditions and kept in the boxes provided until used to protect it from exposure to light.

### **7.7 Concomitant Pharmacological Treatments**

Information on concomitant pharmacological treatments includes information on the treatments administered at the time of being included in the study (recorded in the “Ongoing systemic therapies” section of the CRF).

Any pharmacological treatment taken for diseases unrelated to that of the study and considered necessary for the patient’s health which does not interfere with the food supplement of the study, may be administered as deemed fit by the Investigator and the GP.

All treatments should be recorded in the CRF, indicating the dosage and date of administration as required.

In addition, if, due to the onset of symptoms and/or signs after commencing administration of the food supplement, the administration of any type of medication should become necessary, this should be reported in the “Ongoing systemic therapies” section of the CRF.

## **7.8 Accepted Therapies**

During the experimental treatment period, any medications considered essential for the treatment of any concomitant diseases may be administered.

Any changes in dosage and/or new concomitant therapies will only be introduced if strictly necessary, and must be reported on the Patient's Medical Record and the study CRF.

## **7.9 Non-accepted therapies**

The administration of medications that may interfere with the assessment of oral Citicoline supplements or which could alter the evaluation of efficacy parameters, and in particular medications with potential effects on retinal or macular function or nerve conduction along the optic pathways, such as:

- cerebral vasoactives
- neurotrophics
- lutein, zeaxanthin, retinals
- docosahexaenoic acid
- ubiquinone and/or its derivatives, may not be administered.

Any previous treatment with Ubiquinone, L-Carnitine, Citicoline and/or its derivatives in other formulations and/or dosages must have been discontinued at least 6 months prior to inclusion in the study.

## **7.10 Compliance with treatment**

The importance of taking the Citicoline supplement on a regular basis should be emphasised by the Investigator to the patient, who, pursuant to signing the informed consent, must also be instructed to return unused and/or excess samples (vials) at follow-up visits to the visit at which they were consigned, in line with the study timing.

The Investigator can thus compile, both during the control visits and at the end of the study, the section of the CRF in which compliance with the prescription is recorded, calculate compliance with the treatment and provide for the storage of the returned material in a place accessible to authorised persons only.

Compliance assessment will be carried out by counting the dosage units returned by the patient as unused (vials not taken) and calculating the ratio between the number of vials taken and the

number of vials that should have been taken according to the dosage schedule envisaged by the protocol.

Depending on compliance, all patients who have taken at least 80% of the planned dose regimen will be considered compliant with treatment.

### **7.11 Suspension/interruption of treatment**

The food supplement may be discontinued spontaneously by the patient at any time he/she deems appropriate or upon decision of the Investigator (the patient will be considered “drop-out”) should his/her clinical conditions require. Any discontinuation must be fully documented in the CRF by the Investigator.

Treatment may be discontinued prematurely for the following reasons:

- refusal of the patient to continue treatment;
- the occurrence of an Incident that may interfere with the patient's assessment or cause the continuation of the study to be considered inappropriate;
- logistical variations that make it impossible for the patient to participate in the study.

It will be the Investigator's responsibility to follow-up patients after discontinuation for an appropriate period of time (30 days) to assess their clinical conditions and/or verify the occurrence of Incidents even after the end of the trial therapy.

It is also recommended that these patients carry out the check-ups envisaged for the end of the study, since they will equally be considered for the intention-to-treat analysis.

For each of the patients who drops out of the trial, all available documentation will however be collected, where clinically possible, until they leave the study.



## **8. ASSESSMENT OF SAFETY AND TOLERABILITY**

### **8.1 Safety and Tolerability**

Neukron Ofta® mese, used in the present study, is a Citicoline-based food supplement.

The tolerability of Citicoline by the human body is well known since it is an endogenous substance, and has so far been administered intravenously, intramuscularly, orally and topically (Secades 2001). No adverse events attributable to the administration of such Citicoline-based supplement have been reported to date.

