A Primer on the Use of Stem Cells in Ophthalmology

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I recently came across an interesting news release from ***International Stem Cell Corporation*** *(ISCO) announcing that it had formed a new business unit, Cytovis, to focus on stem cell programs in ophthalmology, including CytoCor for the cornea and CytoRet for the retina.*

That got me thinking about how little I knew about what was going on in stem cell research in ophthalmology, despite having written about two developments in the field, ***the London Project to Cure Blindness*** *and the* ***University of California Irvine (UCI) program to develop an artificial retina based on stem cell research****.*

I decided to become better informed by taking a closer look at what was happening in this field, and presenting that story.

Introduction

Commenting on a **EuroRetina Meeting** held earlier in 2008, John Morrow of **Newport Biotech Consultants** noted, as reported by **Ophthalmology Times Europe** in September 2008, "Stem Cells are looked upon as either an ethical train wreck or the gateway to the alleviation of human illness, depending on which side of the political spectrum one resides. This unfortunate notoriety has resulted in unprecedented coverage in the media, but this has not done much to advance the cause of this technology. Yet recent ophthalmologic research suggests that the medical applications of stem cells hold notable promise for the treatment of ocular degenerative conditions and that realization of this potential may come about in the near future."

I think Dr. Morrow's thoughts eloquently sum up the subject. Stem cell research is politically charged but holds tremendous promise for the future, especially in ophthalmology.

What are Stem Cells?

Every organ and tissue in our bodies is made up of specialized cells that originally come from a pool of stem cells in the very early embryo ("embryonic stem cells"). Throughout our lives we rely to a much more limited degree on rare deposits of stem cells in certain areas of the body ("adult stem cells") to regenerate organs and tissues that are injured or lost, such as our skin, our hair, our blood and the lining of our gut.

Stem cells are like a blank microchip that can be programmed to perform particular tasks. Under proper conditions, stem cells develop or "differentiate" into specialized cells that carry out a specific function, such as in the skin, muscle, liver, or in the eye. Additionally, stem cells can grow extensively without differentiating and give rise to more stem cells. These two characteristics,

“pluripotency” and “self-renewal”, distinguish stem cells from other cells in the body and give stem cells their tremendous therapeutic promise for a wide range of degenerative diseases.

The Four Types of Stem Cells

The four most commonly used and described classes of stem cells are "embryonic stem cells" (embryonic SCs, or human embryonic stem cells hESCs), "induced pluripotent stem cells" (ipSCs), "adult stem cells" (adult SCs) and "parthenogenetic stem cells" (hpSCs).

Besides the embryonic and adult stem cells already used by the body, two other classes of stem cells are increasingly used in medical research, the induced pluripotent stem cells and human parthenogenetic stem cells.

Embryonic stem cells are derived from fertilized human eggs ("oocytes") in the very early stages of development. They are truly pluripotent, in principle enabling them to become any body tissue and thus providing their tremendous clinical potential. However, embryonic stem cells are associated with significant ethical, political and religious controversy since a fertilized egg, under the right circumstances, has the potential to develop into a human. Another major (albeit much less published) issue with embryonic stem cells is that, since they essentially are a transplant from one person (the fertilized egg) to another person (the recipient patient) (“allogeneic treatment”), therapeutic cells and tissues derived from embryonic stem cells can be expected to provoke an immune response from the recipient and be rejected.

In contrast, induced pluripotent stem cells are adult and fully differentiated cells (e.g. skin cells) that are chemically, physically, genetically or otherwise driven back to earlier developmental stages. While creation of such cells does not involve the use or destruction of a fertilized egg, it does require dramatic changes in gene expression that may have unknown biological impact and likely will be subject to substantial scrutiny by regulatory authorities before any approval for therapeutic use. Also, due to immune rejection, induced pluripotent stem cells have to be derived from the patient themselves (“autologous therapy”) which significantly limits clinical use and adds time and cost that will be increasingly difficult to implement in cost-contained health care systems worldwide. Finally, induced pluripotent stem cells cannot be used for hereditary diseases therapy because of bearing the same genetic defects.

Adult stem cells are rare cells found in various organs or tissues in a person that have a limited ability to differentiate into cells with specific functions. They are older and less powerful than other types. While these stem cells do not require use or destruction of a fertilized egg or extensive manipulation of gene expression, they are rare and hard to identify and they generally proliferate poorly, thus making it hard to produce therapeutic amounts.

Parthenogenetic stem cells are derived from activated human oocytes. Parthenogenesis is a form of asexual reproduction in some amphibians and plants but does not occur naturally in mammals, including humans. ISCO scientists have discovered a process for chemical activation of human eggs,

similar to what the sperm does in normal fertilization but without any involvement of a male sperm. ISCO claims that this process results in hpSCs that are as pluripotent and proliferate as embryonic stem cells, yet avoid the ethical, political and religious controversy around use or destruction of human embryos with potential for viable human life. Furthermore, since there is no forced change of gene expression patterns, hpSCs are not likely to face the same safety and regulatory hurdle as induced pluripotent stem cells. Most importantly and unique relatively to all other stem cell classes, hpSCs can be produced in a simplified immunogenetic ("homozygous") form that enables each line to be an immune match for many millions of people (ISCO's first line is an immune match for an estimated 75 million people worldwide).

