



HAL
open science

The primate model for understanding and restoring vision

Serge Picaud, Deniz Dalkara, Katia Marazova, Olivier Goureau, Botond Roska, José-Alain Sahel

► **To cite this version:**

Serge Picaud, Deniz Dalkara, Katia Marazova, Olivier Goureau, Botond Roska, et al.. The primate model for understanding and restoring vision. Proceedings of the National Academy of Sciences of the United States of America, 2019, 10.1073/pnas.1902292116 . hal-03127682

HAL Id: hal-03127682

<https://hal.science/hal-03127682v1>

Submitted on 1 Feb 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Companion article to the Sackler Colloquium, "Using Monkey Models to Understand and Treat Human Brain Disorders," to be published in PNAS.

Length: 64,000 characters with spaces. For tables and figures: subtract 2000 characters for a figure that is 1 column wide and 1/3 of page in length.

The primate model for understanding and restoring vision

Serge Picaud^{1*}, Deniz Dalkara^{1*}, Katia Marazova¹, Olivier Goureau¹, Botond Roska^{2-4§}, José-Alain Sahel^{1,5-7§}

¹ Sorbonne Université, INSERM, CNRS, Institut de la Vision, F-75012 Paris, France

² Institute of Molecular and Clinical Ophthalmology Basel, Basel, Switzerland

³ University of Basel, Basel, Switzerland

⁴ Friedrich Miescher Institute, Basel, Switzerland

⁵ Department of Ophthalmology, The University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, United States

⁶ CHNO des Quinze-Vingts, INSERM-DGOS CIC 1423, F-75012 Paris, France

⁷ Fondation Ophtalmologique Rothschild, F-75019 Paris, France

* Equal contribution

§ Co-corresponding authors

Vision: a vital sense

As part of human multisensorial interaction with the world, vision is often perceived as the dominant sensory modality. Eyes provide most of our information about the environment and efficient vision is considered to be vital to our overall health. Its loss has potentially greater effects on daily life than loss of limbs, memory, hearing or speech (1). The reduced independence associated with blindness, leading to social isolation, unemployment and depression, ranks it among the major health concerns, on a level with cancer and Alzheimer's (1). In addition to the personal impact of visual disability, the economic burden on health and social systems is significant (2).

Retinal degenerative diseases caused by photoreceptor cell death are major causes of irreversible blindness in industrialized countries. Photoreceptor cell death leading eventually to impairment of central vision occurs in monogenic diseases such as retinitis pigmentosa, Stargardt disease, and Leber congenital amaurosis, as well as in complex age-related diseases such as age-related macular degeneration (AMD). For these blinding conditions, retinal

implants and gene and cell therapy may allow therapeutic intervention at various stages of the disease (3, 4). This article emphasizes advancements in therapies for retinal degenerative disease and the contribution of the non-human primate (NHP) model to progress in the clinical translation of emerging retinal therapies.

Understanding vision

Since most visual diseases are caused by eye pathologies, the preservation of sight and the curing of blindness are intrinsically linked to understanding vision mechanisms within the eye. Fortunately, the eye has several features that facilitate investigation and experimentation, including direct optical and mechanical access, small volume, internal compartmentalization, stable cell populations, and relative immune privilege. The visual system is one of the best understood sensory systems, due at least in part to the optical transparency of the eye. This allows direct visualization and precise morphological evaluation via non-invasive high-resolution imaging. The straightforward *in vivo* and *ex vivo* functional and anatomical analyses of eye cells and tissues provide highly responsive and reliable models for in-depth functional studies. Furthermore, brain-imaging studies describing visual cortex activity aid our understanding of the visual system and its functions, and also serve as important tools for studying the brain (5–8).

Uniqueness of the primate model for human vision

An important feature of human vision, shared only by primates, is the central visual pathway starting at the macula in the center of the retina, which is an area dominated by cone photoreceptor cells (Figure 1). The distribution of intermediate neurons and of ganglion cells, as well as the large cortical representation of the central visual field, account for the important high-resolution central vision. This has strong implications for normal vision and many disease conditions.

The genus *Macaca*, as the most widespread NHP, diverged from its ape and human ancestors 25 million years ago. It has become a valuable animal model for investigating human biology and validating biomedical research. The genus diversified approximately 5 million years ago and includes over 20 species that share >90% DNA sequence similarity and highly conserved protein sequences with humans. Moreover, humans and macaques share susceptibility genes for AMD and genotype-phenotype correlations for rare forms of inherited

retinal diseases like achromatopsia (9) and retinitis pigmentosa (10). Old World monkeys such as rhesus macaques (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*) are probably the best models for the study of ocular diseases (11). However, due to the low prevalence of spontaneous disease, only a few animals are affected in even the largest research colonies, and their disease phenotypes have been long ignored (9). Reduction in the cost of DNA sequencing has revealed the amount of functional genetic variation in rhesus macaque research colonies and allowed broad surveys of genetic mutations that display a disease phenotype (12). Other NHP species, such as the squirrel monkey (*Saimiri sciureus*), have also been useful for testing experimental therapies. For example, gene therapy was tested on adult squirrel monkeys defective in the L-opsin gene (13). Although such rare inheritable genetic defects in monkeys make them an invaluable model for the development and testing of mutation-specific gene therapies, a current limitation of NHPs is the lack of specific models that fully replicate most human conditions. Most cutting-edge therapies today are directed towards late-stage rod-cone dystrophy and atrophic or wet AMD, where loss of photoreceptors has already occurred. For this, the lack of severe inherited models displaying outer retinal loss has been compensated by the generation of acute models via laser ablation (14–16), chemically induced photoreceptor ablation (16), or the separation of the outer retina in reaction to a subretinally implanted retinal prosthesis (17). Other promising vision restoration strategies such as optogenetics have been tested extensively in normal NHPs, including marmosets (18) and macaques (19, 20).

Another important advantage of NHPs for therapy development is the availability and similarity of ocular phenotyping instruments that reveal extraordinary insight into the state of the retina in living NHPs (21). Routine use is now made of optical coherence tomography (OCT) and its full-field version, as these provide transverse optical imaging of the retina and identification of the different neuronal layers (Figure 1). Directional OCT adds optical contrast to directionally reflective retinal structures and provides information about the orientation of retinal cell components *in vivo*, e.g., in the outer nuclear layer. Pivotal to these technological advances is adaptive optics (AO) that makes neuroretina structures directly observable *in vivo* in a non-invasive, safe and easily tolerated manner. Different multimodal imaging systems are under development to enhance the potential for studying individual cells in the living eye (Figure 1). These revolutionary imaging tools offer unprecedented insight into the human retina in health and disease; reviewed in (22, 23). Many psychophysical (e.g., microperimetry), electrophysiological (e.g., multifocal and full-field electroretinogram), real-life performance-based tests, and computer models can be combined with eye movement tests

Companion article to the Sackler Colloquium, "Using Monkey Models to Understand and Treat Human Brain Disorders," to be published in PNAS.

to understand cognition and behavior. OCT and adaptive optics scanning laser ophthalmoscopy can be performed *in vivo*, allowing the identification of slight structural changes in the retina, exploration of interactions between rods and cones, and measurement of responses to therapies in targeted cells (24, 25).