The only warnings are not to take Neukron Ofta® mese during pregnancy and lactation, as the possible effects on the fetus and/or newborn are not fully known.

For this reason, pregnant or breast-feeding women cannot participate in the study; at the same time, women of childbearing age must agree to the use of adequate contraception methods throughout the duration of treatment with Citicoline envisaged by the study; for the safety of patients it should be emphasised that scientifically accepted birth control methods do not provide absolute protection: some women have become pregnant even with the regular use of these types of methods.

However, despite our experience and that of others (Parisi et al. 1999, Porciatti et al. 1998, Campos et al. 1995, Campos 1997, Virno et al. 2000, Rejdak et al. 2003 leading us to assume that no patient should suffer adverse events, throughout the duration of the study, particular attention will be paid to patients' descriptions of their general conditions (e.g. increase/decrease in systemic blood pressure, gastric pyrosis, skin rashes, etc...)

The safety and tolerability of the food supplement will be evaluated at the end of the study, analysing all the information collected.

All this information will be recorded on the appropriate CRF, at the times and in the manner described in the dedicated sections of this protocol.

### **8.2 Incidents or Near-Incidents**

Clinical tolerability to treatment will be assessed by recording the occurrence of incidents or near-Incidents reported by the Patient or observed by the Investigator. This registration will be made from the moment the patient signs the informed consent.

All Incidents occurring during the clinical trial must be reported on the CRF.

### **8.2.1 Definitions**

#### **a) Incident**

Also taking into account the European guideline on vigilance "incident" means the condition in which any malfunction or deterioration in the characteristics and/or performance, as well as any inadequacy in the labelling or the instructions for use of a food supplement has led, directly or indirectly, to the death of a patient or user or to a serious deterioration in their state of health.

Serious deterioration of state of health means: a life-threatening illness or injury; an impairment of a bodily function or injury to a body structure; a condition that requires medical or surgical intervention to prevent an impairment of a bodily function or injury to a body structure; a condition that causes hospitalization or the extension of hospitalization.

#### **b) Near-Incident**

A near-incident means: (a) the condition in which any malfunction or deterioration in the characteristics or performance, as well as any inadequacy in the labelling or instructions for use of a food supplement, could have caused, directly or indirectly, if the food supplement had been used, a serious deterioration of the state of health or death of the patient or user;

(b) the condition in which any malfunction or deterioration in the characteristics or performance, as well as any inadequacy in the labelling or instructions for use of a food supplement, could have caused, during use or as a result of use, a serious deterioration in the state of health or death of the patient or a user, had the health care professional not intervened.

### **8.2.2 Incident or near-incident reporting**

Investigators involved in the clinical trial are required to complete the standard section for gathering information on Incidents or near-Incidents in the CRF of the study.

#### **a) Methods of communicating the Incident or Near-Incident**

Upon the occurrence of an incident or near-incident, the Investigator must complete the CRF page for reporting all Incidents and near-incidents and the appropriate form (Incident or near-incident report by health care professionals to the Ministry of Health) relative to the reporting of Incidents caused by taking food supplements.

This Form, accompanied by a duly compiled submission form, must be sent by fax (064424800) within 24 hours of knowledge of the incident to the

Hospital Department of Visual Neurophysiology and Neurophthalmology, Bietti-IRCCS

Foundation, which will directly inform the persons responsible for monitoring medical devices of the Ministry of Health.

**b) Further Investigations**

The Investigator and the other persons responsible for Patient health must conduct any further investigation of Incidents or Near-Incidents, based on their clinical judgement of the likely causal factors.

**c) Regulatory aspects**

The Bietti-IRCCS Foundation is responsible for reporting to the competent Health Authorities any information relating to the safety of its medications. It is therefore essential for the Investigator to promptly report any Incident or Near-Incident, in order to allow it to comply with such requirements.

These responsibilities are accepted by the Investigator as per the conditions set out in this protocol.

