**Cone photoreceptor neuroprotection**

Calibri 11 or equivalent, single spaced, 2cm margins, numbered pages

**PDF format and cannot exceed a four-page limit.** CVs on-line.

**Preproposals must fulfil the two main evaluation criteria** : « Quality and scientific aim » and « Organisation and implementation of the project ». **Applicants are advised to consult the document** [**AAPG2019**](http://www.agence-nationale-recherche.fr/AAPG2019) **for further information about different sub-criteria related to the chosen funding instrument**.

# Pre-proposal’s context, positioning and objective(s)

**This paragraph refers to the evaluation criterion « Quality and scientific aim ». The following information should be detailed here:**

* project’s objectives and research hypothesis ;
* position of the project as it relates to the state of the art;
* methodology to reach the objectives ;
* Innovative nature of the project, ambitiousness and originality of the objectives and the methodology
* ability of the project to address the research issues covered by the chosen research theme

T**he criterion « Quality and scientific aims» will be the determining one: only the projects having received an « A » will proceed to the second step of the evaluation process.**

**Objectives and research hypothesis**

The loss of cone photoreceptors is causing blindness in diseases like age-related macular degeneration (ARMD) and retinal dystrophies (e.g. retinitis pigmentosa). **We here propose to screen neuroprotective molecules on a cell line for a subsequent validation on purified cone photoreceptors and animal models of these retinal diseases.** The screening relies on the identification of a new pathway for cone photoreceptor degeneration. This pathway is initially activated by a membrane receptor. The cell protection by an enzymatic blocker within this pathway has confirmed its induction for cone cell degeneration. This pathway was first identified in isolated cone photoreceptors and confirmed in vivo. Its presence in a cell line provides an adequate model for the rapid screening of molecules. Our first objective is therefore to **screen efficient receptor antagonists or improve the affinity of our known enzymatic blocker** on the cell line with the subsequent and immediate validation on isolated cone photoreceptors. The second objective is to **demonstrate that the identified pathway is involved in vivo in cone photoreceptor degeneration using animal models of the diseases.** This objective will enable to validate the in vivo efficacy of our identified drugs. The third objective is to activate this pathway in living primates to provide an animal model of cone degeneration and macular dystrophy. To achieve these objectives, Serge Picaud, a specialist of retinal physiopathology at the Vision Institute, is teaming up with Dr Christophe Rochais and Pr Patrick Dallemagne (Dean), who have an established record in drug development at the CERMN, where further an important chemolibray (19,000 original compounds) is available.

**State of the art**

In ARMD, the molecular mechanisms leading to cone photoreceptor degeneration remain very enigmatic. Risk factors of the disease include tobacco, light and genes. The two first risks underline the importance of oxidative stress in the physiopathology of the disease (Hernandez-Zimbron *et al.*, 2018). Polymorphisms in the complement factor H have indicated that the immune response is likely to increase the severity of the disease (Calippe *et al.*, 2017). **Currently, there is no treatment to limit the degeneration of photoreceptor in ARMD except for the supplementation of macular pigments** and treatment to suppress neovascularization (Hernandez-Zimbron *et al.*, 2018). These macular pigments can limit blue light exposure due to their blue light absorption and further reduce oxidative stress by their antioxidant properties. If blue light damage in ARMD is often attributed to A2E photosensitization of retinal pigment epithelium, we recently found that cone photoreceptors are even more sensitive to blue light than A2E-loaded RPE cells. These results suggest further that photoreceptors could be the first damaged cells in ARMD.

In retinal dystrophies like retinitis pigmentosa, photoreceptor degeneration often relies on a mutation in genes solely express in rod photoreceptors. However, the initial loss of rod photoreceptors is followed by a secondary cone photoreceptor degeneration. This secondary degeneration of cone photoreceptors is attributed to the loss of the Rod-derived Cone Viability factors (Leveillard *et al.*, 2004; Ait-Ali *et al.*, 2015; Byrne *et al.*, 2015). Suppression of RdCVF increases the susceptibility to oxidative stress in photoreceptors (Cronin *et al.*, 2010) which is consistent with the reported importance of oxidative stress during cone degeneration (Campochiaro & Mir, 2018). If RdCVF gene therapy is likely to enter into clinical trials in the coming years (Byrne *et al.*, 2015), there are still no treatment to rescue cone photoreceptors in retinal dystrophies.