International Stem Cell Corporation has kindly provided the table below that describes the characteristics of the various types of stem cells.

Table 1. Characteristics of the Four Types of Stem Cells

	ISCO parthenotes	**Embryonic stem cells**	**iPS cells**	**Adult stem cells**
Pluripotent	Yes	Yes	Possibly	No
Proliferation Expansion to clinical volumes	Strong	Strong	Varies	Weak
Ethical Concerns Embryo us or destruction	None	Significant	None	None
Gene Manipultation	None	None	Substantial	None
Immune Matching ● Rejection ● Universal cell source	● No rejection ● Practical	● Reject ● Impractical	● Reject or autologous ● Impractical	● Autologous ● Impractical

Provided by International Stem Cell Corporation, September 2010

Table 1. Characteristics of the Four Types of Stem Cells

(Editors Note: Please take into account that the company specializes in Parthenogenetic stem cells.)

What are the Applications for Stem Cells in Ophthalmology?

The Front of the Eye

Scarred and degenerative corneas represent one prime area of research for the use of stem cells. Because of a lack of donated human cornea bank corneas for transplantation, especially in populous nations such as India and China (and the third World countries), the use of stem cells to regenerate damaged corneal tissues could become lifesavers in those countries where blindness due to damaged corneas is prevalent.

The Middle of the Eye

There are only a few research programs using stem cells for the middle areas of the eye, specifically in treating glaucoma. NeoStem has said that they are working with Schepens Research Institute in using the company's VSELs (very small embryonic-like stem cells) in the treatment of glaucoma (and AMD), and Stemedica claims to be working with the Fyodorov Eye Institute in Moscow on a glaucoma program.

I know of no programs targeting the lens.

The Back of the Eye

Most of the research efforts appear to be focused on the back of the eye, specifically retinal tissue and diseases. Areas of interest that I have identified include regeneration of retinal epithelial (RPE) cells for the treatment of both dry and wet forms of age-related macular degeneration (AMD); replacement of damaged photoreceptors; the growth of artificial retinas, again for treating AMD; and direct treatments for diseases such as retinitus pigmentosa (RP), retinopathy of prematurity (ROP), diabetic retinopathy (DR), Stargardts disease (Stargardt Macular Dystrophy) (SMD), and retinal veinr occlusions (RVO).

Who is Involved and What are They Doing?

In the following section, are short reviews of the seven companies identified as doing research with stem cells in ophthalmology, including who they are collaborating with, what type of stem cells they are using, and what ophthalmic diseases/eye structures they are attempting to treat.

The table at the end of this section summarizes the results.

Advanced Cell Technology Incorporated (Santa Monica, CA)

Advanced Cell Technology (ACT) is currently focused on using its proprietary technologies to generate stable cell lines including retinal pigment epithelium (RPE) cells for the treatment of retinal diseases such as age-related macular degeneration (AMD).

ACT has demonstrated the ability to rescue visual function in rats through implantations of RPE cells derived from hESCs, in collaboration with Raymond Lund at the University of Utah. The rats, blinded because of RPE degeneration, were injected with embryonic stem RPE cells into the subretinal space, which resulted in the restoration of their ability to see light and attained approximately 70% of the spatial acuity of a normal, healthy rat. In addition, the hESC derived RPE cells did not appear to cause any side effects in the animals.

The company subsequently entered into a sponsored research agreement with Oregon Health and Science University (OHSU). The company is collaborating with Drs. Lund, Richard Weleber and Peter Francis at the Casey Eye Institute to conduct preclinical studies for its RPE program. The company is also in discussion with the OHSU team to conduct a Phase I human clinical trial in this area.

Furthermore, after discussions with the FDA, ACT has contracted with a leading contract research organization to commence work on an extensive preclinical program. The company has conducted safety studies necessary for the initiation of a clinical trial. The protocols utilize therapeutic dosage levels of RPE cells, based on the rat studies, for the treatment of retinal disease. The company has been able to produce an unlimited amount of RPE cells for this clinical use. In addition, the company has developed novel methodology to cryopreserve the RPE cell products.

In late July 2010, ACT announced that it had submitted documentation to address FDA concerns with the company's plans to initiate a Phase I/II multicenter study using its line of hESC RPE cells to treat patients with Stargardt's Macular Dystrophy (SMD).

Editors Note: Stargardt's disease (also known as fundus flavimaculatus and Stargardt's macular dystrophy) is the most common form of inherited juvenile macular degeneration. Inherited as an autosomal recessive trait, it is a severe form of macular degeneration that begins in late childhood, leading to legal blindness. Stargardt's disease is symptomatically similar to age-related macular degeneration, and it affects approximately one in 10,000 children.

Unanswered Questions:

I asked a company spokesperson the following questions and received a few answers:

In the study of treating Stargardt's, who will be heading the clinical work?
That information is not currently available.

How many patients will be enrolled?
Twelve.

How many clinical centers will be participating?
Multiple, but the exact number has not yet been announced.

When will the first results be reported?
Again, that has not yet been announced.

I guess that we will just have to await further details.