**d) Instructions for Compiling the Forms Provided**

For the compilation of the CRF page as well as the incident or near-incident report by health care professionals to the Ministry of Health, the Investigator should refer to the instructions provided to him/her together with such forms.

**8.2.3 Overdose**

Cases of overdose (accidental or intentional) leading to Incidents or near- incidents should be managed according to emergency procedures. This includes reports of food supplement intake for suicidal purposes with consequent overdose of such food supplement.

Reports of overdose not associated with incidents should also be reported to the

**Hospital Department of Visual Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation,**

(although not requiring notification to Regulatory Authorities), in order to obtain information on symptoms, corrective treatment and the outcome of such an overdose.

## 9. STATISTICAL ANALYSIS

### 9.1 Sample size

The sample size is estimated based on the primary objective: 'assessment of stabilisation and/or improvement of visual acuity' (see section 2.1.).

This calculation is based on known preliminary data (Parisi et al, 2008), in which visual acuity was assessed through Early Treatment Diabetic Retinopathy Study (ETDRS) cards and the acquired value has been expressed in LogMAR (logarithm of the minimum angle of resolution).

Based on these known preliminary data (Parisi et al, 2008), and considering a power value = 90% ( $\alpha = 5\%$ ) it is deemed reasonable to include in the study  $N = 5$  observations (eyes tested) per group.

The sample size has been calculated based on the difference in visual acuity measured in patients with NAION after 60 days of treatment with Citicoline in oral suspension compared to the baseline of 0.35 LogMAR (baseline visual acuity: mean 0.621 LogMAR, a standard deviation: 0.122 LogMAR; visual acuity at 60 days: mean 0.271 LogMAR, a standard deviation: 0.107 LogMAR ).

However, based on the secondary objective-point 2 (see section 2.2) 'to determine in patients with NAION the potential for neuroenhancement related to the administration of Citicoline oral solution. This will be achieved through functional condition assessments (PERG/VEP) measured after treatment with Citicoline compared to the condition at the time of recruitment', it is considered appropriate to assess the sample size taking into account the known preliminary data (Parisi et al, 2008) in which they were analysed:

a) changes in the functionality of retinal ganglion cells by measuring the Amplitude P50-N95 (measured in microvolts) obtained by recording the ERG from Pattern (PERG).

b) changes in optic nerve function by measuring Implicit Time P100 (measured in milliseconds, ms) obtained by recording Visual Evoked Potential (VEP).

Based on known preliminary data (Parisi et al, 2008), considering the P50-N95 amplitude of the PERG and a power value = 90% ( $\alpha = 5\%$ ) it is reasonable to include in study  $N = 12$  observations (eyes tested) per group. The sample number has been calculated based on the difference in the P50-N95 amplitude of the PERG measured after 60 days of treatment with Citicoline for oral suspension compared to the baseline of 0.38 microvolts (Baseline P50-N95: mean 0.92 microvolts, a standard deviation: 0.29 microvolts; P50-N95 at 60 days: mean 1.30 microvolts, a standard deviation: 0.25 microvolts).

Based on known preliminary data (Parisi et al, 2008), considering the implicit time P100 of the VEP and a power value = 90% ( $\alpha = 5\%$ ) it is deemed reasonable to include in the study N = 11 observations (eyes tested) per group. The sample number has been calculated based on the difference in the implicit time P100 of the VEP measured after 60 days of treatment with Citicoline for oral suspension compared to the baseline of 15 ms (Baseline P100: mean 141 ms, a standard deviation; P100 at 60 days: mean 126 ms, a standard deviation: 9.8 ms).

To summarise, the size from the sample estimated on the basis of the primary objective (visual acuity) is N= 5; the size from the sample estimated on the basis of secondary objectives (PERG and VEP) is N=12 and N=11, respectively.

Considering the larger sample size (N=12, PERG), and assuming the presence of outliers and the potential voluntary withdrawal from the study by patients, the sample is increased by 30%, so it is considered appropriate to reach N=16 observations (eyes tested) per group (NAION Treated with Citicoline and NAION not treated).