In this project, **we want to develop a treatment targeting cone photoreceptor degeneration based on our recent identification of a death pathway in cone photoreceptors**. When this pathway is expressed in cells, it has been related to oxidative stress underlying its importance for cone photoreceptor degeneration. **The project will demonstrate the importance of this pathways in animal models of the diseases while screening for new proprietary molecules.**

**Preliminary results**

Using porcine cone photoreceptors purified by lectin-panning (Balse *et al.*, 2005), we screened for pathways inducing cell death. **This screen resulted in the identification of a new pathway inducing cone photoreceptor degeneration.** Different pharmacological agonists of a receptor induced the degeneration of isolated cone photoreceptor following 3 days in culture. Similarly, with one of the receptor agonist, we induced cone photoreceptor degeneration in porcine retinal explants, which represents a more integrated model of the retinal tissue. **The highly regular distribution of cone photoreceptors has been disrupted in treated explant**. In vivo ? Manon?? Very preliminary tests in primates also indicate a degeneration of cone photoreceptors in non-human primates. To verify the implication of the considered cell pathway, we have tested molecules known to inhibit the downstream enzymatic processes. **Application of an inhibitor on this downstream pathway suppressed very significantly cone degeneration in our purified cell model.** To increase the speed of our drug screening, we have identified a cell line, which was reported to express the same receptor pathway. Using this cell line, we confirmed that the receptor agonist is inducing cell death while the inhibitor of the downstream enzymatic step is able to prevent this cell death.

* **Methodology:**

1. **Aim 1: Production of receptor antagonists and enzymatic inhibitors**

The aim of the project is to select small original compounds preventing cone photoreceptor degeneration. Using known ligands at our identified receptor responsible for cone degeneration, **we will design antagonists. In parallel, we will also design proprietary molecules targeting the downstream enzymatic molecular target.** For these molecular production, we will use a ligand-based drug design approach for the screening of the CERMN's chemolibrary. Indeed, the later has the largest academic library in France (~ 19,000 original and druggable compounds). In addition, **we will take advantage of the CERMN's molecular modelling platform to generate an in silico screenings to produce molecules to be tested at the Paris Vision Institute.** Following the hit selection, we will generate molecules with a higher affinity and a greater drug ability. **The selection of druggable lead molecules will be achieved through a hit-to-lead chemistry approach,** carried on to optimize both the pharmacodynamic and pharmacokinetic properties of the selected compounds. Several iterations in this process are expected to generate the most interesting molecules with efficacy in vitro and in vivo.

1. **Aim 2: Screening of efficient molecules**

**A cell line expressing the same cell pathway as cone photoreceptors** was selected and subsequently validated on the drug screening platform at the Vision Institute. Therefore, this platform will be used to screen molecules selected from the ligand-based drug design approach out of the CERMN’s library. **The molecules will be tested for cell death and then for oxidative stress production**. **Molecules selected from the screen will be validated on isolated porcine cone photoreceptor in culture and on porcine retinal explants**. Purified cone photoreceptors can be kept in culture for several days or weeks. At the end of the experience (3 days in vitro), living cone photoreceptors in 96 well plates are quantified by calcein fluorescence on the whole well surface. To further confirm the neuroprotective value of the most promising molecules, they will be tested on porcine retinal explants produced as for other species (Vallazza-Deschamps *et al.*, 2005). For this preparation, cone photoreceptors are labelled by opsin immunolabeling and then quantified on the whole explant.

1. **Validation on in vivo models of retinal diseases.**

To assess the in vivo efficacy of molecules, we will quantify cone cell death on rodent models of retinal diseases treated or not. **The first model will be the rd1 retina, which represent a well-known and classic model for retinitis pigmentosa**. Mice will be daily treated from one month to three months because visual acuity measured by the optomotor response indicates a progressive decline up to 100 days. Photoreceptor neuroprotection will be assessed in vivo using OCT measurement and eye fundus imaging. Cone photoreceptor will then be counted on the isolated retina following their immunostaining for cone arrestin. **In parallel, we will measure neuroprotection on ??, a model of age-related macular degeneration???.** The evaluation will follow a similar protocol as for rd1 mice. This third aim will start by evaluating our first active molecule, the enzymatic inhibitor downstream in the death pathway, to subsequently assess the different highly efficient molecules issued from the screen.