AstraZeneca (London, UK and Wilmington, DE (US))

AstraZeneca and University College London (UCL) have announced a research partnership, to develop medicines that use stem cells to repair damaged eyesight in people with diabetes. Under the three-year partnership, funded by the drugmaker, researchers from AstraZeneca will team up with scientists at the UCL Institute of Ophthalmology to work on new medicines that use the regenerative capacity of stem cells. They hope to come up with a compound in three to five years, which could then undergo clinical development and possibly be on the market in 10 years' time.

Dr Marcus Fruttiger of the UCL Institute of Ophthalmology, who is leading the project, said: "These tools could be used either to manufacture transplantable material or to directly stimulate new cell growth in the eye to help restore or improve the vision of those with diabetic retinopathy (DR)."

AstraZeneca's U.S. rival Pfizer also has a partnership with Professor Pete Coffey of the UCL Institute of Ophthalmology, aimed at another eye condition, macular degeneration. Coffey said: "It's great that 'Big Pharma' is considering regenerative medicines as a serious possibility." He added: "This is British science being developed into a commercial entity with the pharmaceutical industry. It's a good example why the government shouldn't cut funding for biomedical research."

While this is the first time that AstraZeneca has worked on medicine for retinopathy, diabetes has been an area of focus. The company has a new diabetes treatment on the market called Onglyza, which was developed with Bristol-Myers Squibb, and the companies are developing a second diabetes drug that could be submitted to regulators for approval later this year.

International Stem Cell Corporation (Oceanside, CA)

On August 19, 2010, International Stem Cell Corporation announced that its stem cell therapeutic programs, focused on protective, transparent corneas (CytoCor) in the front of the eye and the light-sensitive retinal tissue (CytoRet) in the back of the eye would be formalized into a new business unit, Cytovis. Together these programs will leverage external and internal development, regulatory, and commercial expertise in cellular ophthalmology to form a focused portfolio of complementary product candidates designed to address high unmet medical needs with apparent pharmacoeconomic and quality of life benefits.

CytoCor is the brand name for ISCO's corneal tissue that can be derived from the company's proprietary parthenogenetic stem cells or commonly used embryonic stem cells. Research and development with partners Absorption Systems in the US, Sankara Nethralaya in India and Automation Partnership in the UK will continue for the purpose of optimizing the tissue for

transplantation in the 10 million people worldwide suffering from corneal vision impairment and as an alternative to the use of live animals and animal eyes in the $500+M market for safety testing of drugs, chemicals and consumer products. ISCO's goal in the coming months is to establish funding and infrastructure in India for accelerated development of CytoCor for the therapeutic application and to advance and implement the chemical testing application with partners in the US and Europe.

CytoRet is the brand name for ISCO's stem cell-derived retinal tissue. ISCO is using its parthenogenetic stem cells to develop individual retinal pigmented epithelial ('RPE') cells and layered retinal structures internally and in collaboration with the laboratory of Dr. Hans Keirstead, Professor of Anatomy and Neurobiology at the University of California, Irvine. ISCO recently commenced a new research collaboration with UC Irvine to launch the next phase of its retinal studies with that institution, including preclinical trials. Potential therapeutic applications include retinitis pigmentosa, an untreatable inherited disease affecting about 100,000 Americans, and the dry form of age-related macular degeneration, a major cause of blindness in the elderly of the Western world. ISCO's goal is to establish functional proof of concept for RPE cellular therapy in models of human disease in the next twelve-eighteen months.

(Editors Note: An extensive writeup about the work being done in building an artificial retina from human embryonic stem cells by the team at UC Irvine, is contained in ***AMD Update 12.*** *That writeup describes the use of human embryonic stem cells as the starting point. It is not known at this time who is the supplier of the hESCs used by UCI for this program, since ISCO does not supply this type of stem cells.)*

Jointly referred to as Cytovis ('cyto' for cellular, 'vis' for vision), these two cellular ophthalmology programs share a number of features and benefits. First, with the aging of the population worldwide and the growing number of work-related eye injuries in India, China and other major countries, the market opportunity is growing steadily. Second, there are strong pharmacoeconomic and quality-of-life rationales for full or partial vision restoration or delay of vision impairment diseases. Third, delivery of cells and tissues to the confined anatomy of the eye inherently provides for better safety and efficacy than, for example, the systemic circulation or the central nervous system. This will likely result in lower regulatory barriers and shorter and less costly development paths compared to that of anatomically deeper and more widespread diseases. Fourth, a number of eye diseases cannot be treated with surgery or traditional small molecule or protein therapeutics, yet cell and tissue therapy is proven to work but currently limited by availability of safe and sufficient cells and tissue from human donors. Finally, eye care development programs like CytoCor and CytoRet share a number of regulatory, development and commercial aspects that make it feasible for a relatively small team to produce substantial clinical outcomes and achieve competitive presence in the marketplace alone or in collaboration with dedicated partners.

Brian Lundstrom, ISCO's President, said, “ISCO's proprietary parthenogenetic stem cell technology continues to form the foundation for the company's long term regenerative medicine therapy programs. In the nearer term, CytoCor and CytoRet's unique benefits in the field of cellular

ophthalmology offer the potential for partnering and funding at a relatively early stage. Combined with the current and future revenue of Lifeline Cell Technology and the revenue potential of Lifeline Skin Care, scheduled for launch in the 4th quarter, Cytovis adds significantly to ISCO's diversity and value creation potential for its investor base in a cost-efficient fashion."