The primate model for understanding vision

Vision is initiated in the retina and images collected by the eye are interpreted in the brain. The functional anatomy of the visual system is the key to interpreting the complex process of “seeing” as a target for vision restoration therapies. Shared essential anatomical features and organization of the visual pathways make NHPs an ideal model to reproduce the human visual system and human eye diseases, and to study the principles of brain function. A significant body of evidence reveals features of the primate eye and brain that are distinctly different from those of other mammals, and many behavioral adaptations are consequences of high retinal acuity; reviewed in (26). Notably, a macula located temporal to the optic nerve is a distinctive attribute of the primate retina (Figure 1). Primate visual perception with the macula and the fovea, located within the macula at the optical center of the retina, is unique among mammals. Moreover, photoreceptor cell organization within the macula is similar in humans and NHPs (27), with densely packed cones in the fovea of up to 160,000-200,000 cones/mm²; cone photoreceptor packing density decreases faster with increasing eccentricity from the foveola. This photoreceptor distribution profile directly reflects the specialized functions of the macula and macular cones that ensure sharp central vision in bright light conditions and color vision. As the foveal region covers about 2° of visual angle, fast and precise saccadic eye movements in primates are essential for visual perception bringing new visual information to the foveal region. Further common features of Old-World primates include trichromatic color vision and forward-facing eyes with overlapping visual fields and a high degree of binocular convergence. As in other mammals, the primate retina has three nuclear layers: the photoreceptor layer, the inner nuclear layer and the ganglion cell layer. Axons of ganglion cells connect to the brain via the optic nerve and reach two main targets: the lateral geniculate nucleus (LGN), which projects to the primary visual cortex (V1), and the superior colliculus.

The visual system is part of the central nervous system and is most developed in primates, where approximately 55% of the cortex is specialized for visual processing, compared to 3% for auditory processing and 11% for somatosensory processing (28). The

NHP brain closely resembles the human brain in developmental trajectory and anatomy, including encephalization, the number and density of cortical neurons, a large prefrontal cortex, greater myelination, etc. The evolutionary proximity of NHPs and humans is also reflected in their similar perceptual capacities and ability to perform complex tasks. Alone or in combination with other sensory stimuli, visual signals are an important component of NHP behavior. Primates share other important aspects of their lifestyles (such as diurnality, terrestriality, omnivory) and sensory/perceptual abilities (such as color vision, greater reliance on vision than olfaction and other senses, the use of hands and thumbs, etc.); reviewed in (29). Because primates learn through observation and perform tasks in constrained experimental conditions, investigation of brain responses to visual stimuli provides unique insight into high-level perceptual skills and distinct primate behavioral adaptations such as precise manual motor behavior (reaching and grasping) and complex social interactions. For instance, optical imaging has enabled the definition of ocular dominance maps (5). Calcium imaging has revealed the orthogonal micro-organization of orientation and spatial frequency maps in layer II/III of the visual cortex (6). Recently, we defined retinotopic maps and presented functional evidence for ocular dominance columns within the depth of the visual cortex using ultrafast ultrasound imaging in behaving NHPs (8). Furthermore, anatomical tracing and histological techniques have provided access to the connectome between higher visual centers (7, 30) and structural correlates to functional maps such as ocular dominance columns (31–34). Primates also present opportunities to study visual attention. One of the most critical functions of sensory and motor systems is preparation for upcoming events. In sensory systems, this preparation confers perceptual and behavioral advantage. Given the location of an upcoming event, subjects respond with shorter reaction times and greater accuracy, an internal state often referred to as spatial attention. Attention has been studied in humans for over a century (35), based on behavioral tests as well as non-invasive methods such as electroencephalography and magnetic resonance imaging. The neural circuits that give rise to the perceptual and behavioral advantages conferred by attention have been studied in NHPs for decades, focused at first on single neurons in the visual cortex (36) that change their responsiveness or gain (37, 38). With advances in technology, investigation of attention in NHPs has revealed how populations of neurons may shift the neural code and improve perception (39–42).

Translation of therapies targeting the fovea (gene therapy, optogenetics)

High-acuity vision is dependent on the cone photoreceptor visual pathway in all species with a macular function. In human retinal disease, visual acuity loss is commonly the result of cone photoreceptor dysfunction such as observed in age-related degeneration. In addition, mutations that disrupt cone function have been identified in more than 40 genes (43). NHP models are vital for the development of therapeutics targeting the cones that restore and maintain high-acuity vision in humans. Unfortunately, there are only a few NHP models of macular disease. Examples of individual NHPs with validated inherited retinal disease have been described (44–46) but the genetic origin is known for only one rare form of achromatopsia (9). These animals, with all the characteristic signs, symptoms, and functional deficits of phosphodiesterase 6C (PDE6C)-related achromatopsia, will be decisive in the development of gene or cell therapies targeting the fovea.

Finally, the identification of further NHPs with spontaneously occurring inherited retinal dystrophies (IRDs) will generate new resources for further preclinical validation of the stream of future therapeutics expected to enter clinical testing. We anticipate that NHP models of retinal disorders will reduce the time required for translation of gene- or cell-based therapies to human clinical trials, such as the ones described below, and dramatically improve the success rate.

NHP – Models and therapies for complex diseases

NHPs have also been used for modeling and developing treatments for complex diseases such as diabetic retinopathy, choroidal neovascularization, wet AMD (47, 48) and glaucoma (49, 50). Since neovascularization is a common symptom in several ocular diseases, including diabetic retinopathy, age-related macular degeneration, retinopathy of prematurity, and sickle cell disease; an NHP model presenting this feature was created using intraocular administration of AAV2 vectors encoding human vascular endothelial growth factor (VEGF) (47). Overexpression of VEGF was achieved and led rapidly to features of diabetic retinopathy or macular edema, depending on the targeted cell type/mode of production of VEGF and diffusion of VEGF. NHP models were also useful in testing long-term safety and efficacy of AAV mediated treatments for blinding neovascular diseases such as wet AMD, as mentioned previously (48). Glaucoma being an important retinal neurodegenerative disease affecting over 70 million people worldwide, NHP models recapitulating symptoms of this condition were also sought. The intraocular pressure -a common risk factor for glaucoma- is tightly regulated by aqueous humor production and outflow pathways. One of the tissues that

contribute significantly to this complex regulation is the trabecular meshwork, which is fully developed only in humans and NHPs (51). Laser- and bead-induced ocular hypertension model in NHPs have been proposed to test and develop treatments for glaucoma (49, 50). These models are similar to the human disease as presenting with reduced outflow facility, nerve fiber layer defects and progressive enlargement of the cup-to-disc ratio and, thus, it is highly predictive for drug efficacy in humans. The trabecular meshwork also represents a potential target for gene therapy in glaucoma, as genes delivered into the anterior chamber of the NHP eye preferentially reach this tissue allowing permanent secretion of therapeutic factors (52). Early studies used Ad recombinant vectors to transduce the trabecular meshwork (53). Later studies used AAV vectors (54) and Lentiviral vectors (55) to deliver potentially therapeutic genes targeting the Rho/ROCK and prostaglandin pathways in NHPs. For example, single intracameral injection of AAVs ensured a stable and long-term expression (>2 years) of a reporter gene in the trabecular meshwork of living *Cynomolgus* monkeys with an early onset and a safe clinical profile (54). Intracameral delivery of exoenzyme C3 transferase (C3) vector to monkey eyes lead to a safe and persistently lowered the intraocular pressure for 112 days as a result of inactivated RhoA in trabecular meshwork, disruption of the actin cytoskeleton and altered cell morphology (56). Lentiviral-mediated delivery of the prostaglandin F synthase (PHFS) gene to the anterior segment in NHP has been shown to lower intraocular pressure for a period of 5 months (57). Furthermore, experimental studies in NHPs provided strong evidence that the optic nerve head is the primary site for disturbances in axonal transport (reviewed in (58, 59)) contributing to our understanding of basic mechanisms of disease. High-resolution *in vivo* imaging techniques, along with histological studies were able to precisely quantify the effects of changes in the intraocular pressure on optic nerve head and surrounding tissues, identifying the peripapillary connective tissue, lamina cribrosa and neural tissue as structures susceptible to intraocular pressure (59–61). The NHP model revealed distinct structural differences of optic nerve head in primates when compared to rodents. Specifically, in the primate eye, retinal ganglion cell axons pass through a meshwork of astrocyte-covered, capillary containing, connective tissue beams known as the *Lamina cribrosa* (reviewed in (58)), similar to human optic nerve head structure.

