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## Critique of Experimental Procedures Targeting Retinitis Pigmentosa

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CRITIQUE OF EXPERIMENTAL PROCEDURES TARGETING RETINITIS  
PIGMENTOSA

by

Jenna Goss

A Thesis Submitted in Partial Fulfillment  
of the Requirements for a Degree with Honors  
(Zoology)

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## ABSTRACT

Retinitis Pigmentosa (RP) is a group of hereditary genetic disorders, all causing degradation of the retina through loss of rod photoreceptor cells, ultimately causing loss of vision and in many cases complete blindness. Despite the prevalence of Retinitis Pigmentosa disorders, safe and effective treatment methods have not yet been approved to stop or regain vision loss in patients diagnosed with RP. However, the ophthalmic field as a whole is working on several new technologies and procedures in order to both slow loss of vision as well as potentially regain lost sight. In this thesis, I describe the genetic causes and effects of retinitis pigmentosa, followed by an analysis of current experimental treatments. Possible treatments were screened based on the following criteria; potential release date, adverse effects, ability to treat children, convenience, and the exclusion of patients for each therapy. Finally, I lend to support my claim that stem cell therapy by mesenchymal stem cells through intravenous infusion appears to be the most effective and safest method of experimental treatments available today.

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## CHAPTER 1

### INTRODUCTION

#### Retinitis Pigmentosa

Retinitis Pigmentosa or RP refers to a group of inherited conditions all resulting in a gradual progressive reduction of vision through the loss of light-sensing cells of the retina (Zhang, 2016). Disorders of the eye, related to RP can be caused by variations in any of 60 genes that affect the retina, these variations lead to the eventual death of photoreceptor cells and pigmentation abnormalities. The differences in the degree of vision loss experienced by an individual correlates to the number of retinal cell deaths, with complete blindness being uncommon (Daiger, 2007). Retinitis pigmentosa disorders are hereditary. The gene affected often influences the speed of photoreceptor cell loss, level of progression, and displayed symptoms (Zhang, 2016). However, the typical progression regardless of other factors is as follows; loss of night vision (night blindness), blind spots in peripheral vision, loss of peripheral vision, and effects to central vision which often impairs tasks such as reading, driving, and recognizing faces (Birtel et al., 2021). Some patients can retain cone photoreceptor cells if disease progression is slow enough as to not induce catastrophic damage to the rest of the eye, additional some patients' photoreceptor loss is slow enough that they will not reach blindness before end of life.

## Structures Affected

The disease primarily affects three related components of the eye; the general retina, photoreceptor cells, and the retinal pigment epithelium (RPE). The retina is located at the back of the eye and is responsible for the detection of both light intensity and color. It is constructed of millions of light-sensitive cells called photoreceptors. Once fully developed, the photoreceptor cells cannot regenerate if damaged, leading to a progressive loss of function in each affected photoreceptor. With the substantial loss of photoreceptor cells the retina becomes unable to function properly leading to an inability to process and transmit sight information to the brain (Zhang, 2016). There are two types of photoreceptor cells: rods, which allow for light signaling, and cones which are responsible for fine detail and color, together they perform phototransduction which converts light to signals that can stimulate biological processes (Molday & Moritz, 2015). These structures are supported by the pigment epithelium, a pigmented layer of cells next to the retina which serves as a barrier between photoreceptors and a layer of blood vessels. The role of the RPE is to nourish the nerve tissue of the retina and maintain its health (Klimanskaya, 2006). When photoreceptors die, imbalances within the eye begin to occur, including oxidative damage. This oxidative damage can harm the pigment epithelium and cause significant risk for photoreceptor cell death due to lack of nourishment.

## Phototransduction

The above structures are critical to an early stage of vision that contributes to our visual processing system termed phototransduction. In this process, photoreceptor cells serve as intermediate messengers assisting in the process of sending signals of light

