Let There Be Light

Optogenetic Treatment of Blindness

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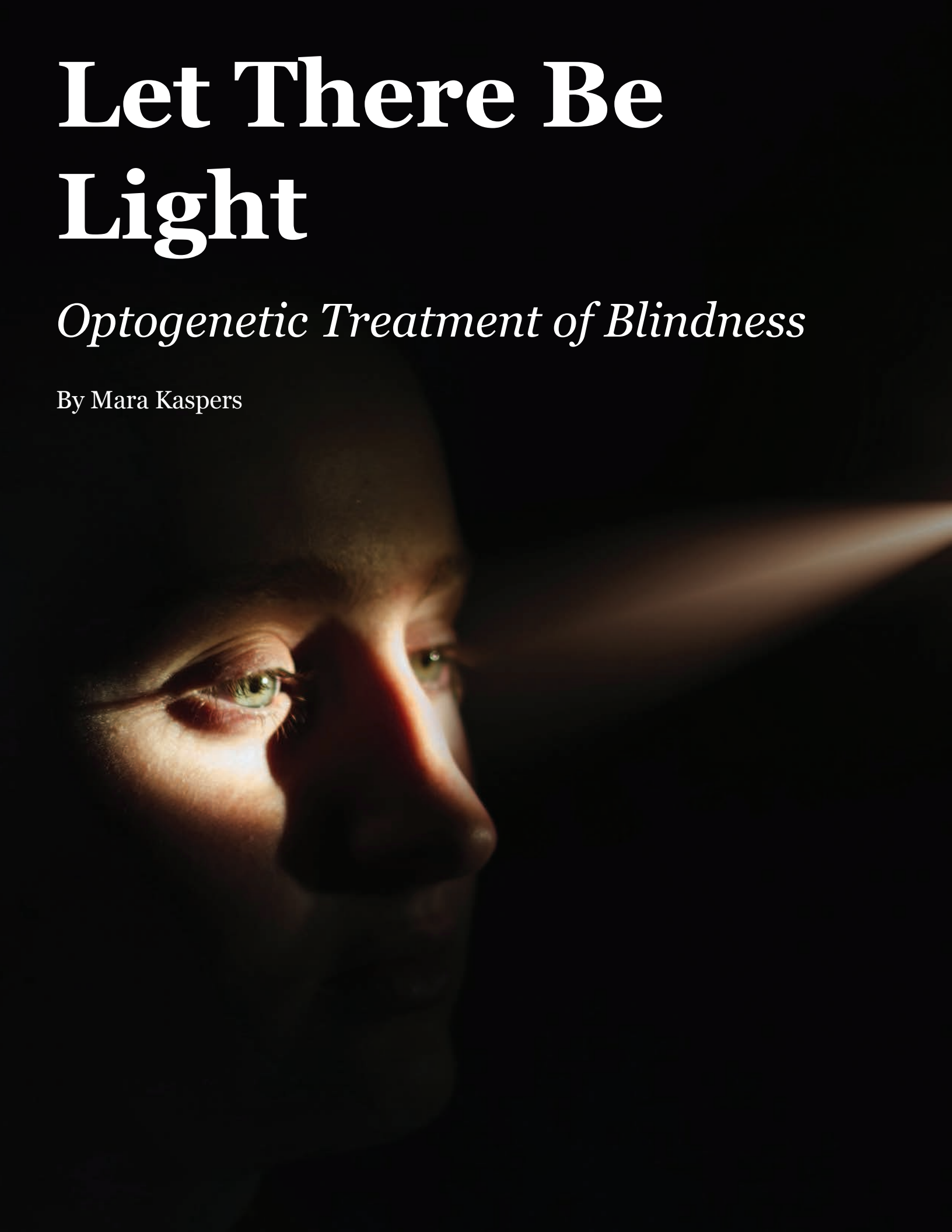

We are moving to a world where a single injection into the eye can restore vision of the blind. In September of 2018, a patient suffering from retinitis pigmentosa, a common disease resulting in complete blindness, was the first to receive optogenetic treatment designed to restore their vision. This patient at the UPMC Eye Center in Pittsburgh is part of a pioneer clinical trial carried out by biopharma company GenSight Biologics. What are some of the recent developments in molecular biology that have made this therapy possible? What is so novel about this technique and what are some things to consider before starting large scale implementation?

