MACULAR DEGENERATION

1. MACULAR DEGENERATION PROTOCOL

A. Clinical response: Patients may experience improvements in function and quality of life parameters. This could include improvements in eye sight as measured by visual field analysis and visual activity measurements. Delivering ASCs into the damaged areas of the posterior pole, these regions may become populated with cells that may have the ability to restore the normal function of the eyes.

B. Objective: To provide the patient with a treatment that stimulates his / her immune system, promote cellular regeneration and improve symptoms associated with Macular Degeneration. The endovascular/intravenous Ad-SVF Containing Adult Stem Cell Procedure should serve to compliment the patient's current treatment regimen or to promote healing when current treatment is not responding.

2. PRELIMINARIES

A. Background: A number of common eye diseases such as age-related macular degeneration are associated with ocular perfusion abnormalities. Although this is well recognized there is not much possibility to improve blood flow to the posterior pole of the eye in these diseases. Age-related macular degeneration (AMD) is a disease associated with aging that gradually destroys sharp, central vision. AMD affects the macula, the part of the eye that allows you to see fine detail. In some cases, AMD advances so slowly that people notice little change in their vision. In others, the disease progresses faster and may lead to a loss of vision in both eyes. AMD is a leading cause of vision loss in Americans 60 years of age and older [1]. In these common eye diseases, the blood flow in the posterior pole of the eye is often restricted [2]. Current pharmaceuticals do not address this issue. There are two types of Age-related Macular Degeneration including:

- ***Dry age-related macular degeneration:*** When RPE cells lose their color; waste accumulates and causes the rods and cones to deteriorate [3].
- ***Wet age-related macular degeneration:*** Caused from the growth of new blood vessels underneath the macula. The new blood vessels leak fluid and/or blood, which inhibit the macula from receiving nutrients and cause the rods and cones to begin to break down [3].

B. Causes of Macular Degeneration:

- ***Genetic Susceptibility***: i.e. family history of macular degeneration
- ***Environmental Risk Factors***: i.e. long-term exposure to sunlight
- ***Lifestyle Risk Factors:*** i.e. cigarette smoking

C. Treatment Options: [3]

- ***Drug Therapies***: i.e. family history of macular degeneration
  - Pegaptanib (Macugen)
  - Ranibizumab (Lucentis)
- ***Surgical and Other Procedures***:
  - Photocoagulation (laser surgery)
  - Photodynamic therapy
- ***Nutrition:***
  - AREDS formula (vitamin C, vitamin E, beta-carotene, and zinc, plus copper)
  - Lutein & zeaxanthin
  - Leafy greens & Omega-3 fatty acids
  - Herbs: Ginkgo & Bilberry

3. AD-SVF CONTAINING ADULT STEM CELLS TREATMENT OPTION

A. Ad-SVF Containing Adult Stem Cells Procedure

- ***Initial patient evaluation***: A physician reviews the medical information, lab work, and diagnostic imaging provided by the patient in order to determine the stage of the medical condition and any other secondary conditions.

- ***Pre-op Evaluation / post-op medical consultation***: A medical specialist to the specific condition to be treated provides a medical consultation at the location where the procedure will be performed. During pre-op evaluation informed consent is obtained from all patients and medical records are updated, including patient's most recent physical exam, most up-to-date lab results and imaging studies. Physician then performs surgical risk assessment.

- ***Harvesting of adipose tissue***: Adipose tissue acquisition can be summarized as three step process:
  - ***Application of anesthetic / injection of tumescent solution***
  - ***Waiting time***
  - ***Acquisition of adipose tissue:*** An area of the body with sufficient adipose tissue is selected; this is usually the periumbilical area. With the patient supine, the physician infiltrates a small amount of local anesthetic. A tissue sample is then obtained using 60 cc syringe(s) to aspirate 50 to 100 cc of adipose tissue. Immediately following lipo-aspiration, adipose tissue sample is processed (minimally manipulated) to separate stem cells for use as graft.