Surrogate models on the development path

As the step from rodents to humans appears too distant to ensure the success of therapies in patients with retinal degenerative disorders, therapeutic strategies must be validated in NHP

models after proof-of-concept in rodents (62). However, prior to *in vivo* studies in NHPs, different *ex vivo/in vitro* primate models are advantageous for screening promoters, genes, viral vectors, and molecular targets on NHP or even human cells. For instance, retinal cells have been cultured from various species, including NHPs or postmortem human retina (63, 64). The survival of human retinal cells in culture has been exploited to characterize their electrophysiological properties (33). The morphology of dissociated retinal cells changes and the lack of synaptic contact may also alter cell physiology and gene expression profiles. Retinal explant cultures that retain tissue architecture with cell synaptic and neurocrine interactions were developed to assess retinal cell neuroprotection in different animal models of retinal disease (65–68). Furthermore, retinal explant cultures have been produced from primate and postmortem human retina (69–72). These *in vitro* preparations allowed us to demonstrate functional expression of genes such as microbial opsins in cone photoreceptors (69) and retinal ganglion cells (73) in the development of optogenetic therapy for restoring vision. We also used *ex vivo* NHP retinal explants to assess the efficacy of the PRIMA photovoltaic retinal prosthesis (17). The recent development of retinal organoids from induced pluripotent stem cells (iPSCs) has extended the interest in *in vitro* studies by providing an unlimited source of human retinal cells, with the additional possibility of generating tissues from patients affected by specific gene mutations (72, 74–77). Such *in vitro/ex vivo* retinal models will allow an essential validation step prior to *in vivo* NHP studies and thus reduce the number of animals in preclinical studies.

Despite their value for drug development, viral vector selection, and neuroscience research, there are major limits to modeling *in vivo* conditions using retinal explants and self-forming retinal organoids derived from human embryonic stem cells (hESCs) or human iPSCs. First, the heterogeneity in production, composition and maturation of organoids is problematic, with differentiation and maturation efficiencies varying not only between iPSC lines but even between organoids originated at the same time. Second, these *in vitro* tissues lack vascularization and will thus obscure how metabolic and oxygen supplies may affect the degenerative process and therapeutic intervention. Third, such preparations are often separated into neural retina and retinal pigment epithelium (RPE) at the expense of physiological interactions between photoreceptors and RPE. A recent report suggests that these shortcomings can be overcome using a new microphysiological model of the human retina enhanced with a vasculature-like perfusion system in addition to a support system with RPE (78). These favor the establishment of *in vivo*-like physiological processes such as outer segment phagocytosis and calcium dynamics in retinal cells, significantly expanding the

relevance of such a system. However, even such sophisticated retinal organoids have limitations, for example for investigations that probe vision, which is a result of interplay between the visual cortex and the retina. They are also not appropriate for studies in which pharmacodynamics and immunology are important factors, as these *in vitro* tissues do not represent the whole eye connection to the body and the immune system.

Therapy development involving non-human primates *in vivo*

A. Gene therapy

Gene therapy approaches today fall into four broad categories. The **first** is gene replacement therapy, also referred to as gene addition or gene supplementation. This employs cDNA encoding a healthy copy of a recessive mutant gene. After an initial success in the treatment of type 2 Leber congenital amaurosis, a dozen further clinical trials used this strategy against well-characterized monogenic recessive diseases of the retina, including choroideremia, Leber's hereditary optic neuropathy, retinoschisis, Stargardt disease, Usher 1B, and achromatopsia (79). In the absence of representative genetic models of these conditions in NHPs, most of the clinical trials followed regulatory safety and toxicity studies on healthy NHPs. However, it is perhaps in inherited cone dystrophies that an NHP model will provide the most accurate readouts, with the emergence of a model for testing gene therapies directed towards PDE6C supplementation (9). Such work may allow comparison of outcomes using a model with identical genotypes/phenotypes to humans and also lead to gene replacement studies that have already restored cone cell function in mouse models with CNGB3 achromatopsia (80–82), as well as canine and sheep models of achromatopsia with mutations in CNGA3 (83, 84).

As well as for gene replacement, PDE6 mutant NHPs may be a good model for testing the **second** form of gene therapy based on CRISPR/Cas9 modification of disease-causing mutations. CRISPR/Cas9-mediated base editing or homology-directed repair (CRISPR-HDR) can be used to produce a functional protein. Since no other genetically characterized, spontaneously occurring IRD models exist in NHPs, PDE6C-mutant macaques may be a unique model to test CRISPR targeting of this specific mutation, with the possibility of functional readouts. As for other gene-editing attempts to target various disease-causing

mutations, a productive rate of editing of the target gene can be tested with a comparable surrogate NHP vector (85) to determine whether a therapeutic threshold is met or not under any given circumstances. This approach has demonstrated the ability of CRISPR/ Cas9 to edit somatic primate cells *in vivo* in a study aimed at restoring vision loss in Leber congenital amaurosis type 10 (86).

The **third** approach to gene therapy uses secretable factors to hamper adverse symptoms of retinal disease. These factors typically include soluble anti-VEGF molecules for the treatment of wet-AMD (48, 87, 88) and trophic factors like RdCVF (25, 89), GDNF (90, 91), and Nrf2 (92). Secretable factors such as soluble fms-like tyrosine kinase (sFLT) have been proposed, together with therapeutic options alleviating the need to re-administer anti-VEGF antibodies in some AMD patients. Preclinical studies in macaques have tested both the safety and the dosage thresholds required for the follow-up clinical trial (48). There is an ever-increasing list of known disease-causing mutations in monogenic inherited retinal diseases. Thus, the identification of a generic gene-based treatment that can alleviate photoreceptor loss is a priority. Secretable factors widely applicable in inherited retinal diseases such as rod-cone dystrophy have been intensively researched in rodent models of rod-cone dystrophy. Several promising candidate survival factors are now in the pre-clinical stages of testing, with regulatory studies foreseen in NHPs (24).