According to Lundstrom, both CytoCor and CytoRet are currently at the discovery research stage with proof-of-concept testing ongoing in various model systems in India and the US. The company has not yet met with the FDA or similar regulatory bodies overseas, and there are no clinical paths as yet.

NeoStem Incorporated (New York, NY)

On August 10, 2010, NeoStem Inc. announced that it had entered into a sponsored research agreement with the Schepens Eye Research Institute, an affiliate of Harvard Medical School. NeoStem will collaborate with the Schepens Institute and sponsor research in the laboratories of principal investigators Drs. Michael Young, Ph.D., Director of the Institute's Minda de Gunzburg Center for Ocular Regeneration, and Kameran Lashkari, M.D. The focus of the research will be on the development of therapies for both age-related macular degeneration (AMD) and Glaucoma.

The research will examine in animal models the regenerative potential of NeoStem's VSEL technology in the visual system through the engraftment of the very small embryonic-like stem cells. VSELs (adult stem cells) are small embryonic-like stem cells are a heterogeneous population of stem cells found in adult bone marrow that have properties similar to those of embryonic stem cells. NeoStem has shown that their VSELs can be mobilized into the peripheral blood, enabling a minimally invasive means for collecting what NeoStem believes to be an important population of stem cells that may have the potential to achieve the positive benefits associated with embryonic stem cells without the ethical or moral dilemmas or the potential negative effects associated with embryonic stem cells.

"Our research team is looking forward to leveraging our adult stem cell expertise to advance the understanding and development of very small embryonic like stem cells for the treatment of age-related macular degeneration and glaucoma through our collaboration with the Schepens Institute," said Robin Smith, M.D., Chairman and CEO of NeoStem. "We are excited to gain access to the expertise in ocular regeneration offered by Drs. Michael Young, Kameran Lashkari and the Schepens Institute through this important project."

Unanswered Questions:
(Posed to the company and awaiting answers.)

Since this is one of the first programs to study stem cells in the treatment of glaucoma, exactly what is going to be done?

What kind of glaucoma will be treated – open-angle or closed-angle?

Also, will this research be done in animal or human models?

Finally, what will Schepens be doing in the treatment of AMD?

Pfizer Regenerative Medicine (Cambridge, UK)
Pfizer Ophthalmics (San Diego, CA)

As reported in the June 2010 issue of **Retina Today**, Pfizer, Inc. launched the Pfizer Regenerative Medicine research unit in 2008 to lead investigative efforts in stem-cell therapies throughout the company. Building on Pfizer's experience in the field of regenerative medicine, this independent research organization aims to discover and develop a new generation of medicines for major medical needs across several therapeutic areas. Specific to ophthalmology, Pfizer's Ophthlamics division hopes to develop stem-cell therapies for patients with severe vision loss due to late-stage age-related macular degeneration (AMD) and other retinal diseases. Additionally, Pfizer's research unit is sampling stem cells in an effort to understand how retinal diseases progress.

Pfizer is partnering with academia and industry to understand new technologies and accelerate innovation in the area of retinal regenerative medicine. Pfizer and its most recent partner, University College London (UCL), are examining how human embryonic stem cells differentiate into retinal pigment epithelium, with the goal of developing stem-cell-based therapies for wet and dry AMD. The collaboration marries the pioneering work of Professor Peter Coffey, UCL Institute for Ophthalmology and Director of the London Project (The London Project to Cure Blindness), and colleagues in the field of cell-based therapies with Pfizer's expertise in the design and delivery of therapeutics.

Under an agreement between the company and the University, Pfizer will provide funding to UCL to enable research into the development of human embryonic stem-cell-based therapies for AMD and other retinal diseases. Pfizer's contributions will include expertise in the design and execution of clinical studies, interaction with global regulators, and product manufacturing techniques – specifically the membrane containing the stem cell structure that will be implanted into the retina..

*(Editors Note: As discussed in the introduction to this Primer, I have written extensively about the London Project – see my writeups beginning with **AMD Update 5** in April 2009, followed by the latest information about the London Project in my **AMD Update 7** in April 2010. Professor Coffey expects to begin human clinical trials in early 2011, based on safety studies being submitted to the UK's National Health Service later this year.)*

Pfizer is also investing in EyeCyte, Inc., a company that is advancing adult stem-cell approaches developed at the Scripps Research Institute in La Jolla, CA. EyeCyte's regenerative medicine technologies are under development to treat acquired and inherited retinal diseases that include diabetic retinopathy, retinopathy of prematurity, retinal vascular occlusive disease, AMD, and retinitis pigmentosa.

Pfizer officials realize that it will take time to develop a practical and effective stem-cell therapy in ophthalmology, as there are many scientific and clinical barriers that must be overcome, Dr. Eveleth, Vice President of Pfizer Ophthalmics, said. "In order to achieve the best outcomes and minimize the risk of stem-cell transplant rejection, researchers are working to develop culture and differentiation protocols that produce the purest possible populations of stem cells." Such protocols are not yet established; however, researchers are making progress.