- ***Preparation of Platelet Rich Plasma (PRP):*** Using a standard phlebotomy technique the patient's own blood sample is obtained. After collection of whole blood, sample is centrifuged to obtain PRP aliquot. The regenerative potential of PRP is based on the release of growth factors / cytokines upon platelet rupture. PRP also enhances stem cell proliferation.

- ***Autologous implant of Ad-SVF:*** To do the implant procedure, the cells are suspended in PRP and directly injected intravitreally into your eye with a small needle. You will be closely monitored throughout the procedure.

B. Risks: There are possibilities for unwanted effects related to the local anesthesia, harvesting procedure, and injection of stem cells. Even with the most established protocol, adequate technique, and careful administration; a medical team may encounter uncontrollable events. Although there is no guarantee of perfect results, excellent results can be attained. The surgeon provides services in the most responsible, professional and diligent manner, always considering that surgeries imply risks. The risks of complications of adipose tissue harvesting and stem cell infusion are very low. Possible risks include but are not limited to:

- Pain at site of injections
- Bleeding at injection site
- Malaise
- Low-grade fever
- Hot flashes
- Itching at injection site
- Vascular spasm or obstruction
- Bruising
- Nerve or muscle injury
- Allergic reaction
- Dizziness
- Nausea
- Vomiting

C. Benefit: ASCs are a novel therapy for patients suffering from degenerative eye diseases like Macular Degeneration. By injecting ASCs into the eye, these regions may become populated with the ASCs, thereby potentially angiogenesis and blood flow. ASCs are a patient-derived ("autologous") cell transplantation technology that is delivered to the patient intravitreally. By delivering ASCs into the damaged areas of the posterior pole, these regions may become populated with cells that may have the ability to restore the normal function of the eyes. Recent studies have focused on adult stem cells that are angiogenic in nature and can help produce new blood vessels in areas of low perfusion [4].

In addition, mesenchymal stem cells have been shown to differentiate into various cell types from the mesodermal and ectodermal lineages. These cells also have differentiation potential into the neuroectodermal lineage, yielding cells with phenotypic characteristics of RPE cells [5]. Recent studies have identified adipose tissue as a new source of mesenchymal stem cells; some of which may be suitable for the restoration of eye function [6].

D. Follow-Up Plan:

- ***Pre- Ad-SVF implant:***
  - Patient Status
  - Review of Medical History
  - Review of Medication History
  - Visual field analysis
  - Visual acuity measurements (Snellen Digital Projector)

- ***3 months after Ad-SVF implant:***
  - Patient Status
  - Review of Medical History
  - Review of Medication History
  - Visual field analysis
  - Visual acuity measurements (Snellen Digital Projector)

- ***6 months after Ad-SVF implant:***
  - Patient Status
  - Review of Medical History
  - Review of Medication History
  - Visual field analysis
  - Fluorescein-angiography
  - Intraocular pressure
  - Visual acuity measurements (Snellen Digital Projector)
  - Optical Coherence Tomography

MD – Physician Schedule of Events

	Baseline Evaluation and Data Review	Three-Month Follow-up	Six-Month Follow-up
	Visit 1	*Visit 2*	*Visit 3*
Informed Consent	X		
Adverse Event Review/Status	X	X	X
Medical History Review	X	X	X
Medication History Review	X	X	X
Physical examination including vital signs	X	X	
Pregnancy test[1]	X		
Laboratory determinations	X		
Visual field analysis	X	X	X
Visual Acuity Measurements	X	X	X
Fluorescein-angiography	X		X
Optical Coherence Tomography	X		X
Intraocular pressure	X		X
Liposuction and ASC isolation			
Adipose-derived stem cell implantation			

1 For female patients of childbearing age.

MD – Adult Stem Cells Schedule of Events

1. **Initial Patient Evaluation:** A physician reviews the medical information, lab work, and diagnostic imaging provided by the patient in order to determine the stage of the medical condition and any other secondary conditions.