What Is Optogenetics?

Optogenetics is a very young lab technique first developed in 2005 by a group of researchers at Stanford University and spearheaded by Dr. Karl Deisseroth. The technique allows researchers to control neurons, the building blocks of the brain, with the use of light by changing the genetic code, or blueprint, of a living organism. Dr. Deisseroth and his team were inspired by a certain type of algae that has the ability to detect light with an endogenous light-sensitive protein called Channelrhodopsin-2 (ChR2). These algae use this information to swim towards the light, which is beneficial for certain biological processes and like all proteins, Channelrhodopsin-2 is coded for in the algae's DNA. Dr. Deisseroth and his colleagues were successful in extracting that exact piece of DNA and placing it into neurons of living organisms like mice and eventually people. When expressed in neurons, these algae-derived opsins operate much like a switch, allowing the researcher to activate or silence the neurons simply by shining light on them. This switch is very fast and can change brain activity within milliseconds. This technique was so innovative and promising for the neuroscientific field that it was awarded with "Method of the Year" by Nature Methods in 2010 and received "Breakthrough of the Decade" by Science BotD in 2013. However, for medical application in people, turning all neurons in the brain on and off at the same time would not be helpful. Rather, we want to be able to control certain groups of neurons in certain parts of the brain selectively. Luckily, we have a lot of knowledge about the genetic blueprints of mice, humans, and many other organisms so we can choose which *type* of neuron and *where* in the brain this biological switch should be produced.

It didn't take long for researchers to use this extremely exciting technique in people as optogenetics has now been used to expand our understanding of brain mechanisms involved in neurological conditions like depression, Parkinson's, Schizophrenia, autism, aggression, and addiction. This research then quickly developed into the use of optogenetics in clinical treatment of these disorders. However, since the opsins need direct light to activate a cell, treatment of these disorders would require people to walk around with a power cord through their skull, emitting light directly on their brain. While this is certainly not impossible and has been successful in other living organisms like mice, it is not the ideal starting place. An easier first step into optogenetic treatment of human diseases is targeting neurons that are more easily accessible with light, like the ones found in your eye. When produced in neurons in the eye, the switch is easily accessible for light and treatment would be minimally invasive. This is why patients suffering from genetically determined blindness, like patient Pittsburgh, are the first to receive this cutting-edge optogenetic treatment, which will have an incredible impact on the neuroscientific community if proven successful.

Restoring Vision of the Blind

Optogenetic treatment provides hope for over 1.5 million people across the world that suffer from degenerative retinal diseases like retinitis pigmentosa. Retinitis pigmentosa is a heritable disease causing the first line of cells in the eyes, the photoreceptors, to die. Photoreceptors are the only light-sensitive cells in the eye and degeneration therefore results in complete blindness. Rosalinda Barrero, a retinitis pigmentosa patient advocate, recalls not wanting to go out for trick or treating when she was younger because it was already hard for her to see in the dark. At a board meeting of the California Institute for Regenerative Medicine, her husband shares that they met because she accidently got into his car instead of her date's. This funny anecdote of the beginning of their relationship eludes to the fact that by the time she was in her twenties, Rosalinda had lost nearly all vision. She is now a mother of three and has never been able to see her husband or kids. The family shares their excitement about optogenetic research and the beginning of the clinical trials.

As illustrated by Rosalinda's story, onset of the retinitis pigmentosa often begins during childhood and develops into early adulthood. It often starts with loss of peripheral vision as the sides of the visual field become blurry.