The **fourth** gene therapy category is based on optogenetics, i.e., the use of light-sensitive proteins to transform any neuron of the retina into an artificial photoreceptor (93), and restore vision in rod-cone dystrophy following the loss of cones in outer segments (94). Optogenetics holds promise for biomedical applications particularly in the retina and research over the past decade, first in rodents and then in NHPs, has led to translation into two clinical trials using this strategy (93). Most of the studies to date used microbial opsins from algae or bacteria, which have specific wavelength and light-intensity requirements and the ability to respond to light in the absence of chromophore replenishment by the RPE. The activity of these microbial opsins can thus be evaluated in normal primate retinas *ex vivo*, where fading of the endogenous light responses allows specific recording of the optogenetic activity (19, 20, 69, 95). These strategies have been tested in normal primates including marmosets (18), which have a fovea but a very small eye, making dose-related extrapolations difficult. For this reason, macaques have been used for most mandatory dose-extrapolation and functional studies of adeno-associated virus (AAV)-mediated delivery of optogenetic sensors prior to

clinical translation (19, 20). In fact, the use of viral vectors, particularly AAV, to deliver therapeutic genes is the common denominator of the majority of human gene therapy studies. The efficacy of therapeutic DNA as a pro-drug depends largely on efficient targeting of the cells of interest and induction of adequate expression levels; each case is a unique scenario. This challenge is discussed below.

- AAV screen

NHPs are a potential model not only to evaluate treatment of affected NHPs but also to optimize viral-mediated treatment modalities by subretinal and intravitreal injections. Intraocular injection of AAV vectors has been an evident route for delivering genes to retinal cells. However, gaps in our understanding of AAV transduction patterns within the anatomically unique environments of the subretinal and intravitreal space of the NHP eye, which differ from small animal models and unfoveated large animals, has slowed the establishment of noninvasive and efficient gene delivery important for the clinical application of gene therapy. Intraocular (subretinal or intravitreal) injections of AAVs encoding fluorescent proteins and the resulting expression can be followed using noninvasive employment of a retinal fundus camera to visualize neurons expressing the transgene (96–100). The similarity to the ocular anatomical barriers of humans is a major advantage of working with NHPs (101). Surgical methods, pharmacological parameters such as dosage, and the access of AAV vectors to various retinal neurons can be rigorously evaluated before going to clinical trials. Testing of new vector-promoter combinations for cone transduction, particularly in the fovea, can only be conducted in NHPs (20, 98, 102, 103). Based on these studies, AAV9 and its variants have been shown to lead to efficient foveal cone transduction when injected into the subretinal space (20, 98). An AAV9 variant (AAV9-7m8) injected several millimeters away from the fovea, without detaching this delicate region, induced significant gene expression in this region. An engineered AAV2 variant (AAV2-7m8) delivers genes to foveal cones with a well-tolerated dose when administered intravitreally. Although a healthy NHP is an excellent model to determine the adequacy of AAV capsids and doses in combination with appropriate injection routes (96, 101, 104, 105), studies in mouse models, human iPSC-derived organoids (20, 106), and *post-mortem* human retinal explants (69, 70, 95) are required to complement findings in normal macaques. Although use of macaques can efficiently predict the distribution of AAV in a normal retina, viral transduction patterns change dramatically in retinal disease (107–109), making it necessary to add further model systems. Tests in several model systems provide the most accurate insight

into the behavior of AAV vectors across species in order to validate the safety and efficacy required for gene therapy in neurodegenerative disorders.

- Promoter screen

Specific promoters can be used to restrict expression of genes to defined cell types. A large-scale promoter screen recently performed in mouse, NHP and human retinas (71) showed that particular promoters rarely targeted the same cell types in mice and humans; however, the correspondence between NHPs and humans was much better. Since a promoter screen in humans can only be performed *in vitro*, use of NHPs can determine whether the selected promoter is also active and selective *in vivo*.

B. Implants

Retinal prostheses aim at restoring vision in blind patients lacking photoreceptors by activating the residual retina with electrical currents delivered by a multielectrode array. The current state of the art is represented by the Argus II implant, which received the CE (Conformité Européenne) mark and FDA approval (humanitarian exemption) for commercialization in Europe and USA, respectively (110) and was discontinued in 2019. After the clear demonstration that electrical currents can activate the retina (111), an initial prototype with 16 electrodes chronically implanted in blind patients showed that electrical stimulation can provide visual perception with maintained activity over time (112). Patients could already perform simple shape detection and movement perception with this 4x4 array. The current Argus II device with a 60 electrode array allowed some patients to recognize letters and even read simple words (113). Prior to their clinical use, these implants were mainly tested in normal and blind dogs (114, 115). However, the first photovoltaic implants introduced into the subretinal space at the original position of the photoreceptors were instead tested in cats (116). Such a subretinal implant has obtained the CE mark following positive results in blind patients (117). It was discontinued in 2019. We recently tested the new photovoltaic prosthesis PRIMA invented by Daniel Palanker on NHPs to demonstrate the efficiency and high resolution that would allow transfer to clinical trials. PRIMA was found to provide high visual acuity in blind rats (118). Prior to transfer of these implants into clinical trials, PRIMA devices were first fabricated with a clinical grade process. To demonstrate their potential superiority to the existing devices, we assessed their activity on the primate retina. First, we showed that post-mortem human

retina can be activated by these implants. We then removed photoreceptors by sectioning the NHP retina with a vibratome and demonstrated that individual retinal ganglion cells but not implant neighbors were activated by a single electrode. Finally, to mimic the patient situation, PRIMA implants were introduced into the subretinal space, eliciting photoreceptor degeneration and creating a blind spot at the implant position, as previously reported (116). Taking advantage of this blind spot, we showed that NHP eyes generated visual saccades in response to activation of a single implant electrode by infrared activation of the photovoltaic implant. PRIMA implants are now in clinical trials in patients with advanced atrophic age-related macular degeneration in France and in the USA.

C. Stem cells

- Transplantation studies

Cell therapy is an alternative method to repair degenerated retina. Transplantation of retinal cells is a potential vision restoration strategy for retinal degenerative diseases, particularly in disease stages associated with significant cell damage. This therapeutic approach aims at replacing lost retinal cells using progenitor cells or stem cell-derived retinal cells (76, 77, 119–121). The advantage of cell therapies is that they are mutation-independent and can be used in a wide range of conditions. Furthermore, culture systems for the production of 3D tissue organoids from pluripotent stem cells (PSCs), which reflect a developmental environment more closely related to the *in vivo* situation, now allow the generation of specific cell-types with high precision and amounts. This was demonstrated in self-organizing organoids (74, 122) to address critical issues for intraocular cell transplantation. However, determination of the most appropriate surgical approach, the application of treatment in eyes of size and structure similar to the human eye, including the presence of a macula, and a thorough understanding of the immunological considerations for graft survival and the consequences of grafted rejection can only be assessed in transplantation studies conducted with NHPs. Promising therapies, such as those based on transplantation of iPSC-derived retinal sheets (15, 16) or RPE (123, 124), conducted in acute outer retinal degeneration models in NHPs contributed greatly to their clinical translation. Indeed, the first-in-human safety and tolerability clinical trial to evaluate subretinal injection of human ESC-derived RPE cells into patients with dry AMD and Stargardt disease is ongoing (125). Cell transplantation experiments in NHPs are expected to address several

outstanding questions, such as which type of cells are best to transplant, and whether transplanted precursors differentiate *in vivo* into functional photoreceptors and form functional synaptic connections with downstream neurons. NHP models of photoreceptor loss will also be useful for evaluating cell transplantation in an allogenic context based on the specific use of macaque iPSCs. Indeed, it is very difficult to know whether xenograft models - generally used to validate cell transplantation benefits in specific diseases - are truly predictive of transplantation efficacy in humans. Photoreceptor transplantation studies focusing on cones can now be tested in the genetic model of achromatopsia, taking advantage of the cone-silent state and relatively stable architecture of the macula in these NHPs (9). Cone replacement has suffered from challenges in the production of cone photoreceptors *in vitro* from human iPSC-derived retinal organoids, though some strategies have been reported to bias cells to adopt this fate (126, 127). There is now renewed interest in investigating human cell transplantation to the retina (128) as advances in the last decade using mouse models appear to be in part secondary to material transfer between transplanted cells and endogenous cells (129, 130).