In this calculation of the sample, it is not possible to estimate a priori the sample number relative to the secondary objective – point 3 (see Point 2.2), as data in the literature are not currently available regarding possible changes in the structure of nerve fibres and the optic nerve (assessed by OCT) following treatment with Citicoline.

The calculation procedure was carried out using the Minitab v.12.7 and Gpower 3.1 programmes, assuming that the averages and standard deviations of all the parameters considered come from two independent samples: Patients with NAION in baseline condition and patients with NAION after 60 days of treatment with Citicoline in oral suspension (Parisi et al, 2008).

## **9.2 General methodology**

Data from repetitions of visual acuity tests, PERG, VEP, OCT will be expressed as the average difference between two recordings obtained during separate sessions + / - the standard deviation of this difference. The 95% confidence limits of the test-retest variability will be established by assuming a normal distribution of the data, after verification using the Anderson-Darling and Kolmogorov-Smirnov tests, calculating the average of the differences and the relative standard deviations.

For each group (NAION Control eyes and NAION eyes Treated with Citicoline), differences in visual acuity values, PERG and VEP and OCT responses measured at 6 and 9 months from

baseline will be assessed using ANOVA and the  $p < 0.05$  value will be considered significant. Multiple comparisons will be made using the Tukey and Games-Howell methods, establishing the value of the error rates at 5%.

Correlations between the electric-physiological measurements (PERG/VEP) and field of view measurements, normalized in logarithmic value, both at Baseline and at each follow-up time, for each group will be performed using Pearson's test and the  $p < 0.05$  value will be considered significant.

## **10. EXPECTED RESULTS AND NEUROFUNCTIONAL CONSIDERATIONS**

### **10.1 Expected results and their impact in clinical practice**

Reducing the loss of visual acuity achieved through stabilization or improvement of optic nerve and retinal function could ultimately lead to a reduction in visual disability secondary to non-arteritic ischaemic neuropathy, with a significant reduction in related costs for the National Health Service.

In NAION patients treated with Citicoline oral solution, one of the following conditions may be observed at the end of follow-up:

- 1) further loss of retinal nerve fibres (OCT with reduced RNFL thickness) and functional reduction of RGCs (PERG reduced in amplitude) and of the Optic nerve (increase in implicit time P100 of VEP);
- 2) further loss of retinal nerve fibres (OCT with reduced RNFL thickness) and functional maintenance of RGC (PERG with unchanged amplitude) and Optic nerve (implicit time P100 of the VEP unchanged);
- 3) maintenance of retinal nerve fibres (OCT with unchanged RNFL thickness) and functional maintenance of RGC (PERG with unchanged amplitude) and Optic nerve (implicit time P100 of the VEP unchanged);
- 4) maintenance of retinal nerve fibres (OCT with unchanged RNFL thickness) and functional increase of RGC (PERG with increased amplitude) and optic nerve (implicit time P100 of VEP reduced);
- 5) improvement of retinal nerve fibres (OCT with increased RNFL thickness) and functional increase of RGC (PERG with increased amplitude) and Optic nerve (implicit time P100 of

VEP reduced);

The results of NAION patients treated with Citicoline for oral suspension will be compared with those of NAION patients given no treatment to assess the natural evolution of the morpho-functional condition. The following conditions may occur in such patients:

- 1) further loss of retinal nerve fibres (OCT with reduced RNFL thickness) and functional reduction of RGCs (PERG reduced in amplitude) and of the Optic nerve (increase in implicit time P100 of VEP);
- 2) further loss of retinal nerve fibres (OCT with reduced RNFL thickness) and functional maintenance of RGC (PERG with unchanged amplitude) and Optic nerve (implicit time P100 of the VEP unchanged);
- 3) maintenance of retinal nerve fibres (OCT with unchanged RNFL thickness) and functional maintenance of RGC (PERG with unchanged amplitude) and Optic nerve (implicit time P100 of the VEP unchanged);

## **10.2 Neuro-functional considerations**

Whatever the result obtained (see points 1-5 of the expected results), the neurodegeneration model used by us (NAION) will provide important information on the potential of neuroenhancement and neuroprotection of Citicoline, i.e. the therapeutic opportunity to increase the function of RGCs and of the optic nerve in the presence or absence of structural changes. These results may open up new neuropathophysiological considerations regarding degenerative processes that may be present, with different etiologies, in the various diseases that afflict the optic nerve with consequent significant reduction in visual capacity.