Within the span of a couple years, this loss of vision will gradually expand to the middle of the visual field until all photoreceptors are lost and no amount of light can elicit visual perception. While this first layer of crucial photoreceptors will be completely lost over time, the other cell layers of the retina remain intact and provide a perfect target for optogenetic treatment. By making the remaining healthy cells produce the extracted algae opsins, researchers can restore the eye's sensitivity to light. This way, most of the visual system's healthy wiring remains intact and the patient should be able to produce a picture of the world by simply "skipping" the dead photoreceptors.

The Pittsburgh patient received one injection into one of their eyes. This injection contained the algae DNA and targeted the retinal ganglion cells, the cell layer following the photoreceptors, to express channelrhodopsin proteins. The newly engineered light-sensitive ganglion cells should now cause the visual pathway to be activated when exposed to light and hopefully restore some vision of the patient (Figure 1). However, this therapy will not restore vision perfectly because channelrhodopsins are not as complex as photoreceptors. There are many photoreceptor functions that these opsins simply cannot mimic like adapting to different light intensities or optimal detection of contrast. Therefore, we cannot expect vision to be perfectly restored, but hopefully the treatment will allow patients to observe large objects and move through the world independently again. Sean Ainsworth, CEO of biopharma company RetroSense says he hopes the patients will be able to "*see tables and chairs*" and maybe even read large letters with the help of light-enhancing goggles. The neuroscience community is waiting in suspense as these first transgenic patients are being assessed for safety. If proven successful and safe, the implications for the future of brain medicine are incredibly promising.

Clinical Trials

Two major companies, GenSight and RetroSense, are dedicated to advancing the field of optogenetic treatment of blindness and are currently running clinical trials studying a total of 39 patients suffering from retinitis pigmentosa,. Dr. Jose Sahel, co-founder of GenSight, explains how they are already able to partially restore impared vision with the use of microchips placed in the back of the eye. These microchips work much like a scanner as they read the visual input of the world and translate it into a code that the brain can understand. The most successful microchip treatment allowed blind patients to read about 10 words per minute, which is slow compared to the healthy speed of 200 words a minute. Nevertheless, this was a great success, but Dr. Sahel also explains how there is a limit to the restorative abilities of these implants. With current technological limitations, the chips will never be able to produce a resolution resembling healthy vision. In other words, the restored vision will always be blurry. Therefore, GenSight decided to switch to a biological technique and use optogenetics to:

"Transform any type of remaining neuron in the retina into a photoreceptor"

This was the beginning of the current clinical trials that patient Pittsburgh is a part of.

While RetroSense has yet to have published any results regarding their first trials, GenSight has confirmed the safety of the first treated patients in three centers across the United States, United Kingdom, and France in May of 2019. Bernard Gilly, another co-founder of GenSight, is pleased to be able to move forward to the second phase of their trials and says that they *"look forward to confirming the safety of GS030 at higher doses and to demonstrate efficacy in restoring useful visual functions in RP patients"*. They have since injected the next three patients with a higher dose of the treatment (GS030) and hope to release their preliminary results in late 2020. Since human testing is still in the safety testing phase and no treatment efficacy assessments have been made, we are basing

our excitement for possible vision restoration on preliminary animal research. What are the findings that are driving these human trials?

Preclinical Research

Mice lend themselves well for initial testing of genetic treatment as we know a lot about their genetic blueprint and have some widely applied ways to test their behavior. About 8 years ago, a team of researchers located across the United States collaborated to achieve one of the first successful optogenetic vision restorations in blind mice. After treatment, they compared how long it took for healthy, blind, and treated blind mice to find a platform in a maze of water. As you might expect, the healthy mice were rather quick to find the platform, whereas the blind mice were swimming around seemingly aimlessly before accidentally stumbling upon it. What was amazing about this study is that the blind mice that were treated with optogenetics were just as fast as the healthy mice to find the platform, suggesting that their vision was restored to the quality of healthy mice, and the treatment worked. In a TED Talk around that same time, a leader in the field, Dr. Ed Boyden, shares his excitement about this particular study and says that the results bode hope for potential therapeutic application in people. The data presented in this study were monumental for the current clinical trials on optogenetic treatment of blindness.