Dr. Eveleth admits that there is still much to learn about how and when these new cells integrate and grow, but he is confident that Pfizer will be a leader in developing stem-cell therapies for ophthalmology. "With the expertise of Pfizer's Regenerative Medicine group, the clinical and disease area knowledge of Pfizer Ophthalmics, the talents of our academic and industry partners, and the scientific know-how and resources of the world's largest biopharmaceutical company, we have the experience and the staying power required to develop practical applications of this exciting new science."

StemCells Incorporated (Palo Alto, CA)

StemCells, Inc. hopes to build upon the promising results of its research through the initiation in 2012 of clinical trials for patients with retinal degenerative diseases. The company has already engaged the FDA in discussions regarding a pathway to clinical testing of its human neural stem cells for retinal indications and additional preclinical studies are underway in pursuit of that goal.

StemCells is developing its HuCNS-SC product candidate (purified human neural stem cells) as a potential therapeutic product to treat several disorders of the central nervous system (CNS). These tissue-derived “adult stem cells” are currently in clinical development for two fatal neurodegenerative diseases in children; Neuronal Ceroid Lipfuscinosis (NCL or Batten disease) a lysosomal storage disorder, caused by inheritance of a recessive genetic mutation, and Pelizaeus-Merzbacher disease (PMD) a myelination disorder caused by a mutation in the gene controlling the production of proteolipid protein (PLP), which is integral to the formation of myelin. StemCells recently completed a Phase I clinical trial of its HuCNS-SC cells in NCL. Data from this trial demonstrated the safety and tolerability of these cells, and the company plans to initiate a second NCL trial later this year. StemCells is also currently conducting a Phase I trial in PMD at the University of California, San Francisco (UCSF) Children’s Hospital.

The human safety data that StemCells is accumulating for its HuCNS-SC product candidate through these clinical trials is expected to facilitate future clinical testing in other CNS disorders including spinal cord injury and retinal degenerative diseases such as AMD and retinitis pigmentosa. The status of its HuCNS-SC product development programs is shown below:

- NCL (Phase I Clinical Trial completed; Second Trial planned for second half of 2010)
- PMD (Phase I Clinical Trial underway)
- Spinal cord injury (Phase I Clinical Trial planned for 2011)
- Retinal disorders (Phase I Clinical Trial planned for 2012)

Work in the Eye

In January 2008, StemCells entered into a research collaboration with the Casey Eye Institute at Oregon Health & Science University (OHSU) to evaluate it neural stem cells as a potential treatment for retinal diseases. These studies showed that, when transplanted into the sub-retinal space of the RCS (Royal College of Surgeons) rat, a well-established animal model of retinal degeneration, the company's human neural stem cells protected the retina from progressive degeneration and preserved visual function long term as measured by two separate visual tests. The transplanted cells also exhibited robust, long-term protection of both rod and cone photoreceptors. The ability to protect cones, in particular, is significant in regard to AMD, since it is the progressive deterioration of these specific cells that ultimately results in the devastating vision loss caused by this disease. The protection of both rods and cones is important in considering the potential of using human neural stem cells as a treatment for retinitis pigmentosa and other retinal degenerative disorders.

In May 2009, preclinical data showing the ability of the company's human neural stem cells to protect the retina from progressive degeneration were presented at the **Association for Research in Vision (ARVO) Annual Meeting**.

More recently, additional preclinical data showing photoreceptor protection and ability to preserve long term visual function was presented at the **Society for Neuroscience 2009 Annual Meeting** and at the **International Society for Stem Cell Research (ISSCR) 2010 Annual Meeting.**

"We have long recognized that a number of eye disorders may be suitable candidates for stem cell-based therapies," stated Stephen Huhn, MD, FACS, FAAP, vice president and head of the CNS program at StemCells, Inc. "The demonstrated ability of our human neural stem cells to preserve cones is very meaningful, because it is the progressive deterioration of these specific cells that ultimately results in vision loss in AMD. These data support our hypothesis that our neural stem cells may provide neuroprotection to existing cells, and it is our hope that we will be able to replicate these promising results in the clinic."

The encouraging results of its latest studies follow previously reported data showing that StemCells' neural stem cells engraft, survive long term, and can protect the retina from progressive degeneration in the RCS rat. StemCells is pursuing additional preclinical studies of its neural stem cells in the hope of one day achieving a breakthrough in treating AMD.

Stemedica (San Diego, CA)

I first learned about Stemedica's involvement in the use of stem cells to treat retinal disease in January 2007, upon seeing a news release announcing the company's collaboration with Lumenis to use the latter's new retinal laser to produce a non-damaging "wound" the retina to create a signaling proteins focal source to attract stem cells to "heal" the retinal disorder. The work was to be undertaken at the Fyodorov Eye Institute in Moscow.

That collaboration fell through, but Stemedica continued to study the use of stem cells (and lasers) in treating retinal diseases at the Fyodorov Eye Institute.

In July 2009, Stemedica announced a breakthrough in the use of human stem cells and stem cell factors for the potential treatment of retina and retinal pigmented epithelium degeneration, including diseases such as Retinitis Pigmentosa. The results of these studies were presented at several major conferences.