Discussion

NHPs are used in biomedical research as a highly relevant model because of their similarities to humans in physiology, neuroanatomy, reproduction, development, cognition, and social complexity. Indeed, over the past decades, research involving NHPs has played a vital role in key medical and scientific advances in the fields of neurodegenerative diseases (e.g., Alzheimer's and Parkinson's), neurologic and cognitive conditions (e.g., stroke and anxiety), HIV/AIDS, development of vaccines, studies of the reproductive system and *in vitro* fertilization, etc. Being the species closest to humans, NHPs also have a high degree of sentience; thus, ethical principles require NHP use in research to be strictly justified. The laws regulating research with animals aim at securing laboratory animal welfare while allowing research to continue. In the US, research with animals follows a complex set of federal and state laws, regulations, guidelines and institutional policies. In Europe, the principle of the 3Rs –Replacement (avoid/replace the use of animals), Reduction (minimize the number of animals used), and Refinement (minimize animal suffering and improve welfare) - must be considered when selecting approaches to the regulatory testing of human and veterinary medicinal products (131). Wherever possible, at an early stage in potentially translational

Companion article to the Sackler Colloquium, "Using Monkey Models to Understand and Treat Human Brain Disorders," to be published in PNAS.

research, a significant reduction in animal studies should be achieved by alternative cellular and organotypic *in vitro*, *in vivo* and *in silico* models and methods. The proper design of studies and the implementation of mathematical models for outcome prediction could also contribute significantly to a marked reduction of animals in research. However, in various areas of medicine, including neurosciences and vision research, translation to the clinic often needs the use of primates in order to achieve results that unequivocally confirm benefits for human health. Given that only primates have a fovea, there is no realistic alternative to NHPs for translational studies in genetic and age-related blinding disorders associated with macular pathology that are a major health and social burden. NHP research in the emerging visual prosthetics field is also essential. In these cases, NHP research alone can provide precise and conclusive data that either facilitate the pathway “from bench to bedside” or stop further steps in research and/or translation to the clinic of inadequate treatment candidates, thus saving animals, effort and money. In other words, NHP research secures a greater success rate in clinical studies.

Figure legend: Anatomy of the rodent and primate eye. Upper panel shows the gross anatomy of the mouse, macaque and human eye with their corresponding axial lengths. The middle panel shows fundus and OCT images of the back of the eye in each species. The bottom panel shows adaptive optics images of rod and cone dominated regions of the primate retina.

Acknowledgments: The authors would like to thank Olivier Marre for valuable comments, Philippe Hantraye, Celine Jaillard and Pierre Pouget for their contributions to primate studies, and Kate Grieve and Céline Jaillard for contributing the images in Figure 1.

Funding: Programme Investissements d’Avenir IHU FOReSIGHT (ANR-18-IAHU-01), Programme Investissements d’Avenir LabEx LIFESENSES (ANR-10-LABX-65), ERC Synergy "HELMHOLTZ", Banque publique d’Investissement, Foundation Fighting Blindness, ERC StG (REGENETHER, 639888).

Competing interests: SP: consultant for Pixium Vision, GenSight Biologics; personal financial interests in Pixium Vision, GenSight Biologics and ICONEUS; DD has received grants and personal fees from GenSight Biologics and is co-inventor on patent #9193956 (Adeno-associated virus virions with variant capsid and methods of use thereof), with

royalties paid to Avalanche Biotechnologies; KM and OG: no competing interests; JAS: consultant (with no consulting fee) for Pixium Vision, GenSight Biologics and SparingVision; personal financial interests in Pixium Vision, GenSight Biologics, Sparing Vision, Prophesee and Chronolife; BR is the chair of the Scientific Advisory Board of GenSight Biologics.

References

1. Scott AW, Bressler NM, Ffolkes S, Wittenborn JS, Jorkasky J (2016) Public Attitudes About Eye and Vision Health. *JAMA Ophthalmol* 134(10):1111.
2. Chakravarthy U, et al. (2017) The Economic Impact of Blindness in Europe. *Ophthalmic Epidemiol* 24(4):239–247.
3. Scholl HPN, et al. (2016) Emerging therapies for inherited retinal degeneration. *Sci Transl Med* 8(368):368rv6.
4. Roska B, Sahel J-A (2018) Restoring vision. *Nature* 557(7705):359–367.
5. Blasdel G, Campbell D (2001) Functional retinotopy of monkey visual cortex. *J Neurosci* 21(20):8286–301.
6. Nauhaus I, Nielsen KJ, Disney AA, Callaway EM (2012) Orthogonal micro-organization of orientation and spatial frequency in primate primary visual cortex. *Nat Neurosci* 15(12):1683–1690.
7. Donahue CJ, et al. (2016) Using Diffusion Tractography to Predict Cortical Connection Strength and Distance: A Quantitative Comparison with Tracers in the Monkey. *J Neurosci* 36(25):6758–6770.
8. Kevin Blaize, Marc Gesnik, Fabrice Arcizet, Harry Ahnine, Ulisse Ferrari, Thomas Deffieux, Pierre Pouget, Frederic Chavane, Mathias Fink, Jose-Alain Sahel, Mickael Tanter SP (2019) Functional ultrasound imaging of deep visual cortex in awake non-human primates. *bioRxiv*.
9. Moshiri A, et al. (2019) A nonhuman primate model of inherited retinal disease. *J Clin Invest* 129(2):863–874.
10. Ikeda Y, et al. (2018) Discovery of a Cynomolgus Monkey Family With Retinitis Pigmentosa. *Investig Ophthalmol Vis Sci* 59(2):826.
11. Ribka EP, Dubielzig RR (2008) Multiple ophthalmic abnormalities in an infant rhesus macaque (*Macaca mulatta*). *J Med Primatol* 37:16–19.
12. Xue C, et al. (2016) The population genomics of rhesus macaques (*Macaca mulatta*) based on whole-genome sequences. *Genome Res* 26(12):1651–1662.
13. Mancuso K, et al. (2009) Gene therapy for red–green colour blindness in adult primates. *Nature* 461(7265):784–787.
14. Merigan WH, Pasternak T, Zehl D (1981) Spatial and temporal vision of macaques after central retinal lesions. *Invest Ophthalmol Vis Sci* 21(1 Pt 1):17–26.
15. Tu H-Y, et al. (2019) Medium- to long-term survival and functional examination of human iPSC-derived retinas in rat and primate models of retinal degeneration. *EBioMedicine* 39:562–574.
16. Shirai H, et al. (2016) Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration. *Proc Natl Acad Sci* 113(1):E81–E90.
17. Prévot P, et al. (2019) Functional efficacy of stimulations with a photovoltaic sub-retinal prosthesis in non-human primates. *Nat Biomed Eng* in press.
18. Ivanova E, Hwang G-S, Pan Z-H, Troilo D (2010) Evaluation of AAV-Mediated Expression of Chop2-GFP in the Marmoset Retina. *Investig Ophthalmol Vis Sci*