These findings were confirmed by many other behavioral studies of mice in the years after, among which were research groups in France associated with GenSight. One GenSight study tested the efficacy of optogenetic treatment by comparing how long it took for healthy, blind, and treated mice to respond to light at the end of a tube and return to the safety of darkness. Again, the researchers were pleasantly surprised to see the treated mice to behave in the exact same way as their healthy counterparts and run away from the light as quickly as possible, suggesting the treatment was effective.

While these results were important and exciting, preliminary research had to be done on human retinas to further strengthen the translatability of the therapy. This led the GenSight groups in Paris to test optogenetics in human and monkey retinas. They were able to successfully make the remaining healthy cells in the retina produce channelrhodopsin proteins and found that the cells were activated in response to light. This data showed efficacy and safety of optogenetic treatment in deceased human retinas. This was the last bit of data necessary for the human clinical trials to be launched and the first patients to be enrolled in the GenSight study. If this treatment shows to be safe and effective in people suffering from retinitis pigmentosa, it will have great implications for optogenetic treatment of other brain disorders including epilepsy, post-traumatic stress disorder, anxiety, depression, and many more.

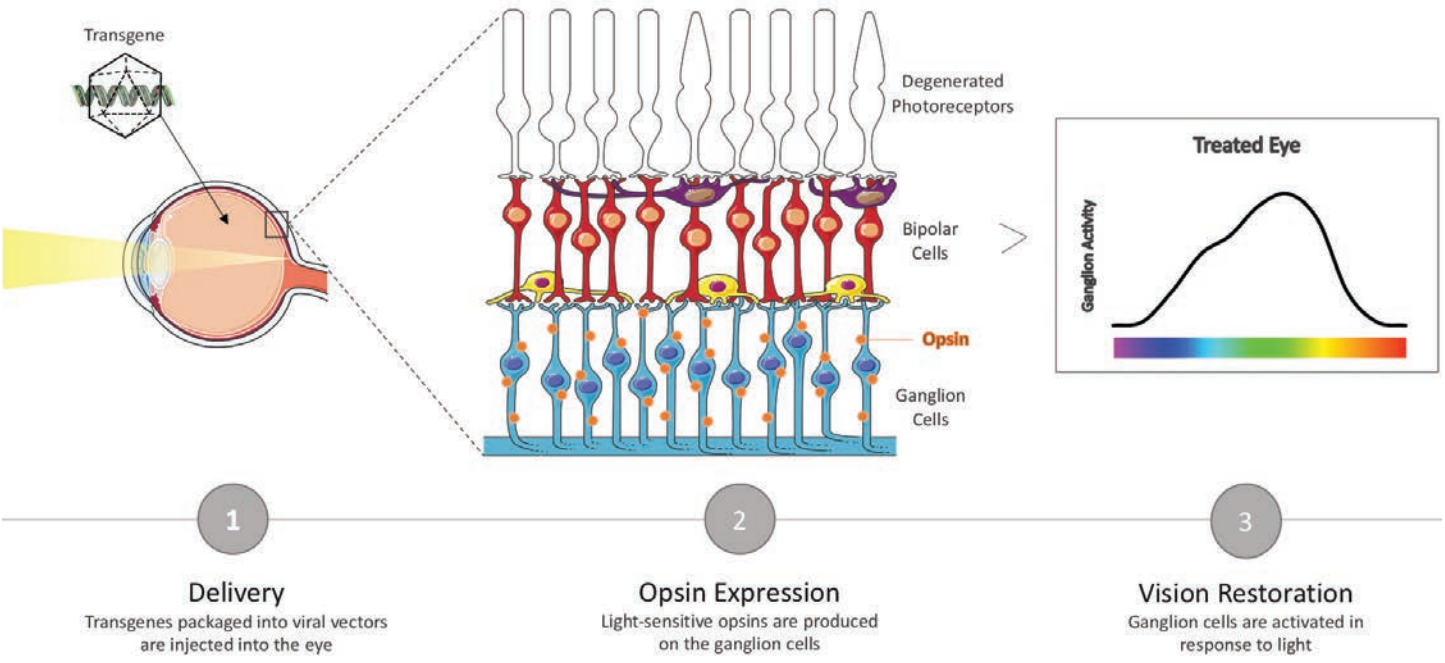

Figure 1. Infographic illustrating optogenetic treatment of an eye suffering from retinitis pigmentosa. "Cataract", DNA", "Retina" by Servier Medical Art by Servier (CC BY 3.0)."Spectrum" by Megabeckett27 from Wikimedia (CC BY-SA 3.0). Graph was adapted from figure 6D from Sengupta, et al. (2016)..Adapted by Mara Kaspers.