According to one of the study's Principle Investigators, Dr. Paul Tornambe, "The results from this pre-clinical experiment are exciting. It allows researchers and clinicians to push the envelope in the quest to use stem cells to modulate diseases like Retinitis Pigmentosa." There is currently no medical treatment that can completely cure Retinitis Pigmentosa - an eye disease that affects approximately 1,500,000 people on a worldwide basis each year.

The 18 month pre-clinical study was implemented at the Fyodorov Eye Institute using Stemedica's proprietary multiple cell technology. Three different types of adult human stem cells (hSC) were used in the study - retinal pigment epithelium (RPE), neural (NSC) and cilliary body (CB) - all obtained from human donor tissue. Cells were injected into rats with hereditary pigmented degradation of retina. One eye of each participating rat served as the treatment eye and the other eye served as the control eye. Healthy non-dystrophic and non-treated (normally dystrophic) animals were also used as independent control groups. Electroretinography (ERG) and immunohistochemical (ICH) analysis was performed on both eyes.

The research team compared the efficacy of each of the three cell types. The study showed statistically significant gain (77%) in the treated eye (with RPE cells) over the control eye of the same animal. Of interest, both the treated eye and the control eye were approximately 10 times more active (response to ERG) compared to non-treated (normally dystrophic) control animal. It was also shown that the RPE and NSC cells were effective in preserving the thickness of the outer nuclei layer of the retina.

A contra lateral effect was observed between the test and control eyes. As a result, both eyes exhibited significant improvement. It is believed that the positive outcome in the control eye was achieved through the systemic release of cytokines; growth and other important factors; peptides; and, molecules from stem cells transplanted into the treated eye. This phenomenon is referred to by Stemedica as "The Factor Release Effect" and branded by the company as StemedicaFRET. These factors, circulating in the blood flow, effect and mobilize endogenous stem cells. Stemedica believes improvement in the contra lateral eye is a 'Factor Release Effect' rather than a Sympathetic Ophthalmic effect which is very rare.

At the **Laser Florence 2009 Meeting**, Drs. Alexei Lukashev and Eugene Baranov (Stemedica); Natalia Gavrilova (Fyodorov Eye Institute); Irina Saburina and Alexander Revischin (Russian Academy of Medical Science); and Paul Tornambed (Retina Specialists, San Diego) presented a paper, "**Combination of a Laser and Stem Cells in Posterior Eye Ophthalmology**" (**AIP Conf.**

Proc. -- May 31, 2010 -- Volume 1226, pp. 82-90), that described the use of the combination of laser and stem cells in treating the retina of a rabbit model.

An argon laser at 514nm and a dye laser at 577nm were used to provide a controlled damage on the rabbit retina. Two type of human progenitor stem cells(hPSC) were tested: Mesenchymal and Neural. Four cell delivery methods were compared: Retrobulbar, Introvitreous, Subconjuctival and Suprachoroidal injections. Electroretinography(ERG) was used as a diagnostics of retina functionality. Selective immunohystochemical analysis was performed to assess cells migration and viability.

Controlled laser damage on the retina provided a strong attracting signal for the stem cells. The team concluded that, the application of laser light enhances the results of stem cells injection in the posterior eye and may have benefits for treatment of different types of retinopathy and macular degeneration.

According to the company's website, both clinical and pre-clinical work is currently underway at the Fyodorov Eye Institute in Moscow, in using stem cells (and lasers) In treating diabetic retinopathy, macular degeneration, retinitis pigmentosa, and in the treatment of glaucoma.

Some questions posed to company management:

What is the current status of these clinical studies?

We have not started ophthalmic clinical studies (on humans) using stem cells as an IND (Investigational New Drug) approved by FDA. We plan to do this under an existing IND using allogeneic bone marrow derived stem cells for intravenously administration in patients with retinal ischemia with and without laser treatment of retina.

Are they being conducted in human eyes?

Eight human subjects with different kinds of retinopathy were treated by human adult progenitors stem cells manufactured by Stemedica (clinical case studies). Three years follow up show the safety and preliminary efficacy of adult stem cells for ophthalmic applications, and warrant further investigation in clinical trials which are planned for initiation in the U.S.

And, exactly what is being done in the glaucoma work? This is the second time I have heard of stem cells being applied to glaucoma. Can you provide some further explanation?

We are double checking and analyzing our preliminary results, preparing our intellectual property (IP) filings, but this is not yet ready for public release.

Table 2. Stem Cell Companies Active in Ophthalmology

Company	Collaborator(s)	Cell Type	Applications
Advanced Cell Technology Inc. (ACT)	Oregon Health & Science University (OHSU)	hESCs	RPE cells for retinal diseases, including AMD and Stargardt's
AstraZeneca	University College London (UCL)	hESCs	Diabetic Retinopathy
International Stem Cell Corp. (ISCO)	CytoCor - ● Absorption Systems - US ● Sankara Nethralaya - India ● Automation Partnership - UK CytoRet - UC Irvine	hpSCs hpSCs	Corneal tissue for transplantation into degenerated corneas RPE cells and layered retinal structures for AMD
NeoStem Inc.	Schepens Eye Research Institute	Adult SCs (VSELs - very small embryonic-like from bone marrow)	In animal models for treating glaucoma and AMD
Pfizer Regenerative Medicine/Pfizer Ophthalmics	● Univ. College London (UCL) (London Project to Cure Blindness) ● EyeCyte Inc. with Scripps Research Inst.	hESCs Adult SCs (From bone marrow)	RPE for wet and dry AMD and other retinal diseases Treating Retinal diseases including diabetic retinopathy ROP, RVO, AMD, and RP
StemCells Incorporated	Oregon Health & Science University (OHSU) with Casey Eye Institute	Adult SCs (HuCNS-SCs - purified human neural stem cells)	For retinal degenerative diseases, including photoreceptor protection to preserve visual function in AMD and retinitis pigmentosa
Stemedica	Fyodorov Eye Microsurgery Center, Moscow	Adult SCs	Stem cell injection following spot laser damage of retina, for RP, AMD, DR Glaucoma