   A. Pre-Examination:
   - You will have a physical exam, which will include measuring your blood pressure, temperature and heart rate (vital signs).
   - Your doctor will discuss your medical history and any medications that you are taking.
   - Your doctor will assess how well you can perform your daily activities
   - If needed, you will have a urine or blood pregnancy test.
   - Blood will be taken.

   B. Additional Tests: should be done during or soon after this visit
   - Visual field analysis
   - Fluorescein-angiography
   - Intraocular pressure

   A. Review Results: After your doctor has reviewed the results of these tests, he or she will assess whether you are a good candidate for stem cell therapy. If you decide to obtain this therapy you will sign a consent form. A medical specialist to the specific condition to be treated provides a medical consultation at the location where the procedure will be performed. During pre-op evaluation informed consent is obtained from all patients and medical records are updated, including patient's most recent physical exam, most up-to-date lab results and imaging studies. Physician then performs surgical risk assessment.

   B. Schedule Stem Cell Procedure

2. **Pre-Operation / Stem Cell Procedure:**

   A. Two Weeks Before Procedure:

   - No Aspirin or medicines that contain aspirin or Ibuprofen since it interferes with normal blood clotting.
   - You may take Tylenol or generic forms of this drug.
   - Discuss with your primary physician to discontinue anticoagulant drugs at least 1 week before the procedure.
   - Please discontinue all herbal medications as many have side effects that could complicate a surgical procedure by inhibiting blood clotting, affecting blood pressure, or interfering with anesthetics.
   - Please discontinue all diet pills whether prescription, over-the-counter or herbal.
   - NO SMOKING because nicotine reduces blood flow to the skin and can cause significant complications during healing.
   - Purchase a compressive garment to wear after the lipoaspiration procedure.

B. Morning of the Procedure:

- Have a light breakfast.
- Take your regular prescribed medications
- Wear comfortable, loose-fitting clothes that do not have to be put on over your head.

3. Stem Cell Procedure:

A. Preparation & Harvesting of Adipose Tissue:

- ***Application of anesthetic / injection of tumescent solution***
- ***Waiting time (~15 – 20 minutes)***
- ***Acquisition of adipose tissue:*** An area of the body with sufficient adipose tissue is selected; this is usually the periumbilical area. With the patient supine, the physician infiltrates a small amount of local anesthetic. Immediately following lipo-aspiration, adipose tissue sample is processed (minimally manipulated) to separate stem cells for use as graft.

B. Preparation of Platelet Rich Plasma (PRP): Using a standard phlebotomy technique the patient's own blood sample is obtained. After collection of whole blood, sample is centrifuged to obtain PRP aliquot. The regenerative potential of PRP is based on the release of growth factors / cytokines upon platelet rupture. PRP also enhances stem cell proliferation.

C. Autologous implant of Ad-SVF: To do the implant procedure, the cells are suspended in PRP and directly injected intravitreally into your eye with a small needle. You will be closely monitored throughout the procedure.

4. Recommended Post-Operation / Stem Cell Therapy Schedule:

A. Post-Op Medical Instruction - **(Please follow these instructions closely!)**

- ***Post-op medication*** will be given to you the day of your surgery. They will consist of an antibiotic and a painkiller:
  - *Antibiotic*: Cephalexin/Cipro, please take as directed beginning the day after surgery
  - *Painkiller:* Please take as directed and only as needed for pain

    * If you are unable to take any of these medications, please contact your patient coordinator so we can arrange for other medications.
- ***Resume previous medication*** as directed by the physician
- ***Report any symptoms of feeling unwell***, fever, eye pain or swelling, or decrease in visual acuity. Patients should be seen promptly by an ophthalmologist for full evaluation should any of the above symptoms be encountered.
- It is recommended that the ***patient have a companion stay with him or her*** for at least 24 hours after discharge.