perception to the brain. This is accomplished as photoreceptor cells are stimulated by the intensity of light. After light is absorbed by a photoreceptor its cell membrane hyperpolarizes thereby reducing the rate of transmitter release. In dark conditions, the photoreceptors are depolarized, and the rate of transmitter release is high. Change in polarization is facilitated in the cell by ion channels permeable to  $\text{Na}^+$  and  $\text{Ca}^{++}$  in the outer segment of the membrane. As light is introduced, the channels close, allowing hyperpolarization to occur, thus reducing transmitter release to the photoreceptor synapse. cGMP levels regulate the opening and closing of the channels. In darkness, cGMP levels are high, keeping channels open whereas when exposed to light, levels drop, and channels begin to close. Photons create the cascade that reduces cGMP levels mediating the rate of the photo transmitter release. The photon will first be absorbed by a photopigment, rhodopsin, containing a light-absorbing chromophore, in the receptor disk of the cell. After absorbing the photon, a conformational change will occur triggering a series of alterations in the protein component of the molecule. The alteration activates the intercellular messenger transducin. Transducin then activates a phosphodiesterase that hydrolyzes cGMP lowering its concentration therefore reducing its binding ability to the channels, which keep them open (Stryer, 1983). The transmitters previously mentioned signal the numbers of photons absorbed thereby communicating the quality and quantity of light by graded membrane polarization to bipolar cells also housed in the retina, bipolar cells can then communicate with the ganglion and amacrine cells of the brain (Li et al., 2012). When photoreceptors are damaged or lost due to retinitis pigmentosa conditions, this photoreceptor-bipolar-ganglion cellular pathway is first dampened and then destroyed. After cells have become dormant or begun apoptosis, they can no longer

serve phototransduction functions. RP-related diseases often attack the rod cells first which lie on the periphery of the eye, leading to loss of peripheral vision and producing tunnel vision in early stage patients. Since both rod and cone cells complete phototransduction, a patient can still have some vision even after all rod cells are lost. However, complications of rod cell death often trigger the death of cone cells as well, leaving no remaining cells with the ability to photo-transduce signals.

### Hereditary Development

Retinitis Pigmentosa, because of its serious hereditary influences on retinal development, greatly complicates any possible treatments. First, the disease can arise from a number of genes, many of which have not yet been identified, leading to complications in the diagnostic process and application of appropriate treatments. Additionally, retinitis pigmentosa can arise through several modes of inheritance making diagnoses increasingly difficult, as well as result from several different mutations on the same gene which add to the incredibly large number of variations the disease may take on. Each gene associated with the disorder is directly linked to photoreceptor cells, affecting structure and or function of the cell in some capacity. When mutations arise within these genes a gradual loss of photoreceptor cells in the retina begins, therefore characterizing the disorder (Fahim et al., 1993).

The current modes of inheritance causing disorders connected to RP include; autosomal dominant which can result from a single inherited dominant mutated allele, autosomal recessive or requiring a mutated allele from both parents, X-linked which stem from mutation on an expressed X chromosome, or mitochondrial occurring through maternal mitochondrial DNA. Mutations can also arise with no previous family history

indicated which are categorized as isolate (*Phelan, Mol Vis 2000; 6:116-124.*, n.d.). This heterogeneity makes not only determining which gene is affected incredibly difficult, but also genetic counseling and approaches for gene therapy.

Research on this topic has exponentially increased in recent years with several dozen teams across the world attempting to identify the genetic roots of RP. However, only 56 of the estimated 70 or more genes which may cause these disorders have been identified, making it possible to detect the specific mutations in only 30-80% of cases (*Phelan, Mol Vis 2000; 6:116-124.*, n.d.) (Daiger et al., 2013). Utilization of deep sequencing or next-generation sequencing (NSG) has aided researchers in the discovery of new genes causing RP. As well, several of the diseases overlap with one another and single gene mutations have been found to cause multiple diseases, this is exemplified by the fact that some identical mutations have been found to manifest and produce different clinical findings. The heterogeneous nature of RP causes greater confusion as RP can arise from over 3,100 mutations across more than 50 genes. RP is defined as genetically, allelically, phenotypically, and clinically heterogeneous, this creates substantial confusion for clinicians and researchers desperately attempting to diagnose and create new treatment approaches (Daiger et al., 2013).