## **11. DIRECT ACCESS TO ORIGINAL DOCUMENTS**

The Investigator/Institution must allow national and foreign Regulatory Authorities and personnel designated by the Independent Ethics Committee direct access, and verification thereof, to all original study documentation, including Informed Consent forms signed by the subjects in the study or their Legally Recognized Representatives and hospital records and/or outpatient records. Those who have direct access to such documentation must take all reasonable precautions to keep the identity of the subjects confidential in compliance with applicable regulatory provisions.



## **12. QUALITY CONTROL AND ASSURANCE PROCEDURES**

### **12.1 Data collection or Case Report Form**

The Case Report Form (CRF) is the hard copy document that is managed by the Investigator  
**Hospital Department of Visual Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation.**

#### **a) Presentation of the CRF**

The CRF consists of numbered pages which show in sequence the information required at each visit/assessment of the Patient.

Each individual page of the CRF will be identified by the centre no., Patient initials, Patient screening number, randomization number, trial time and date of the visit.

#### **b) Instructions for compiling the CRF**

The CRF must be compiled in Italian, with legible handwriting, in capital letters, using a black ink ballpoint pen. Each individual page must be compiled immediately after the assessments to which it refers, and must be signed by the Responsible Investigator or other qualified person duly authorized, immediately after checking the data transcribed. CRFs should remain available for monitoring and for any audit and/or inspection visits.

If a determination is not available, the corresponding space for its registration must not be left blank, but the wording NA ("Not Available") must be reported; in the case of tests not performed, the corresponding space for registration must not be left blank, but the wording NP ("Not Performed") must be written.

Any correction must be made cancelling the wrong data by crossing it out and entering the correct data near it. Deleted or corrected data should not be erased, masked or corrected, but should remain legible. The correction made must be initialled and dated by the Investigator or by a qualified person designated by him, possibly justifying with a comment, if necessary, the appropriateness of the same.

The copy of each page will remain at the Study Centre as documentation of the clinical case. It will be the responsibility of the Investigator to report on the appropriate form the normal ranges of laboratory parameters provided by the analysis laboratory.

## **12.2 Clinical Monitoring**

The clinical monitoring activities of the study will be entrusted to the **Hospital Department of Visual Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation,**

Monitoring activities will be conducted in accordance with the Guidelines of the European Union of Good Clinical Practice adopted by Ministerial Decree of 15 July 1997.

## **12.3 Audits**

The Investigator/Institution must allow audits to be carried out as an integral part of the quality assurance system. The Audit is an independent inspection, separate from the monitoring of the study activities and documents to verify whether the relevant study activities have been conducted and whether the data has been recorded, analysed and transmitted in accordance with the protocol, the GCP, SOPs and applicable regulatory provisions.

## **12.4 Inspections**

The Investigator/Institution must allow national and foreign Regulatory Authorities to carry out Inspections.

The Inspection, by one or more Regulatory Authorities, shall consist of the official review of documents, facilities, records and any other resources considered by such authorities as related to the clinical trial.

## **13. CLINICAL DATA MANAGEMENT**

The management of clinical data is entrusted to the

**Hospital Department of Visual Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation.**

All statistical analyses will be carried out by personnel of the Bietti Foundation using the SAS software, version 9.2 (SAS Institute, Cary, NC, USA).