- 51(10):5288.
19. Chaffiol A, et al. (2017) A New Promoter Allows Optogenetic Vision Restoration with Enhanced Sensitivity in Macaque Retina. *Mol Ther* 25(11):2546–2560.
 20. Khabou H, et al. (2018) Noninvasive gene delivery to foveal cones for vision restoration. *JCI Insight* 3(2). doi:10.1172/jci.insight.96029.
 21. Rossi EA, et al. (2017) Imaging individual neurons in the retinal ganglion cell layer of the living eye. *Proc Natl Acad Sci* 114(3):586–591.
 22. Burns SA, Elsner AE, Sapoznik KA, Warner RL, Gast TJ (2019) Adaptive optics imaging of the human retina. *Prog Retin Eye Res* 68:1–30.
 23. Paques M, et al. (2018) Adaptive optics ophthalmoscopy: Application to age-related macular degeneration and vascular diseases. *Prog Retin Eye Res* 66:1–16.
 24. Sahel J-A, et al. (2013) Functional rescue of cone photoreceptors in retinitis pigmentosa. *Graefes Arch Clin Exp Ophthalmol* 251(7):1669–77.
 25. Ait-Ali N, et al. (2015) Rod-Derived Cone Viability Factor Promotes Cone Survival by Stimulating Aerobic Glycolysis. *Cell* 161(4):817–832.
 26. Mitchell JF, Leopold DA (2015) The marmoset monkey as a model for visual neuroscience. *Neurosci Res* 93:20–46.
 27. Volland S, Esteve-Rudd J, Hoo J, Yee C, Williams DS (2015) A Comparison of Some Organizational Characteristics of the Mouse Central Retina and the Human Macula. *PLoS One* 10(4):e0125631.
 28. Felleman DJ, Van Essen DC (1991) Distributed Hierarchical Processing in the Primate Cerebral Cortex. *Cereb Cortex* 1(1):1–47.
 29. Phillips KA, et al. (2014) Why primate models matter. *Am J Primatol* 76(9):801–827.
 30. Nassi JJ, Lyon DC, Callaway EM (2006) The Parvocellular LGN Provides a Robust Disynaptic Input to the Visual Motion Area MT. *Neuron* 50(2):319–327.
 31. Kennedy C, et al. (1976) Metabolic mapping of the primary visual system of the monkey by means of the autoradiographic [¹⁴C]deoxyglucose technique. *Proc Natl Acad Sci* 73(11):4230–4234.
 32. Hubel DH, Wiesel TN, Stryker MP (1978) Anatomical demonstration of orientation columns in macaque monkey. *J Comp Neurol* 177(3):361–379.
 33. Picaud S, Hicks D, Forster V, Sahel J, Dreyfus H (1998) Adult human retinal neurons in culture: Physiology of horizontal cells. *Investig Ophthalmol Vis Sci* (13):2637–2648.
 34. Takahata T, Miyashita M, Tanaka S, Kaas JH (2014) Identification of ocular dominance domains in New World owl monkeys by immediate-early gene expression. *Proc Natl Acad Sci* 111(11):4297–4302.
 35. Carrasco M (2011) Visual attention: The past 25 years. *Vision Res* 51(13):1484–1525.
 36. Moran J, Desimone R (1985) Selective attention gates visual processing in the extrastriate cortex. *Science* (80-) 229(4715):782–784.
 37. Reynolds JH, Pasternak T, Desimone R (2000) Attention Increases Sensitivity of V4 Neurons. *Neuron* 26(3):703–714.
 38. Williford T, Maunsell JHR (2006) Effects of Spatial Attention on Contrast Response Functions in Macaque Area V4. *J Neurophysiol* 96(1):40–54.
 39. Cohen MR, Maunsell JHR (2009) Attention improves performance primarily by reducing interneuronal correlations. *Nat Neurosci* 12(12):1594–1600.
 40. Mitchell JF, Sundberg KA, Reynolds JH (2009) Spatial Attention Decorrelates Intrinsic Activity Fluctuations in Macaque Area V4. *Neuron* 63(6):879–888.
 41. Snyder AC, Morais MJ, Smith MA (2016) Dynamics of excitatory and inhibitory networks are differentially altered by selective attention. *J Neurophysiol* 116(4):1807–1820.
 42. Snyder AC, Yu BM, Smith MA (2018) Distinct population codes for attention in the

- absence and presence of visual stimulation. *Nat Commun* 9(1):4382.
43. Roosing S, et al. (2014) Causes and consequences of inherited cone disorders. *Prog Retin Eye Res* 42:1–26.
 44. Hummel M, Li ZG, Pfaffinger D, Neven L, Scanu AM (1990) Familial hypercholesterolemia in a rhesus monkey pedigree: molecular basis of low density lipoprotein receptor deficiency. *Proc Natl Acad Sci* 87(8):3122–3126.
 45. Dray BK, et al. (2018) Mismatch repair gene mutations lead to lynch syndrome colorectal cancer in rhesus macaques. *Genes Cancer* 9(3–4):142–152.
 46. Dominik Fischer M, et al. (2012) Detailed functional and structural characterization of a macular lesion in a rhesus macaque. *Doc Ophthalmol* 125(3):179–194.
 47. Leberherz C, et al. (2005) Nonhuman Primate Models for Diabetic Ocular Neovascularization Using AAV2-Mediated Overexpression of Vascular Endothelial Growth Factor. *Diabetes* 54(4):1141–9.
 48. MacLachlan TK, et al. (2011) Preclinical Safety Evaluation of AAV2-sFLT01— A Gene Therapy for Age-related Macular Degeneration. *Mol Ther* 19(2):326–334.
 49. Burgoyne CF (2015) The non-human primate experimental glaucoma model. *Exp Eye Res* 141:57–73.
 50. Lambert WS, et al. (2019) Towards A Microbead Occlusion Model of Glaucoma for a Non-Human Primate. *Sci Rep* 9(1):11572.
 51. Rohen JW (1965) Morphology of the uveal tract. *Int Ophthalmol Clin* 5(3):581–667.
 52. Nilsson SFE (1997) The uveoscleral outflow routes. *Eye* 11(2):149–154.
 53. Borràs T, Gabelt BT, Klintworth GK, Peterson JC, Kaufman PL (2001) Non-invasive observation of repeated adenoviral GFP gene delivery to the anterior segment of the monkey eye in vivo. *J Gene Med* 3(5):437–449.
 54. Buie LK, et al. (2010) Self-complementary AAV Virus (scAAV) Safe and Long-term Gene Transfer in the Trabecular Meshwork of Living Rats and Monkeys. *Investig Ophthalmology Vis Sci* 51(1):236.
 55. Barraza RA, et al. (2009) Prolonged Transgene Expression with Lentiviral Vectors in the Aqueous Humor Outflow Pathway of Nonhuman Primates. *Hum Gene Ther* 20(3):191–200.
 56. Tan J, et al. (2019) Lentiviral Vector-Mediated Expression of Exoenzyme C3 Transferase Lowers Intraocular Pressure in Monkeys. *Mol Ther* 27(7):1327–1338.
 57. Lee ES, et al. (2014) Prospects for Lentiviral Vector Mediated Prostaglandin F Synthase Gene Delivery in Monkey Eyes In vivo. *Curr Eye Res* 39(9):859–870.
 58. Tamm ER, et al. (2017) Biological aspects of axonal damage in glaucoma: A brief review. *Exp Eye Res* 157:5–12.
 59. Patel N, McAllister F, Pardon L, Harwerth R (2018) The effects of graded intraocular pressure challenge on the optic nerve head. *Exp Eye Res* 169:79–90.
 60. Yang H, et al. (2009) Deformation of the Normal Monkey Optic Nerve Head Connective Tissue after Acute IOP Elevation within 3-D Histomorphometric Reconstructions. *Investig Ophthalmology Vis Sci* 50(12):5785.
 61. Strouthidis NG, Fortune B, Yang H, Sigal IA, Burgoyne CF (2011) Effect of Acute Intraocular Pressure Elevation on the Monkey Optic Nerve Head as Detected by Spectral Domain Optical Coherence Tomography. *Investig Ophthalmology Vis Sci* 52(13):9431.
 62. Danesh-Meyer H V., Levin LA (2009) Neuroprotection: Extrapolating from Neurologic Diseases to the Eye. *Am J Ophthalmol* 148(2):186–191.e2.
 63. Hicks D, Forster V, Dreyfus H, Sahel J (1994) Survival and regeneration of adult human photoreceptors in vitro. *Brain Res* 643(1–2):302–305.
 64. Gaudin C, Forster V, Sahel J, Dreyfus H, Hicks D (1996) Survival and regeneration of