1. **Production of non-human primate model of maculopathy**

To define if the cell death pathway is also expressed in humans, we will assess if the receptor agonist can induce photoreceptor degeneration in non-human primates. Preliminary injections indicated a thinning of the photoreceptor layer as indicated by OCT and a degeneration process on the isolated fixed retina immunolabeled for cone photoreceptors. Our objective will be to inject **the agonist in the macular area and follow the degenerative process in a longitudinal study on 3 non-human primates lasting 3 months**. Controls animals will be injected in parallel with the same vehicle solution in the macular area. Cone photoreceptor degeneration will be documented in vivo by OCT/SLO imaging and multifocal electroretinogram. Then the fixed retina will be immunlabelled for cone photoreceptors using cone arrestin and opsin antibodies while microglial cells will be revealed by iba1 immunostaining.

* **Innovative nature of the project, ambitiousness and originality**

The ambitiousness relies on the objective, **prevent blindness**, and the **very large populations with the targeted retinal diseases such as age-related macular degeneration (ARMD, more than a million in France**) and rare retinal dystrophies. The treatment would be suited for all patients with ARMD because it aims at preventing cone photoreceptor loss. For rare diseases, the patient population is clearly more limited but patients may have to take the treatment from very early age.

**The innovative aspect of the project relies on the identification of a cell death pathway in cone photoreceptor**s thanks to a unique preparation of isolated cone photoreceptors. Living cells in this preparation are cone photoreceptors at a 98% density, the applicants have also identified a cell line expressing the same pathway. No publication has reported this pathway in cone photoreceptors. Therefore, **production of proprietary molecules should generate intellectual property for the prevention of cone photoreceptors in retinal diseases**.

* **ability of the project to address the research issues covered by the chosen research theme**

### The project aims at developing neuroprotective molecules for cone photoreceptors to prevent blindness in diseases like ARMD and retinitis pigmentosa. The consortium has developed all the required models for the screening (cell line, cone culture), for the in vivo validation on rodents and they propose a strategy for producing a non-human primate model of the disease. The consortium has also access to a very large chemical library, allowing *in silico* and/or *in vitro* screenings, in order to select, according to various target-based and ligand-base drug design approaches, some hits. The latter will be then pharmacomodulated using hit-to-lead chemistry methodologies and finally optimized to ensure their pharmacokinetic behaviour. Therefore, this project should enable to provide a proof of concept for a druggable compound. The only missing elements prior to a clinical trial would be regulatory toxicity studies that are beyond the scope of this project.

# Partenariat

* **For a collaborative research project (PRC, PRCE),** 
  + **scientific coordinator**

Serge Picaud has recently coordinated several projects including a DARPA research grant and an ERA-Net program. He has contributed to several translational projects with biotech companies leading to clinical trials such as the SightAgain project (Bank public of investment: 18M€). This project lead to a clinical trial on retinal implant (NCT03333954) and a clinical trial on optogenetic therapy (NCT03326336). His expertise in the field is recognized by more than 100 publications. He demonstrated that the excessive activation of cGMP-gated channels can induce cone photoreceptor degeneration. He showed that oxidative stress is a key factor in cone degeneration on a mouse model of Usher syndrome, a disease leading to deafness and blindness. Discovered that the retinal toxicity (cone and retinal ganglion cell degeneration) of an antiepileptic drug was consecutive to an induced depletion in retinal taurine.

Serge Picaud will be leading the project together with Valérie Fradot (IE INSERM) and Manuel Simonutti (IE INSERM), involved in cell culture and animal phenotyping, respectively. The screening on the cell line and on isolated cones will be performed on the screening platform developed at the Vision Institute by Marc Lechuga.

The automated platform is dedicated to large scaled screenings including a fast and sterile automate (Agilent customized Biocel1800), an automated microscope (Thermo-Cellomics Arrayscan) and a wide panel of standards and custom algorithms for the quantification of complex biological events, such as protein translocations or differentiations. The whole system of instruments is connected to our Laboratory Information Management System for sample traceability, robotic management and data mining. The platform is able to carry out from 85,000 (384-well plates) to 362,000 tests (1536-well plates) at the same time, in sterile conditions (transfer of compounds, transfection protocols, seeding, long term incubation, and sterile conditionning process).

* + **consortium and its complementarity** : quality and complementarity nature of the consortium to reach the objectives, identity of the scientists involved, their institution and all other information providing a framework for judging the quality and complementarity of the partners and of the effectiveness of the partnership.

The two partner teams are highly complementary with a specialist in retinal physiopathology (Serge Picaud) and medicinal chemists specialized in drug design (Dr Christophe Rochais and Pr Patrick Dallemagne). An MTA has already been signed with an agreement on shared Intellectual property to allow exchange of information on the molecular targets so that the chemical screen can already be initiated.

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