## **14. ETHICS CONSIDERATIONS**

All the parties involved in the study agree and will make sure that this trial is conducted in accordance with the ethical principles deriving from the Declaration of Helsinki, Good Clinical Practice (GCP) guidelines and applicable regulatory provisions.

### **14.1 Ethical Authorisations**

A clinical study may only be started after written approval from the Independent Ethics Committee (IEC), which the Study Centre reports to. The Investigator must then receive the positive opinion of the IEC of the facility where the trial will be conducted before starting to recruit subjects for the study. The Investigator must provide the IEC with all the documents required for the approval application.

A copy of the IEC approval must be available at the Scientific Directorate of the Bietti Foundation before commencing the study.

This study must be submitted for the authorisation of the Local Regulatory and Health Authorities in accordance with the rules and laws currently in force. The study may not commence without first obtaining a copy of the study authorisation document from the Local Regulatory and Health Authorities, as required by the applicable regulatory provisions. This documentation must be available prior to providing the Citicoline supplement samples for the study and starting recruitment.

### **14.2 Informed Consent**

Before commencing the study, the Written Information and the Informed Consent form to be provided to subjects must be submitted for review and approval by the Independent Ethics Committee, together with the protocol.

Informed Consent must be requested, obtained and documented by the Investigator in compliance with applicable regulatory provisions, GCP and ethical principles deriving from the Helsinki Declaration.

For details on the procedure for requesting and obtaining Informed Consent, please refer to ICH-GCP E6, paragraphs 4.3.3, 4.3.4, 4.8.2, 4.8.3, 4.8.4, 4.8.5, 4.8.6, 4.8.7, 4.8.8, 4.8.11, 8.3.2, 8.3.11, and the procedure for requesting Informed Consent in special situations the following paragraphs also 4.8.9, 4.8.12, 4.8.13, 4.8.14, 4.8.15.

## **15. ADMINISTRATIVE PROCEDURES**

### **15.1 Changes in conducting the study or planned analysis**

Any change in the conducting of the study is defined as an “Amendment to the Clinical Protocol”: this term means any change that is made to the experimental protocol after the final approval of the Investigator.

Amendments to the Protocol are amendments to a document which has legal weight and which as such must be approved and signed in duplicate in original by the signatories to the protocol.

All amendments must be submitted to the Ethics Committee which approved the study protocol before they can be applied.

It is the responsibility of the Investigator to submit the amendment to the relevant Independent Ethics Committee and obtain an approval document.

Written approval will be distributed and filed in the manner provided for by the study protocol.

In the event that the change concerns only changes to administrative or logistical aspects of the trial, the resulting amendment must simply be notified to the Ethics Committee.

Finally, it should be noted that where the amendment substantially alters the design of the study or the potential risks to which the Patient is exposed, each Patient must be informed and must confirm in writing his/her willingness to continue the study. A specific information and consent form will be prepared by the Investigator and approved by the Ethics Committee.

### **15.2 Suspension /interruption of the study**

#### **15.2.1 Complete suspension of the study**

The Bietti Foundation may decide to suspend/discontinue the study in progress at the Study Centre if:

- new toxicological, pharmacological or clinical information make the rationale and experimental design of the study unacceptable;
- the rate of recruitment of Patients proves ineffective;
- the Centre does not comply with the specific requirements of the study protocol, especially regarding the assessment of the inclusion/exclusion criteria of the Patients;
- the Centre is unable to comply with the requirements of the Good Clinical Practice guidelines (GCP-ICH, Ministerial Decree no. 122 of 28/05/98);

- the Centre is not able to apply the regulations in force.

In this case, the Bietti Foundation shall promptly inform the Investigator / Institution and Regulatory Authorities of the premature discontinuation of the study, justifying the decision taken. The Investigator must also inform the relevant Ethics Committee of the Institution to which the Study Centre belongs, justifying the premature discontinuation of the study.

#### **15.2.2 Suspension of the study by the Ethics Committee**

The study may also be discontinued by the Ethics Committee of the health care facility which the Investigator of the Study Centre reports to.