Irv Arons, September 2010 (Revision 2, September 14, 2010)

A Brief History of Stem Cells in Ophthalmology

Stem Cells in Medical Research

Until recently, scientists primarily worked with two kinds of stem cells from animals and humans: "embryonic stem cells" and non-embryonic or "adult" stem cells. Scientists discovered ways to derive embryonic stem cells from early mouse embryos nearly 30 years ago, in 1981. The detailed study of the biology of mouse stem cells led to the discovery, in 1998, of a method to derive stem cells from human embryos and grow the cells in the laboratory. The embryos used in those studies were created for reproductive purposes through in vitro fertilization procedures. When they were no longer needed for that purpose, they were donated for research with the informed consent of the donor. In 2006, researchers made another breakthrough by identifying conditions that would allow some specialized adult cells to be "reprogrammed" genetically to assume a stem cell-like state. This new type of stem cell was called induced pluripotent stem cells (ipSCs).

Stem Cells in the Eye

(With thanks to Dan Roberts, Director, the MD Support Group)

2000

Stem cell work in the eye started with the discovery that stem cells have certain characteristics of photoreceptor cells, reported in the year 2000 by Dr. Derek van der Kooy (University of Toronto) and Dr. Iqbal Ahmad (University of Nebraska).

2001

In 2001, President George W Bush limited government funding for research using embryonic stem cell lines. In the same year, Dr. Michael Young (Schepens Eye Institute) showed that transplanted cells from a mouse retina were able to reproduce, and that some of them contained the photoreceptor-specific protein, rhodopsin, which initiates the visual cycle (phototransduction).

2002

In 2002, the Scripps Research Institute (TSRI) in La Jolla, California reported success in forming new retinal blood vessels in mice with ocular disease. The process uses pluripotent adult stem cells derived from bone marrow and injected into the vitreous of the eyeball. Not only could adult bone marrow stem cells be used to form new vessels, but they could also be used to deliver powerful antiangiogenic drugs to prevent neovascularization. This was promising news to people with wet AMD.

2004

In 2004, Advanced Cell Technology (Alameda, California) announced that they had engineered

human embryonic stem cells which could be used to repair a damaged retina. Dr. Robert Lanza (Scientific Director) said the results illustrate the need to use cloning technology to eliminate the risk of rejection by the patient's immune system.

A month later, the Department of Medical Biophysics (University of Toronto, Ontario, Canada) announced that their researchers had cultured and transplanted stem cells from human retinas into the healthy retinas of young mice. After four weeks, most of the cells had migrated to the new retinas and successfully differentiated themselves into photoreceptor cells.

Later that year, California became the first state to circumvent the federal government's restriction on funding for stem cell research by passing Proposition 71 with a majority vote of 69%. This allowed nearly three billion dollars to be put aside for stem cell research in that state over the next 10 years.

Almost simultaneously, scientists from Harvard's Schepens Eye Research Institute successfully, and for the first time, improved the vision of mice with transplanted progenitor stem cells from day-old mice. The procedure had preserved existing cells and restored health to those that were degenerating--a major step toward the therapeutic use of stem cells for people with all forms of retinal degeneration.

2006

Work with humans began in 2006. In April, a research team in India (Dr Rajender Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences) reported that 50 patients severely affected by age-related macular degeneration or retinitis pigmentosa showed significant improvement in vision after one month of injecting stem cells, and that there was further improvement after a gap of three months.

In August, researchers at the University of Washington used a mix of growth factors to coax embryonic cells into becoming retinal cells. This was the first use of human stem cells using the technique for the retina. The team then began injecting the new cells into the eyes of retina-damaged mice, measuring nerve reactions to see whether there was actual vision improvement.

In September, scientists from Moran Eye Center (University of Utah, Salt Lake City) and Advanced Cell Technology reported that cells grown from human embryonic stem cells safely slowed vision loss when injected into the eyes of rats with a disease similar to macular degeneration.

2007

The year 2007 was full of breakthroughs, as researchers continued to look for ways to avoid harming embryos during the production of new stem cell lines. In January, for example, researchers at the Institute for Regenerative Medicine (Wake Forest University School of Medicine) discovered yet another potential source of embryonic stem cells in the amniotic fluid of the womb. These cells

appeared to be almost as malleable as those in the embryo itself, and the advantage would be that harvesting them would be harmless. On another front, Advanced Cell Technology developed a technique for harmlessly removing a blastomere from an eight-cell human embryo (blastocyst). A blastomere is a cell resulting from the first few divisions of the ovum (egg) after fertilization. In June of 2007, ACT issued a press release announcing successful production of a human embryonic stem cell line (hESC) using that method.