Optogenetics Beyond Blindness

People like Dr. Deisseroth and Dr. Boyden let their minds wander far past optogenetic treatment of blindness. There are endless research and treatment opportunities that this lab technique introduces in the world of neuroscience that has and will change the way we approach modern questions of the brain. Imagine all the things you can explore by having the ability to activate and silence whole parts of the brain, or even types of brain cells, within milliseconds. This ultra-precise control of neurons provides hope for treating previously untreatable disorders including post-traumatic stress disorder (PTSD) or epilepsy.

PTSD is characterized by high levels of anxiety and fear related to a memory. Dr. Boyden and his team were able to reverse this type of fearful memory in a mouse model using optogenetics. They had induced a model for PTSD into the mouse by introducing a shock every time a tone was played. As a result, the mouse would freeze, a behavior expression of fear, every time the tone was played. To treat the mouse of this memory-related fear, they activated the prefrontal cortex, often referred to as the cognitive center of the brain, by shining light on the newly expressed channelrhodopsin. By activating the prefrontal cortex simultaneously with playing the tone, the fear of the tone was reversed, and the mouse was cured of its induced PTSD within only 10 minutes.

Another application that Dr. Boyden mentions with great excitement is the treatment of epilepsy. A symptom of epilepsy is greater risk of seizures, which are characterized by overactive neurons and sporadic brain activity. Seizures can have major effects on the brain and leave permanent damage if not controlled quickly enough. As an optogenetic treatment, we could make neurons in the brain express a different type of light-sensitive opsin which silences cells instead of activating them. During a seizure, you would expose the brain to light, causing the switches to turn the cells off and thus reduce brain activity and restore healthy firing patterns.

As mentioned before, treatment of PTSD and epilepsy are just the tip of the iceberg of all possible optogenetic applications in medicine. However, while the current clinical trials and novel data are promising, they do not come without risk and there are various ethical considerations to be made before wide application is possible. Optogenetic treatment involves editing the human genome and contributes to the transhumanism movement as we introduce exogenous DNA into people. This brings up the ethics of clinical testing, a more philosophical debate of free will, and questions about how comfortable we are disrupting human evolution on a large scale.

OptogenEthics

For obvious reasons, people like Dr. Deisseroth and Dr. Boyden refrain from discussing the ethical implications of gene editing and prefer to focus on the medical benefits. However, editing the DNA of a person to treat a disease remains controversial and should be discussed as the first clinical trials are being conducted.

The primary goal of phase 1 testing in the GenSight and RetroSense clinical trials is safety rather than efficacy. This brings up an interesting ethical debate about phase 1 human trials and risk-benefit ratio for the patient. If the researchers are only assessing safety

and not the medical benefit, what is in it for the patient? And who really benefits from the research? The risk-benefit ratio seems disadvantaged for patients as they are receiving an irreversible treatment that will only be assessed for safety. On top of that, participants are excluded from any future innovative, perhaps more effective, therapeutic trials, increasing the individual risk of enrolling in these trials even more. Including efficacy in phase 1 clinical trials has long been a debate in the medical field and there are many cancer treatment trials that have already accepted the efficacy endpoint as a norm[1]. The highly invasive aspect of optogenetic treatment and skewed risk-benefit ratio are important points of debate when discussing the future of the current optogenetic clinical trials.