#### **15.3 Archiving**

The Investigator/Institution must file the essential study documents as specified by the GCP and in accordance with applicable regulatory provisions. The Investigator/Institution must take the necessary measures to prevent accidental or premature destruction of the same.

The Investigator/Institution shall keep specific essential documents for at least 2 years.

#### **15.4 Use of information and publication of results**

The Investigator acknowledges that all information not made public regarding the study product (indication, patents, chemical formula, synthesis and formulation processes, experimental data or other information) is the property of:

Hospital Department of Neurophysiology and Neurophthalmology, Bietti-IRCCS Foundation:

Scientific Director: Dr. Vincenzo Parisi

and are strictly confidential.

The Investigator may only use this information for the purpose of performing the research. If the Investigator intends to disclose, even partially, the results of the study, without prejudice to reports of Incidents or near-incidents provided for by current legislation on Pharmacovigilance, s/he must notify the Coordinating Centre (Bietti-IRCCS Foundation) in advance, which must respond to the Investigator within two months from the date of receipt of the publication request.

The Coordinating Centre (Bietti-IRCCS Foundation), in agreement with the Scientific Directors of all the Hospital departments involved in the project, will use the data deriving from the clinical study in connection with the development of the constituents of the food supplement and,

therefore, may transmit this information, if necessary, to other Investigators and to the competent Authorities.

### **15.5 Liability insurance cover**

This study is covered by a valid insurance policy and third party liability, stipulated by the Coordinating Centre (Fondazione Bietti), which has undertaken to pay the sums due from the Policy holder, as civilly liable by law, as compensation (capital, interest, expenses) for any type of damage caused by medicinal products, registered or not, administered in hospitals, nursing homes and by health care professionals for clinical trials, as well as for damages caused as a result of administration for pharmacology research and trials with medications and compounds already registered, but with a dosage different from that indicated by the Manufacturers, or with medications in the study phase, as well as for all activities related or connected with such trials, such as the administration of medications and taking of blood samples from the subjects participating in the study.

This guarantee is also valid:

- For liabilities which may derive to the Investigators, as a result of the trials carried out on request and/or on behalf of the Insured;
- For any liability for which the Insured is called upon to respond by law, regulation, internal rules, customs, or uses.

The following shall be excluded:

- Impairment of health or deterioration of the state of health which would have occurred even in the absence of the trial;
- Genetic impairment, consisting of DNA damage, specifically related to damage to somatocytes and germ cells (egg cells, sperm cells);
- Impairment of health resulting from voluntary non-compliance with prescriptions/instructions;
- Impairment related to the AIDS virus: HIV, all AIDS syndromes, those related to HIV ARC viruses, as well as all the consequences attributable to feared or suspected infection.

## **15.6 Trial financing**

As a non-profit study, there is no funding for the trial. The various expenses (e.g. electrodes for the registration of VEP and PERG and other consumables) will be paid for by the Bietti Foundation.



## **16. INVESTIGATOR'S RESPONSIBILITIES**

The Investigator is aware that s/he is responsible for all actions delegated by him/her to the other members of his/her staff designated to conduct the study. Except where specifically required, the term “Investigator” used in this protocol and on the Case Report Forms refers to the Investigator or a qualified person designated by her/him who may thus perform activities related to the clinical trial and sign on his/her behalf the study documents.

The Investigator is required to conduct the study in accordance with the study protocol and in accordance with the Good Clinical Practice Standards (ICH-E6), the principles of the Helsinki Declaration (1964) and subsequent revisions and in compliance with applicable regulations.

## **17. FINAL STUDY REPORT**

At the end of the research, within 6 months of the end of the study, a Final Clinical Report of the study containing clinical comments based on data generated by statistical processing will be prepared by the Investigator and/or by a delegated person. This report will be structured as an “integrated clinical statistical report”, containing clinical comments based on data generated by the statistical report.

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