- adult human and other mammalian photoreceptors in culture. *Investig Ophthalmol Vis Sci* 37(11):2258–2268.
65. Caffé AR, Söderpalm A, van Veen T (1993) Photoreceptor-specific protein expression of mouse retina in organ culture and retardation of rd degeneration in vitro by a combination of basic fibroblast and nerve growth factors. *Curr Eye Res* 12(8):719–726.
 66. Mohand-Said S, et al. (1998) Normal retina releases a diffusible factor stimulating cone survival in the retinal degeneration mouse. *Proc Natl Acad Sci U S A* 95(14):8357–8362.
 67. Vallazza-Deschamps G, et al. (2005) Excessive activation of cyclic nucleotide-gated channels contributes to neuronal degeneration of photoreceptors. *Eur J Neurosci* 22(5):1013–1022.
 68. Froger N, et al. (2012) Taurine Provides Neuroprotection against Retinal Ganglion Cell Degeneration. *PLoS One* 7(10):e42017.
 69. Busskamp V, et al. (2010) Genetic Reactivation of Cone Photoreceptors Restores Visual Responses in Retinitis Pigmentosa. *Science* (80-) 329(5990):413–417.
 70. Fradot M, et al. (2011) Gene Therapy in Ophthalmology: Validation on Cultured Retinal Cells and Explants from Postmortem Human Eyes. *Hum Gene Ther* 22(5):587–593.
 71. Jüttner J, et al. (2019) Targeting neuronal and glial cell types with synthetic promoter AAVs in mice, non-human primates and humans. *Nat Neurosci* 22(8):1345–1356.
 72. Cowan CS, et al. (2019) Cell types of the human retina and its organoids at single-cell resolution: developmental convergence, transcriptomic identity, and disease map. *bioRxiv*:703348.
 73. Sengupta A, et al. (2016) Red- shifted channelrhodopsin stimulation restores light responses in blind mice, macaque retina, and human retina. *EMBO Mol Med* 8(11):1248–1264.
 74. Llonch S, Carido M, Ader M (2018) Organoid technology for retinal repair. *Dev Biol* 433(2):132–143.
 75. Foltz LP, Clegg DO (2019) Patient-derived induced pluripotent stem cells for modelling genetic retinal dystrophies. *Prog Retin Eye Res* 68:54–66.
 76. Gagliardi G, Ben M'Barek K, Goureau O (2019) Photoreceptor cell replacement in macular degeneration and retinitis pigmentosa: A pluripotent stem cell-based approach. *Prog Retin Eye Res*. doi:10.1016/j.preteyeres.2019.03.001.
 77. Jin Z-B, et al. (2019) Stemming retinal regeneration with pluripotent stem cells. *Prog Retin Eye Res* 69:38–56.
 78. Achberger K, et al. (2019) Human Retina- on- a- Chip: Merging Organoid and Organ- on- a- Chip Technology to Generate Complex Multi- Layer Tissue Models. *bioRxiv*:under review.
 79. Bennett J (2017) Taking Stock of Retinal Gene Therapy: Looking Back and Moving Forward. *Mol Ther* 25(5):1076–1094.
 80. Michalakis S, et al. (2010) Restoration of Cone Vision in the CNGA3^{-/-} Mouse Model of Congenital Complete Lack of Cone Photoreceptor Function. *Mol Ther* 18(12):2057–2063.
 81. Dai X, et al. (2017) Long-term retinal cone rescue using a capsid mutant AAV8 vector in a mouse model of CNGA3-achromatopsia. *PLoS One* 12(11):e0188032.
 82. Pang J-J, et al. (2010) Achromatopsia as a Potential Candidate for Gene Therapy. *Advances in Experimental Medicine and Biology*, pp 639–646.
 83. Ezra-Elia R, et al. (2014) Flicker cone function in normal and day blind sheep: a large animal model for human achromatopsia caused by CNGA3 mutation. *Doc Ophthalmol* 129(3):141–150.