Another interesting aspect of the optogenetic trials on blindness is the nature of the disease. Since blindness is not a terminal illness, this treatment is considered to be a non-life-preservative intervention. Some argue that patients suffering from a terminal illness might only have one shot at a potential new treatment, therefore increasing the ethical acceptability of phase 1 safety testing in these individuals. In the case of treating blindness, however, the aim of the treatment is much different. The treatment is designed to improve the quality of life, but adverse outcomes of the treatment could quickly outweigh the potential benefits.

Optogenetic treatment also brings a more philosophical question to the table. Optogenetic headlines often include phrases like "how to take over a brain" or "external control of the brain". While these statements speak to the incredible power of optogenetics, it also introduces an ethical dilemma of free will. You might argue that externally controlling the brain can threaten human autonomy and identity by questioning:

"What becomes of the individual, whose brain can now be manipulated via remote control?"[11]

Are these patients potentially losing a part of their autonomy and identity? Will introduction of algae proteins into a human brain have unforeseen effects on the psyche? Are treated patients under too much control of the environment? With so little knowledge about how the mind is formed from our biological brain structures and interaction with the world, these are questions worth asking.

Finally, altering human genomes with DNA derived from a different organism, could lead to unpredictable effects on human evolution. Richard Dawkins, author of The Selfish Gene, argues that our genetic building blocks, our DNA, are the oldest driver of human evolution. Genes have been passed on for many generations and we are, as Dawkins argues, mere transporters of the genes through time. This is how human evolution has always worked, but with current developments in cheap DNA sequencing, gene editing techniques like CRISPR, and genetic therapeutics like optogenetics, we are at risk of disrupting the natural process of evolution. This could have major effects on humankind that we simply cannot predict. As we enter an age where transhumanism is a rapidly growing field, these are topics necessary to discuss before moving forward with novel genetic therapies like optogenetics.

Nevertheless, these clinical trials are a source of hope for families like patient Pittsburgh's and Rosalinda's. About a decade ago, their doctors would have nothing to offer them, no hope for improvement, just acceptance of living in the dark for the rest of their lives. Now, with the rapid improvement of optogenetic treatment, there is light at the end of the tunnel again. ■

REFERENCES

1. Gilbert, F., Harris, A., Kapsa, A. Controlling Brain Cells With Light: Ethical Considerations for Optogenetic Clinical Trials. *AJOB Neuroscience*, 5(3), 3-11 (2014).
2. California Institute for Regenerative Medicine. A Stem Cell-Based Therapy for Retinitis Pigmentosa: The Patient's Perspective. *YouTube* (2014).
3. GenSight Biologics. Dose-Escalation Study to Evaluate the Safety and Tolerability of GS030 in Subjects With Retinitis Pigmentosa. *ClinicalTrials.gov* (2018).
4. Allergan. RST-001 Phase I/II Trial for Advanced Retinitis Pigmentosa. *ClinicalTrials.gov* (2015).
5. cmurobotics. RI Seminar: José Alain Sahel, MD : ...Artificial Vision, Artificial Retina, Optogenetics. *YouTube* (2017).
6. GenSight Biologics Announces Positive Data Safety Monitoring Board Review and Continuation of PIONEER Phase I/II Clinical Trial of GS030 Combining Gene Therapy and Optogenetics for the Treatment of Retinitis Pigmentosa. *GenSight Biologics* (2019).
7. Doroudchi, M. *et al*. Virally delivered channelrhodopsin-2 safely and effectively restores visual function in multiple mouse models of blindness. *Mol Ther*, 19(7), 1220-1229 (2011).
8. TED. Ed Boyden: A Light Switch for Neurons. *YouTube* (2011).
9. Mace, E. *et al*. Targeting channelrhodopsin-2 to ON-bipolar cells with vitreally administered AAV Restores ON and OFF visual responses in blind mice. *Mol Ther*, 23(1), 7-16 (2015).
10. Sengupta, A. *et al*. Red-shifted channelrhodopsin stimulation restores light responses in blind mice, macaque retina, and human retina. *EMBO Mol Med*, 8(11), 1248-1264 (2016).
11. Starkman, R. Optogenetics: A novel technology with questions old and new. *Huffington Post* (2012).