### Genes Discussed in Experimental Treatments

#### PDE6A and PDE6B Mutations.

Both PDE6A and PDE6B are catalytic subunits of phosphodiesterase 6 (PDE6), a holoenzyme located in the integral membranes of retinal photoreceptors, responsible for the reduction of cytoplasmic levels of cyclic guanosine monophosphate (cGMP). While mutation in both subunits have been seen, the individual properties of PDE6A and

PDE6B are unknown and therefore they are often discussed in tandem (Muradov et al., 2010). When this enzyme is lost due to mutation of the gene producing PDE6A or its subunits, persistent and chronic elevated cGMP levels can occur (STZ eyetrial, 2020). As a result of elevated cGMP increased calcium concentrations begin to flow into the cell causing the hyperactivation of cell death pathways, therefore contributing to the death of photoreceptor cells. Additionally, due to cGMP's role in phototransduction, high levels can lead to problems in the process of visual signal transmission and amplification when incorrect concentrations are present therefore disrupting the phototransduction process and ability to convert light signals into sight (*PDE6A Phosphodiesterase 6A [Homo Sapiens (Human)] - Gene - NCBI*, n.d.).

Rd1 Mice. The Rd1 mouse was created in order to model autosomal recessive RP patients, so researchers could utilize them in retinitis pigmentosa experimental testing. In order mimic this form of RP, the mice are genetically modified for a mutation that affects the “expression of the  $\beta$  subunit of phosphodiesterase 6 (PDE6) leading to accumulation of cGMP that is thought to trigger rod photoreceptor degeneration (Kalloniatis et al., 2016).” These mice are used in several methods of experimental testing including those that target the specific mutation and others such as antioxidant treatments and stem cell therapies which attempt to combat the harmful cascade created in the eye when photoreceptors begin to die.

#### PRGR Gene Mutation

RPGR gene mutations account for about 10 -15% of RP cases and approximately 70% of X-linked RP cases. Despite its prominence, the mechanism which results in photoreceptor cell dysfunction due to modified RPGR genes is unknown (Zhang et al.,

2019). It is currently known that the gene functions to provide instruction for protein creation necessary for normal vision, however, the protein products function is still unknown. Studies suggest that the protein created is integral to cilia function, which assists in the perception of vision. One protein segment created by RPGR is as the ORF15 exon, this exon or protein, is expressed solely in the retina and within photoreceptor cells. It has been suggested that the protein product may regulate the function of cilia which are thought to play a role in maintaining photoreceptors (*RPGR Gene*, n.d.).

## CHAPTER 2

### RETINITIS PIGMENTOSA TREATMENTS

#### Current Treatment

Unfortunately, due to the heterogeneity listed above, learning about both RP and its effective forms of treatment has been extremely difficult. Currently treatment for those who are diagnosed are based on palliative care and helping those with the disease to maintain the best standard of living possible with loss of vision and blindness. No FDA approved treatments have been found to stop disease progression or return lost vision. Until a cure is found clinicians will continue to administer standard palliative treatments recommended by the National Eye Institute including; receiving care from occupational therapists, orientation and mobility specialists, and certified low vision therapists. Patients are also evaluated and administered low vision aids such as special lenses, computer text programs, closed circuit television, and portable lighting. Vitamin A treatments and the implementation of Argus II, an electrode which helps relay information to the brain, have been used in late stage retinitis pigmentosa patients in which the electrodes probable benefits outweigh potential harm from the device (*Retinitis Pigmentosa* | *National Eye Institute*, n.d.).

#### Experimental Treatments

Just as the disorder is complex and diverse, the experimental treatments being explored are as well. Treatments currently being studied range widely and aim for solutions including, preventing disease progression, restoring partial vision, and complete

restoration of vision. Currently, Vitamin A supplements and Argus II implementation are becoming more widely used methods of treatment. However, these treatments are unable to stop the disease progression or restore lost vision. The procedures discussed in this section are working towards vision and photoreceptor restoration. With further exploration, scientific advances could begin to restore lost sight in retinitis pigmentosa patients.

The following treatments were selected for based on both the most recent experimentation while taking into consideration mutation and method diversity. The following studies range in treatment for most retinitis pigmentosa patients to highly targeted methods such as those focused on the Pde6 gene and X-linked cases. Each study also utilizes a different form of potential intervention including antioxidants, optogenetics mediated gene therapy, subretinal injection, sub-tenon injection and intravenous infusion. While only three of these potential treatment methods focus on human studies, due to lack of trials which have reached this phase of testing, animal models such as the Rd1 mice, college surgeon rats, and mutated dogs can serve researchers in gaining valuable insights and data on how these treatments may affect the human eye with similar mutations.