84. Thompson DA, et al. (2015) Advancing Therapeutic Strategies for Inherited Retinal Degeneration: Recommendations From the Monaciano Symposium. *Invest Ophthalmol Vis Sci* 56(2):918–931.
85. Sahel JA, Dalkara D (2019) Gene therapy for retinal dystrophy. *Nat Med* 25(2):198–199.
86. Maeder ML, et al. (2019) Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. *Nat Med* 25(2):229–233.
87. Rakoczy EP, et al. (2015) Gene therapy with recombinant adeno-associated vectors for neovascular age-related macular degeneration: 1 year follow-up of a phase 1 randomised clinical trial. *Lancet* 386(10011):2395–2403.
88. Heier JS, et al. (2017) Intravitreal injection of AAV2-sFLT01 in patients with advanced neovascular age-related macular degeneration: a phase 1, open-label trial. *Lancet* 390(10089):50–61.
89. Byrne LC, et al. (2015) Viral-mediated RdCVF and RdCVFL expression protects cone and rod photoreceptors in retinal degeneration. *J Clin Invest* 125(1):105–116.
90. Frasson M, et al. (1999) Glial cell line-derived neurotrophic factor induces histologic and functional protection of rod photoreceptors in the rd/rd mouse. *Invest Ophthalmol Vis Sci* 40(11):2724–34.
91. Sanftner LHM, Abel H, Hauswirth WW, Flannery JG (2001) Glial Cell Line Derived Neurotrophic Factor Delays Photoreceptor Degeneration in a Transgenic Rat Model of Retinitis Pigmentosa. *Mol Ther* 4(6):622–629.
92. Xiong W, MacColl Garfinkel AE, Li Y, Benowitz LI, Cepko CL (2015) NRF2 promotes neuronal survival in neurodegeneration and acute nerve damage. *J Clin Invest* 125(4):1433–1445.
93. Busskamp V, Roska B (2011) Optogenetic approaches to restoring visual function in retinitis pigmentosa. *Curr Opin Neurobiol* 21(6):942–946.
94. Sahel J-A, Roska B (2013) Gene Therapy for Blindness. *Annu Rev Neurosci* 36(1):467–488.
95. Lin JY, Knutsen PM, Muller A, Kleinfeld D, Tsien RY (2013) ReaChR: a red-shifted variant of channelrhodopsin enables deep transcranial optogenetic excitation. *Nat Neurosci* 16(10):1499–1508.
96. Yin L, et al. (2011) Intravitreal Injection of AAV2 Transduces Macaque Inner Retina. *Investig Ophthalmology Vis Sci* 52(5):2775.
97. Dalkara D, et al. (2013) In Vivo-Directed Evolution of a New Adeno-Associated Virus for Therapeutic Outer Retinal Gene Delivery from the Vitreous. *Sci Transl Med* 5(189):189ra76.
98. Vandenberghe LH, et al. (2013) AAV9 Targets Cone Photoreceptors in the Nonhuman Primate Retina. *PLoS One* 8(1):e53463.
99. Vandenberghe LH, et al. (2011) Dosage thresholds for AAV2 and AAV8 photoreceptor gene therapy in monkey. *Sci Transl Med* 3(88):88ra54.
100. Kay CN, et al. (2013) Targeting Photoreceptors via Intravitreal Delivery Using Novel, Capsid-Mutated AAV Vectors. *PLoS One* 8(4):e62097.
101. Boye SE, et al. (2016) Highly Efficient Delivery of Adeno-Associated Viral Vectors to the Primate Retina. *Hum Gene Ther* 27(8):580–597.
102. Ye G-J, et al. (2016) Cone-Specific Promoters for Gene Therapy of Achromatopsia and Other Retinal Diseases. *Hum Gene Ther* 27(1):72–82.
103. Boye SE, et al. (2012) The Human Rhodopsin Kinase Promoter in an AAV5 Vector Confers Rod- and Cone-Specific Expression in the Primate Retina. *Hum Gene Ther* 23(10):1101–1115.
104. Tshilenge K-T, et al. (2016) Vitrectomy Before Intravitreal Injection of AAV2/2

- Vector Promotes Efficient Transduction of Retinal Ganglion Cells in Dogs and Nonhuman Primates. *Hum Gene Ther Methods* 27(3):122–134.
105. Kotterman MA, et al. (2015) Antibody neutralization poses a barrier to intravitreal adeno-associated viral vector gene delivery to non-human primates. *Gene Ther* 22(2):116–126.
 106. Garita-Hernandez M, et al. (2018) Optogenetic Light Sensors in Human Retinal Organoids. *Front Neurosci* 12(November):1–12.
 107. Kolstad KD, et al. (2010) Changes in adeno-associated virus-mediated gene delivery in retinal degeneration. *Hum Gene Ther* 21(5):571–578.
 108. Vacca O, et al. (2015) Using Adeno-associated Virus as a Tool to Study Retinal Barriers in Disease. *J Vis Exp* 2015(98). doi:10.3791/52451.
 109. Vacca O, et al. (2014) AAV-mediated gene delivery in Dp71-null mouse model with compromised barriers. *Glia* 62(3):468–476.
 110. da Cruz L, et al. (2016) Five-Year Safety and Performance Results from the Argus II Retinal Prosthesis System Clinical Trial. *Ophthalmology* 123(10):2248–2254.
 111. Weiland JD, et al. (1999) Understanding the origin of visual percepts elicited by electrical stimulation of the human retina. *Graefe's Arch Clin Exp Ophthalmol* 237(12):1007–1013.
 112. Humayun MS, et al. (2003) Visual perception in a blind subject with a chronic microelectronic retinal prosthesis. *Vision Res* 43(24):2573–2581.
 113. da Cruz L, et al. (2013) The Argus II epiretinal prosthesis system allows letter and word reading and long-term function in patients with profound vision loss. *Br J Ophthalmol* 97(5):632–636.
 114. Majji AB, et al. (1999) Long-term histological and electrophysiological results of an inactive epiretinal electrode array implantation in dogs. *Investig Ophthalmol Vis Sci* 40(9):2073–2081.
 115. Güven D, et al. (2005) Long-term stimulation by active epiretinal implants in normal and RCD1 dogs. *J Neural Eng* 2(1):S65–S73.
 116. Chow AY, et al. (2001) Implantation of silicon chip microphotodiode arrays into the cat subretinal space. *IEEE Trans Neural Syst Rehabil Eng* 9(1):86–95.
 117. Stingl K, et al. (2017) Interim Results of a Multicenter Trial with the New Electronic Subretinal Implant Alpha AMS in 15 Patients Blind from Inherited Retinal Degenerations. *Front Neurosci* 11:445.
 118. Lorach H, et al. (2015) Photovoltaic restoration of sight with high visual acuity. *Nat Med* 21(5):476–482.
 119. Gasparini SJ, Llonch S, Borsch O, Ader M (2019) Transplantation of photoreceptors into the degenerative retina: Current state and future perspectives. *Prog Retin Eye Res* 69:1–37.
 120. Dalkara D, Goureau O, Marazova K, Sahel J-A (2016) Let There Be Light: Gene and Cell Therapy for Blindness. *Hum Gene Ther* 27(2):134–147.
 121. Reichman S, et al. (2014) From confluent human iPS cells to self-forming neural retina and retinal pigmented epithelium. *Proc Natl Acad Sci* 111(23):8518–8523.
 122. Gagliardi G, et al. (2018) Characterization and Transplantation of CD73-Positive Photoreceptors Isolated from Human iPSC-Derived Retinal Organoids. *Stem Cell Reports* 11(3):665–680.
 123. Kamao H, et al. (2014) Characterization of Human Induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelium Cell Sheets Aiming for Clinical Application. *Stem Cell Reports* 2(2):205–218.
 124. Ben M'Barek K, Monville C (2019) Cell Therapy for Retinal Dystrophies: From Cell Suspension Formulation to Complex Retinal Tissue Bioengineering. *Stem Cells Int*

- 2019:1–14.
125. Zarbin M, Sugino I, Townes- Anderson E (2019) Concise Review: Update on Retinal Pigment Epithelium Transplantation for Age- Related Macular Degeneration. *Stem Cells Transl Med* 8(5):466–477.
 126. Eldred KC, et al. (2018) Thyroid hormone signaling specifies cone subtypes in human retinal organoids. *Science* (80-) 362(6411):eaau6348.
 127. Reichman S, et al. (2017) Generation of Storable Retinal Organoids and Retinal Pigmented Epithelium from Adherent Human iPS Cells in Xeno-Free and Feeder-Free Conditions. *Stem Cells* 35(5):1176–1188.
 128. Garita-Hernandez, M Lampič M, et al. (2019) Restoration of visual function by transplantation of optogenetically engineered photoreceptors. *Nat Commun* in press.
 129. Santos-Ferreira T, et al. (2016) Retinal transplantation of photoreceptors results in donor–host cytoplasmic exchange. *Nat Commun* 7(1):13028.
 130. Pearson RA, et al. (2016) Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. *Nat Commun* 7(1):13029.
 131. DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes Available at: <https://eur-lex.europa.eu/eli/dir/2010/63/oj>.