Meanwhile, research was also beginning in the United Kingdom at the University College London, Moorfields Eye Hospital and Sheffield University, in a cooperative effort called the London Project to Cure Blindness. The London Project is a five year research project that began in June of 2007 to develop stem cell therapy for AMD hopefully by the year 2012. Doctors at Moorfields had some success with human subjects using adult stem cells from the patients' own eyes. Embryonic cells, however, had been shown by the Sheffield scientists to be more malleable and easier to transplant than adult stem cells. Laboratory-grown cells from the blastocyst of a 5-day old embryo require only one injection (a 45-minute procedure), whereas the Moorfields experiments took two hours and two surgical procedures. This protocol would be very expensive and impractical in general practice, so embryos are being used at Sheffield, and that will take a little longer to get into human trials.

Moorfields scientists are also studying the potential transformation of Müller neuroglial cells from the patient's own eyes. These would be removed from the eye, injected with a triggering chemical (either glutamate or aminoadipate, a derivative of glutamate), grown in vitro (i.e. in a petri dish) and transplanted back into the eye as stem cells. Late in 2007, two research groups (one at Kyoto University and the Gladstone Institute of Cardiovascular Disease in San Francisco, and the other at the University of Wisconsin) described a method of creating induced pluripotent stem cells by inserting master regulator genes into the chromosomes of human skin cells. These altered cells appeared to behave like embryonic stem cells, which scientists hoped could eventually eliminate the need for using human embryos for research.

2008

In 2008, President Obama lifted President Bush's restrictions on government funding for using embryonic stem cell lines. This opened the door to the world's first test in people of stem cell replacement therapy.

2009

In February of 2009, Geron, a pharmaceutical company, began enrolling paralyzed patients who could be treated within two weeks of their injury. This move set a precedent for more stem cell research in the low vision field. Then, in August, scientists at the University of Wisconsin-Madison reported that they had reprogrammed skin cells and turned them into different kinds of retinal cells. This added to the growing weight of evidence that stem cells made by reprogramming have similar, if not the same, abilities as embryonic stem cells.

One caveat of the new research, however, was that UW scientists had not yet proved that retinal cells made in a dish can perform all of the functions of those made in the body. But, at least, scientists can now take a skin biopsy from someone with a vision ailment, create retinal cells in a dish, and observe how the disease unfolds and how the cells die over time. Actual stem cell replacement in human retinas is still a little further in the future.

In November, Advanced Cell Technologies filed an IND with the FDA to initiate testing of its embryonic stem cells for treating Stargardt's Macular Dystrophy.

2010

In the May issue of the AIP Conference Proceedings, a paper given at Laser Florence 2009 was published, providing information about the laser and stem cell programs underway with Stemedica and its research partner, the Fyodorov Eye Institute in Moscow. The paper described using lasers to "wound" the retina of rabbit eyes, followed by stem cell injections to "cure" the wounds.

At the end of July, Advanced Cell Technology (ACT) submitted documentation to the FDA in connection with the company's plans to initiate a Phase I/II multicenter study using hESCs derived retinal cells to treat patients with Stargardt's Macular Dystrophy. This followed its filing of an IND in November 2009 to commence treating patients.

In August, International Stem Cell Corp. announced it had reformed its therapeutic stem cell program; with the formation of CytoCor to focus on corneal applications, CytoRet on retinal applications, and all under the wings of Cytovis, the business unit.

Also in August, NeoStem announced that it had entered into a sponsored research agreement with the Schepens Eye Research Institute, whose focus will be on the development of stem cell therapies for both age-related macular degeneration and glaucoma.

In September, AstraZeneca signed a research collaboration with University College London, to develop a stem cell-based treatment for diabetic retinopathy.

Future Promise

As described in the accompanying text, stem cell technology has yet to be tested in controlled human clinical trials. But that test is just around the corner. The hESCs involved in the Pfizer Regenerative Medicine's London Project to Cure Blindness or in the Advanced Cell Technology program to treat Stargardt's disease may well be the first clinical trial program to implant embryonic stem cells into human eyes, and that could take place as early as the fourth quarter of this year or the first quarter of 2011.

As shown in the enclosed table of stem cell companies active in ophthalmology, there are a variety of projects underway to treat a multitude of retinal diseases (and a few for glaucoma and cornea).

If any of these projects are successful, and the hopes are high, we may well be on the verge of a new era, and soon have the technology and means to overcome degenerated eye structures to stave off blindness and poor sight for millions of humans.

I will continue to follow new developments in this exciting field and report on them as they happen.

Resources for Stem Cell Information:

1. **Stem Cells, International Stem Cell Corporation** website.

2. **Stem Cell Facts: The Next Frontier?**, International Society for Stem Cell Research, 2008.

3. **Stem Cell Basics**, The NIH Resource for Stem Cells, April 2009.

4. **Understanding Stem Cell Therapy**, Dan Roberts, and Profs. Claudio Stern and Peter Coffey, MDS Support Library, May 2010.

Citations:

Stem Cells in Ophthalmology, Dr. John Morrow, Newport Biotech, Ophthalmology Times Europe, September 2008.