### Antioxidant Treatments

Oxidative Stress and Retinitis Pigmentosa. As previously discussed, Retinitis Pigmentosa is a genetic disorder, which can be induced by a number of differing gene mutations leading to the loss of photoreceptor cells in the eye. Due to the clinical symptoms, it has been deduced that the rod photoreceptors which account for 95% of all photoreceptors are typically first to become affected, producing the tunnel-like vision

symptoms while the central vision cones are maintained. However, over time complete blindness can occur which is thought to be the result of oxidative stress related to photoreceptor cell loss. A catastrophic cascade is induced when rod cell death begins, rods are responsible for metabolizing excess oxygen and as they are reduced a buildup of oxygen occurs with fewer cells capable of metabolizing it, therefore this loss of cells causes toxic oxidative stress to the entire system. This cascade was summarized as “a cascade of molecular phenomena—such as para-inflammation, synaptic impairment, apoptosis, and cell death—which hugely impact visual function, is triggered (Limoli et al., 2020).” As more rod cells continue to die from both the disease and now present oxidative stress even more excess oxygen is accumulated which eventually begins to cause cone cell apoptosis and total vision loss. While the body does have mechanisms in place to alleviate oxidative stress, the rapid loss of cells creates an insurmountable environment imbalance, often the retinal pigment epithelium is also damaged by the rod loss and subsequent oxidative damage. Transduction pathways and gene expression are also affected by oxidative stress damaging all cellular components including phospholipid membranes, proteins, and nuclear and mitochondrial DNA causing even more damage to the photoreceptors and RPE (Komeima et al., 2007). At this point retinal autophagy to catabolize the oxidatively damaged proteins and organelles may occur, but this can cause a buildup of autophagosomes, which deliver engulfed substrates into lysosomes for degradation. This leads to continued and increasing stress which only harms cells further, leading to apoptosis of all cells within the system, including the cones.

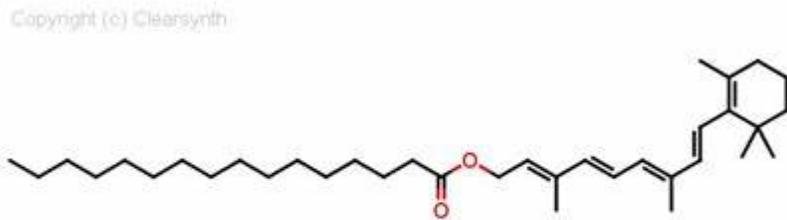
### Antioxidant Treatments in Mouse Models of Retinitis Pigmentosa.

Antioxidant treatments are attractive as they may not require gene specific approaches to help delay the loss of photoreceptors. However, these treatments must begin before any significant loss of photoreceptors to be effective. This treatment could substantially decrease the amount of oxidative pressure on the eye therefore increasing the chances of survival of the remaining rods and cones. In the following study conducted by John Hopkins School of Medicine, antioxidant “cocktails” were administered to several different testing mice to see the effects on different variants of hereditary retinitis pigmentosa (Komeima et al., 2007).

Prior to the above study, the slowing of RP through the use of antioxidant “cocktails” had been achieved in Rd1 mice. These mice are genetically mutated to model rapidly progressive recessive RP. The Rd1 mice treated with antioxidant cocktails produced results which indicated a slowing of the progression of rod cell death and reduction in oxidative damage. Therefore, the treatment created a better environment for the maintenance of cone cells (Sanz et al., 2007). The Hopkins study explored if similar methods could be effective for other models of RP, therefore, they used both Rd10/Rd10 and Q344ter mice. Rd10/Rd10 mice house a mutation on the same gene as Rd1 mice, however they provide a model for more slowly progressive RP, while Q344ter mice carry the mutation of many patients with rapidly progressive dominant RP. While results varied between the mice, both showed positive results. The Rd10/Rd10 mice exhibited a slowing of both cone and rod cell death as well as preservation of photoreceptor function. In the Q344ter mice, there was not significant slowing of rod death, likely due to the dominant mutation which produced toxic gene products fatal to rods, however, cone

density was significantly greater in antioxidant treated mice. This finding suggests that cone death is a result of oxidative damage as well as that it can be mediated with the correct combination of antioxidants (Komeima et al., 2007).