- You should ***expect some of blood-tinged anesthetic solution to drain from the incision sites*** during the first 24 to 48 hours. This will vary from patient to patient. Maxi-pads are recommended for bandages over your incision sites. You may take a shower 24 hours after the procedure.
- ***Compressive garments should be worn*** 24 hours a day for the first week and 12 hours a day for the second week.
- ***Do not shower for the first 24 hours***. ***Do not submerge yourself in any water*** (i.e. taking a bath or swimming) for the 1st week.
- ***If you experience nausea or vomiting it is probably due to the medication***. Please try to take it with food. If it persists, please contact our office.
- ***Diet-meals are not restricted.***
- ***Drink plenty of clear fluids***. We recommend 8 glasses of water or fruit juice every day.
- ***Do not drink any alcohol*** for 48 hours and limit alcohol intake for the first week.

B. <u>Post-Op Medical Consultation Schedule: 3 months & 6 months</u>

- Review of medical history
- Review of medication history
- Visual field analysis
- Fluorescein-angiography (6 months only)
- Intraocular pressure (6 months only)
- Review of any adverse events since the previous visit

Your doctor will contact you by phone within the first week to follow up then future follow up visits will be arranged through your patient coordinator. If you need assistance before do not hesitate to contact us.

MACULAR DEGENERATION – Supporting Studies

Cytotherapy. 2009;11(2):177-88. doi: 10.1080/14653240802714819.

Retinal pigment epithelial phenotype induced in human adipose tissue-derived mesenchymal stromal cells.

Vossmerbaeumer U, Ohnesorge S, Kuehl S, Haapalahti M, Kluter H, Jonas JB, Thierse HJ, Bieback K.

Source
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Abstract
BACKGROUND AIMS:
The non-exudative form of age-related macular degeneration (ARMD) is characterized by a progressive decay of retinal pigment epithelium cells at the posterior pole of the eye. As mesenchymal stromal cells (MSC) have been shown to differentiate into various cell types from the mesodermal and ectodermal lineages, we investigated whether we can induce a phenotype displaying retinal pigment epithelium (RPE) characteristics.

METHODS:
The differentiation of human lipo-aspirate-derived MSC toward the RPE lineage was triggered by exposure to conditioned medium from either human or porcine RPE cells. In a second approach we tested whether adding vasoactive intestinal peptide (VIP) is capable of further modifying differentiation processes. Resulting cell populations were assessed for expression of RPE-specific markers by immunofluorescence, quantitative real time (RT)-polymerase chain reaction (PCR) and Western blotting. The potential for pigment synthesis was assessed by the response to melanocyte-stimulating hormone (MSH).

RESULTS:
Following culture of undifferentiated MSC with RPE-conditioned medium and/or VIP, expression of typical RPE markers bestrophin, cytokeratins 8 and 18 and RPE 65 was induced. MSH induced the formation of pigmented granula in differentiated MSC.

CONCLUSIONS:
MSC are shown to express RPE markers upon induction with either RPE-conditioned medium and/or VIP. The gain of basic functional features of RPE cells was indicated by melanin synthesis. This alludes to a differentiation potential of MSC into the neuroectodermal lineage, yielding cells with phenotypic characteristics of RPE cells.

PMID: 19241195 [PubMed - indexed for MEDLINE]

MACULAR DEGENERATION – Supporting Studies

Histol Histopathol. 2013 Dec;28(12):1577-83. Epub 2013 May 30.

Adipose derived mesenchymal stem cells partially rescue mitomycin C treated ARPE19 cells from death in co-culture condition.

Singh AK, Srivastava GK, García-Gutiérrez MT, Pastor JC.