Vitamin A Palmitate Antioxidant in Human Trials. Vitamin A Palmitate trials are currently being conducted in order to see if its antioxidant properties can produce slowing of rod photoreceptor deaths and survival of cone cells by diminishing oxidative stress. These trials are currently being conducted in humans and have produced a wide variety of results, with differing validity values concerning if rod cell death is being slowed. Clinical researchers appear to have differing opinions on the vitamins ability to slow the effects of RP including retinal loss of function in adults (Berson et al., 2018). No conclusive evidence has been found on its ability to slow rod cell degradation or protect cones from oxidative stress. Studies in children also show no conclusive results (Schwartz et al., 2020).



(Figure 1. Structure of Vitamin A Palmitate)

## Gene Therapy

Optogenetics- Mediated Gene Therapy. Unlike potential antioxidant therapies optogenetic – mediated gene therapy is highly targeted, however, if successful it could bring treatment to a large division of RP patients. This therapeutic approach is currently being investigated in the same Rd1 mice used in antioxidant studies who have a “mutation of rod cGMP- specific 3',5'-cyclic phosphodiesterase subunit beta (PDE6B) gene related to the hydrolysis of cGMP”, a mutation similar to many human patients suffering from autosomal recessive RP (Tomita & Sugano, 2021).

One form of gene therapy is transducing ChR2 or Chlamydomonas- derived channel rhodopsin -2, into differing order neurons. Several studies were summarized each producing greater light stimulation after gene-mediation. When the retinal ganglion cells were targeted in Rd1 mice, visual responses were restored. The same phenomenon occurred when Royal College Surgeons rats, which have similar mutations to Rd1 mice, were treated with an adeno-associated virus- mediated ChR2 gene transferred into ganglion cells. Additionally, reports of restoration of vision have been found in Rd1 mice in which the gene was transduced into the second order neurons, or bipolar cells, which is thought to contribute to the restoration of connection in the phototransduction pathway. While still in preliminary phases, researchers hope to transduce the gene into third-order ganglion neurons, which would restore visual functioning of any surviving retinal neurons. Clinical trials have begun using ChR2 in RP patients in the United States.

Despite the considerable promise of ChR2 – gene therapy, multiple limitations of the therapy were cited. These included low light and wavelength sensitivity which necessitated intense light in order to activate the cells and the high intensity light needed

to activate the cells toxic effect on native photoreceptor cells. Additionally, most objects reflecting wavelengths below 550nm appeared blue to the small animal treatment subjects. Due to these limitations, another gene therapy is being studied using mVchR1 or Volvox- derived ChR1, which has a broader, red- shifted action spectrum potentially allowing RP patients to see a broader range of colored objects. In this form of gene-mediated therapy, blind rats were treated with AVV vectors and stimulus was recorded at 468nm of LED, therefore indicating a broader range of color could be seen with this treatment (Tomita & Sugano, 2021).

AAV- Pde6a Gene Therapy in Large Animals. Researchers at the University of Florida College of Medicine were also interested in AVV vectors, however, they decided to isolate the PDE6A gene in order to produce positive results of photoreceptor survival. Mutations in the alpha or beta subunits of the rod phosphodiesterase (PDE6) result in autosomal recessive RP, and each of these mutations respectively account for approximately 4% of patient cases or 8% together. Ten PDE6A mutant dogs were studied and received subretinal injections using Pde6a delivery by capsid – mutant adeno-associated virus serotype 8 (Mowat et al., 2017). Prior attempts with AVV serotype 5 gene augmentation therapy were unsuccessful, however they prompted the researchers to develop this therapy. It was hypothesized that delivery of the treatment needed to be conducted as the eye was still developing and the use of highly efficient, rapid- onset, viral vectors was necessary (Mowat et al., 2017). Thus, the dogs were injected at a mean of 36 days of age, while their eyes had not yet fully developed, with one eye injected with AAV-Pde6a and the other eye left alone for control.

The dogs were monitored over four months with several forms of retinal testing for thickness and light responses, as well as participating in tunnel mazes with both their injected and uninjected eyes separately. After testing was completed, the dogs were euthanized, and their retinas preserved for further testing unable to be done on a living eye. The study produced positive results in almost every testing field, however, some adverse effects were observed in the treated areas. The results included improvement to dim light vision as measured by electroretinographic testing, preserved photoreceptor layer thickness, evidence of rod function, improved survival of both rods and cones, and return of appropriate rhodopsin localization. Unfortunately, persistent regions of retinal separation and rosette formation occurred in both the AAV-Pde6a and control vector injected regions, suggesting a flaw in administration method rather than the injected gene (Mowat et al., 2017).

Initial X-linked Gene Therapy Results in Humans. Clinical trials have begun using similar methods to those in the previous study, initial findings support that with the use of sub-retinal injected adeno-associated viral vector encoding codon optimized human RPGR (AAV8-coRPGR) gains in visual functioning can occur. However, the requirements for eligibility in this treatment are incredibly specific and only proved positive results for six out of eighteen male participants, all suffering from a form of X-linked retinitis pigmentosa resulting from RPGR gene mutation. While the results of this study were varied and six patients had increased visual performance, it was determined through this study the “mid-dose” range of the vector was most successful without causing negative effects related to inflammation (Cehajic-Kapetanovic et al., 2020).

Stem Cell Therapy

Management of Retinitis Pigmentosa by Wharton's Jelly derived Mesenchymal Stem Cells (MSC'). One of the most promising methods of photoreceptor preservation thus far has been the use of mesenchymal stem cell injection into the sub-tenon space which revives dormant cells and can halt the effects of retinitis pigmentosa symptoms within the retina. In this study, 32 patients in differing stages of retinal decline and suffering from different forms of retinitis pigmentosa, received injections of MSC's. The stem cells were acquired from a single umbilical cord given with the mother's consent and injected into the sub-tenon space of the eye. Only one eye was tested per individual and results were compared between the injected and non-injected eyes. These patients were followed for 6-months receiving an extensive panel of ophthalmic testing at one month intervals. Once injected, the MSC's are assumed to work as an anti-inflammatory agent, help nourish and maintain the retinal pigment epithelium and reactive dormant rod photoreceptor cells. Significant improvements to rate of photoreceptor loss or maintenance of progression were seen in a majority of injected patients. The photoreceptors of multiple patients regained thickness in the inner rings, 1 through 3, and showed improvement in visual functioning, however, the outer fourth and fifth rings showed little to no improvement throughout the testing pool. However, it is known that most forms of RP develop from the outer periphery vision inward so it is likely these cells had already undergone apoptosis. (Emin & uuml et al., 2020).

Intravenous Infusion of Umbilical Cord Mesenchymal Stem Cells. Similar to the prior study, another team of researchers conducted trials with MSC's, however, instead of performing surgery or injection into the sub-tenon space, the cells were introduced into patients through the form of intravenous infusion. This study was composed of 32

individuals, all suffering from late stage clinical RP. Results were similar to those when the treatment was injected, however, the cells effectiveness was for a slightly shorter duration. In this study, 96.9% of patients improved or maintained vision 1 month after infusion. Some patients experienced vision losses, however vision loss is to be expected in individuals with RP, and likely did not indicate negative effects, rather no positive results. The positive effects for most diminished after three – months post infusion when most stopped experiencing visual gains. However, 81.3% patients maintained the results of improved vision throughout the twelve month trial period. With no serious adverse effects in any patients, this study indicated the safety of intravenous MSC injection, as well as the potential positive effects for patients suffering with multiple forms of retinitis pigmentosa (Zhao et al., 2020).

## CHAPTER 3

### RETINITIS PIGMENTOSA TREATMENT DISCUSSION

#### Current Stages of Experimental Treatments

This section covers the current state of treatments concerning Retinitis Pigmentosa followed by my suggestion of a future treatment. While some treatment options may be better than others, each treatment discussed below holds the potential to help those struggling with Retinitis Pigmentosa. Generally, those first approved from clinical trial will likely be used until better treatment methods are found. However, some are far closer than others to clinical release. One antioxidant treatment, Vitamin A palmitate, is currently in use as a treatment option for patients suffering from most forms of retinitis pigmentosa but unfortunately, after years of study, no conclusive or significant positive effects have been indicated. Currently there are no antioxidant cocktails in clinical study. Therefore, of the treatments discussed we are least likely to see them used in the near future. In order to do so researchers will first need to solve problems such as which antioxidants to include, amounts of each, and best method of administration.

Gene therapy for retinitis pigmentosa, conversely, has recently entered very early clinical trial stages in specific retinitis pigmentosa patients. As earlier discussed, some patients housing X-linked PRGR mutations showed positive results when treated with AAV8-coRPGR gene therapy. Mice, rats, and dogs with PDE6A and PDE6B mutations have shown gains in visual functioning through optogenetic mediated and AVV- Vector gene therapy. While it is difficult to assess a timeline of trials still focused on small and

large animal species, the initial trials in x-linked RPGR patients showed that through sub-retinal injection the gene therapy could delay photoreceptor death. Also, with the additional information from the trials indicating the optimal dosage of this treatment it is likely to see great gains in the near future, but is still not yet effective for treating this specific disorder.