Source
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Abstract
Age-related macular degeneration is a retinal disease with important damage at the RPE layer. This layer is considered a target for therapeutical approaches. Stem cell transplantation is a promising option for retinal diseases. Adipose derived mesenchymal stem cells secret growth factors which might play a significant role in RPE maintenance. This study aimed to evaluate human AD-MSCs ability to rescue mitomycin C treated dying ARPE19 cells in co-culture condition. ARPE19 cells were treated with MMC (50µg/ml, 100µg/ml and 200µg/ml) for 2 hours to induce cell death. These treated cells were co-cultured with hAD-MSCs in indirect co-culture system for 3 days and 3 weeks. Then the viability, growth and proliferation of these ARPE19 cells were evaluated by a cell viability/cytotoxicity assay kit and Alamar Blue (AB) assay. Untreated ARPE19 cells and human skin fibroblasts (HSF) were used as controls. MMC blocked ARPE19 cell proliferation significantly in 3 days and cells were almost completely dead after 3 weeks. Cell toxicity of MMC increased significantly with concentration. When these cells were co-cultured with hAD-MSCs, a significant growth difference was observed in treated cells compared to untreated cells. hAD-MSCs rescue capacity was also significantly higher than HSF for treated ARPE19 cells. This study showed that hAD-MSCs rescued MMC treated ARPE19 cells from death. It probably occurred due to undefined growth factors secreted by hAD-MSCs in the medium, shared by treated ARPE19 cells in co-culture conditions. This study supports further evaluation of the effect of hAD-MSCs subretinal transplantation over the RPE degeneration process in AMD patients.

PMID: 23719745 [PubMed - in process]

MACULAR DEGENERATION – Supporting Studies

J Transl Med. 2013 Mar 1;11:53. doi: 10.1186/1479-5876-11-53.

Stem cells: a new paradigm for disease modeling and developing therapies for age-related macular degeneration.

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Abstract
Age-related macular degeneration (AMD) is the leading cause of blindness in people over age 55 in the U.S. and the developed world. This condition leads to the progressive impairment of central visual acuity. There are significant limitations in the understanding of disease progression in AMD as well as a lack of effective methods of treatment. Lately, there has been considerable enthusiasm for application of stem cell biology for both disease modeling and therapeutic application. Human embryonic stem cells and induced pluripotent stem cells (iPSCs) have been used in cell culture assays and in vivo animal models. Recently a clinical trial was approved by FDA to investigate the safety and efficacy of the human embryonic stem cell-derived retinal pigment epithelium (RPE) transplantation in sub-retinal space of patients with dry AMD These studies suggest that stem cell research may provide both insight regarding disease development and progression, as well as direction for therapeutic innovation for the millions of patients afflicted with AMD.

PMID: 23452406 [PubMed - indexed for MEDLINE] PMCID: PMC3599723

MACULAR DEGENERATION – Supporting Studies

Nat Med. 2002 Sep;8(9):1004-10. Epub 2002 Jul 29.

Bone marrow-derived stem cells target retinal astrocytes and can promote or inhibit retinal angiogenesis.

Otani A, Kinder K, Ewalt K, Otero FJ, Schimmel P, Friedlander M.
Source

Department of Cell Biology, The Scripps Research Institute, La Jolla, California, USA.
Abstract

Adult bone marrow (BM) contains cells capable of differentiating along hematopoietic (Lin(+)) or non-hematopoietic (Lin(-)) lineages. Lin(-) hematopoietic stem cells (HSCs) have recently been shown to contain a population of endothelial precursor cells (EPCs) capable of forming blood vessels. Here we show that intravitreally injected Lin(-) BM cells selectively target retinal astrocytes, cells that serve as a template for both developmental and injury-associated retinal angiogenesis. When Lin(-) BM cells were injected into neonatal mouse eyes, they extensively and stably incorporated into forming retinal vasculature. When EPC-enriched HSCs were injected into the eyes of neonatal rd/rd mice, whose vasculature ordinarily degenerates with age, they rescued and maintained a normal vasculature. In contrast, normal retinal angiogenesis was inhibited when EPCs expressing a potent angiostatic protein were injected. We have demonstrated that Lin(-) BM cells and astrocytes specifically interact with one another during normal angiogenesis and pathological vascular degeneration in the retina. Selective targeting with Lin(-) HSC may be a useful therapeutic approach for the treatment of many ocular diseases.
Comment in

Stem cells go for the eyes. [Nat Med. 2002]

PMID: 12145646 [PubMed - indexed for MEDLINE]

MACULAR DEGENERATION – Supporting Studies

J Clin Invest. 2004 Sep;114(6):765-74.

Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hematopoietic stem cells.

Otani A, Dorrell MI, Kinder K, Moreno SK, Nusinowitz S, Banin E, Heckenlively J, Friedlander M.

Source
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Abstract
Inherited retinal degenerations afflict 1 in 3,500 individuals and are a heterogeneous group of diseases that result in profound vision loss, usually the result of retinal neuronal apoptosis. Atrophic changes in the retinal vasculature are also observed in many of these degenerations. While it is thought that this atrophy is secondary to diminished metabolic demand in the face of retinal degeneration, the precise relationship between the retinal neuronal and vascular degeneration is not clear. In this study we demonstrate that whenever a fraction of mouse or human adult bone marrow-derived stem cells (lineage-negative hematopoietic stem cells [Lin- HSCs]) containing endothelial precursors stabilizes and rescues retinal blood vessels that would ordinarily completely degenerate, a dramatic neurotrophic rescue effect is also observed. Retinal nuclear layers are preserved in 2 mouse models of retinal degeneration, rd1 and rd10, and detectable, albeit severely abnormal, electroretinogram recordings are observed in rescued mice at times when they are never observed in control-treated or untreated eyes. The normal mouse retina consists predominantly of rods, but the rescued cells after treatment with Lin- HSCs are nearly all cones. Microarray analysis of rescued retinas demonstrates significant upregulation of many antiapoptotic genes, including small heat shock proteins and transcription factors. These results suggest a new paradigm for thinking about the relationship between vasculature and associated retinal neuronal tissue as well as a potential treatment for delaying the progression of vision loss associated with retinal degeneration regardless of the underlying genetic defect.
Comment in

Bone marrow-derived stem cells preserve cone vision in retinitis pigmentosa.

[J Clin Invest. 2004]

PMID: 15372100 [PubMed - indexed for MEDLINE] PMCID: PMC516263

Exp Eye Res. 2007 Aug;85(2):234-41. Epub 2007 May 6.

Subretinal transplantation of bone marrow mesenchymal stem cells delays retinal degeneration in the RCS rat model of retinal degeneration.

Inoue Y, Iriyama A, Ueno S, Takahashi H, Kondo M, Tamaki Y, Araie M, Yanagi Y.

Source
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Abstract
Because there is no effective treatment for this retinal degeneration, potential application of cell-based therapy has attracted considerable attention. Several investigations support that bone marrow mesenchymal stem cells (MSCs) can be used for a broad spectrum of indications. Bone marrow MSCs exert their therapeutic effect in part by secreting trophic factors to promote cell survival. The current study investigates whether bone marrow MSCs secrete factor(s) to promote photoreceptor cell survival and whether subretinal transplantation of bone marrow MSCs promotes photoreceptor survival in a retinal degeneration model using Royal College of Surgeons (RCS) rats. In vitro, using mouse retinal cell culture, it was demonstrated that the conditioned medium of the MSCs delays photoreceptor cell apoptosis, suggesting that the secreted factor(s) from the MSCs promote photoreceptor cell survival. In vivo, the MSCs were injected into the subretinal space of the RCS rats and histological analysis, real-time RT-PCR and electrophysiological analysis demonstrated that the subretinal transplantation of MSCs delays retinal degeneration and preserves retinal function in the RCS rats. These results suggest that MSC is a useful cell source for cell-replacement therapy for some forms of retinal degeneration.

PMID: 17570362 [PubMed - indexed for MEDLINE]

MACULAR DEGENERATION – References

[1] National Eye Institute – National Institutes of Health. "Research Today, Vision Tomorrow." http://www.nei.nih.gov/health/maculardegen/armd_facts.asp

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