Of the all the treatments discussed, stem cell therapy appears to be the closest to clinical approval. In both forms of clinical treatment, subretinal injection of MSC's and intravenous infusion of MSC's, significant positive results indicating the slowing of photoreceptor cell death and even the revival of dormant cells were seen with very few if any adverse side effects. Based on their relative success and lack of serious adverse effects we will likely see these forms of treatment approved soon.

#### Adverse Effects of Treatments

It is also important to consider the adverse effects of each treatment. It is difficult to discuss those of the antioxidant treatment as the cocktails were studied in mice, and we do not yet know how they will react with the human body. Additionally, while Vitamin A palmitate is not known to have any serious adverse effects, it has also not been proved to be an effective form of treatment. However, other forms of experimental treatment have caused serious side effects which could lead to the loss of vision in some cases, most occurring in gene therapy treatment approaches. Within the study utilizing PDE6A dog's serious complication from injection occurred including retinal separation and rosette formation. The human X-linked trials also had adverse effects, although less serious, reporting blebs, or fluid filled blisters on the surface of the eye, at the injection site. Post injection irritation and inflammation also occurred in a few of the cases. While these

forms of therapy could ultimately restore vision, the risks seem to be greater with the method of administration currently used. However, injection into the sub-tenon space, as completed by the Wharton's jelly derived stem cell trial, could decrease the number of adverse effects seen as the patients in these trials did not experience any severe adverse effects, nor did those administered intravenous MSC's.

Unknown Long Term Effects of All Treatments. It is also important to note that researchers are unsure of the long term effects of all stated experimental trials. Once again, antioxidants are difficult to discuss as these treatment approaches have not yet been tested in humans, but there could be complications due to a buildup of introduced vitamins and foreign materials.

In regard to stem cell therapy treatment, these therapies could cause the development of severe long term side effects as it would currently require regular treatments. It has been stated in both studies that the effects of the initial injection will not remain for extended periods of time, requiring multiple procedures in order to maintain vision. Both methods cited few adverse side effects, however, with repeated and continued use this may not be the case. With subretinal injection evidence indicated benefits were highest up to six months and intravenous up to three. Both papers discussed issues that could arise from the rapid nature of proliferation in stem cells. If these cells were to migrate to undesired areas and build up in inappropriate locations, this could cause a slew of issues for other systems. Also, with additional therapies injection sites become more at risk for complications while sub- tenon injection seems to be a highly beneficial method without the issues of sub-retinal injections experienced, it is unknown the damage repeated exposure could cause.

Similarly, to stem cell therapies, gene therapy if completed successfully would likely require multiple procedures to re-introduce the desired vector. Additionally, patients may experience unpredictable adverse effects several months after treatment, which is currently unknown due to the early stages of clinical trial many gene therapies are in.

### Implications of Early Age Onset and Treatment

One of the difficulties of any treatment targeting Retinitis Pigmentosa is both the young age of onset and rapid nature of photoreceptor loss. Photoreceptor loss due the disease can begin to occur in young adults although age of onset varies widely, often correlating to which gene is affected. With the discovery of more of the genes which induce retinitis pigmentosa diseases, early diagnosis has become far more common. However, once noticeable symptoms begin to be displayed many rod photoreceptors have been lost due to the disease. As discussed earlier, no current treatments exist that have the ability to rescue lost photoreceptors after apoptosis has occurred and only stem cell therapy has been seen to rescue those that have entered dormancy. This issue was exemplified in the gene therapy treatment of the PDE6A dogs as the injections worked most effectively at approximately 36 days post birth and in only still developing eyes. This creates difficulties for researchers as often young patients would benefit most from and be ideal patients for treatments designed to stop photoreceptor death as they would be in early stages of disease progression. However, experimental clinical testing is not often conducted on children, as parents would be unlikely to allow their children who have not yet experienced loss of vision to participate. This calls for a treatment which would be safe for children. Antioxidant treatment stands out, as if an effective cocktail can be

created children could take these supplements orally, removing the need for multiple clinical visits, injections, or surgeries. Intravenous input of stem cells also appears to be a more child friendly approach when compared to the subretinal injection of gene therapy, or sub-tenon injection of stem cells, decreasing the need for doctor visits and invasive procedures.

#### Convenience and Requirement of Recurring Visits

With treatments one must consider the convenience of treatment as well as the requirement of physician administration and therefore doctors' visits in order to administer treatment. In the case of a discovered antioxidant treatment likely they could be taken orally requiring only a prescription appointment and check-in intervals to determine the effectiveness of treatment. This set-up is far different than that of both gene therapy and stem cell therapy treatments, although the duration between each treatment may vary, both will require repeated injection or infusion in order to maintain decrease of photoreceptor loss or visual gains, this means routine doctors' visits for the patient as well as repeated potentially invasive treatments to see sustaining positive effects.

#### Patient Exclusions of Each Therapy

One of the most attractive features of antioxidant therapy is its ability to potentially decrease the loss of photoreceptor cells in all retinitis pigmentosa patients. This treatment targets patients with remaining vision as it strives to mediate a consequence of rod photoreceptor loss, instead of the more complicated cause of loss. In theory, most retinitis pigmentosa diseases do not target the cone cells, but their death is caused by the extremely oxygen saturated environment created when rod cells die. If

cones were able to be preserved through these treatments, patients would only lose peripheral vision as opposed to complete vision loss. If a treatment is completed, every patient suffering from early stage retinitis pigmentosa and oxidative damage may be able to have the remaining vision produced by cone cells. Though, any patients with complete blindness, or substantial loss of cone photoreceptors could not be helped through this treatment.

Similarly, stem cell therapy strives to serve a large percentage of retinitis pigmentosa patients, the treatments can be used for those suffering from multiple different forms of RP resulting from several different gene mutations. This form of treatment is slightly less inclusive than antioxidants. All treatment subjects were required to have no previous history of treatment mediation due to complications that could be produced from interacting with previous interventions. Additionally, yet again, patients already suffering from complete blindness or dramatically reduced vision, are unlikely to see benefits from these treatments due to their inability to restore dead photoreceptor cells.

Conversely, gene therapy approaches are often highly targeted to singular genes and mutations. The genetic nature of the disease makes it a crucial target for gene therapy, however since the group of diseases is so diverse, these methods must be highly targeted and will only serve small percentages of retinitis pigmentosa patients per each targeted therapy. As previously discussed, human trials have begun on patients displaying X-linked PRGR mutations. If the treatment described does get approved by the FDA then it will only benefit those with this mutation which include only relatively young males, who are all evaluated for good health before trial, which means this method will likely

exclude many older patients with more progressive stages of retinitis pigmentosa due to other health concerns.

### Conclusion of Most Promising Therapy

With the lack of a treatment that is currently in use to help Retinitis Pigmentosa patients slow the loss of photoreceptor cells or regain lost site, the success of any of these treatments would be a substantial triumph for patients and researchers alike. However, they are not all created equal and through critical evaluation, stem cell therapy with MCS's through intravenous infusion appears to be the most promising. This treatment can not only service a wide range of patients with few adverse effects, but also will be able to potentially halt the rapid decline of photoreceptor cells. It also appears to be the closest to approval and minimally invasive. It is difficult to dismiss treatment with antioxidants as it could produce similar effects and would likely rank better in all of the categories previously listed. However, its development is behind the other treatments and thus has a more uncertain future. Additionally, gene therapy would likely provide better results and could even restore lost vision, but with its highly targeted nature, can only improve the lives of small portions of the retinitis pigmentosa community per perfected treatment. When comparing the two forms of MSC injection, sub-tenon injection of Wharton's Jelly derived MSC's does produce longer lasting results of photoreceptor death decrease but requires time consuming culture of materials and eye injection, which could lead to increased complications and adverse side effects with continuous injections.

In additional clinical trials, if intravenous stem cell therapy continues to be proven safe and effective, trials will likely expand to broader age ranges and patients with more progressive retinitis pigmentosa. With greater information of long term treatment effects

through testing, more funds dedicated to the culture and distribution of intravenous umbilical cord MSC's, and greater awareness of this form of treatment, I predict this treatment modality could be quickly heading to clinical approval. This would allow a majority of retinitis pigmentosa patients a method of treatment which may halt disease effects and photoreceptor death until a cure for the disease is discovered.

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