ALOPECIA PROTOCOL

**Clinical Response:** Clinical response demonstrates a decrease in symptoms related to Alzheimer's as a result of damaged nerve cells. In addition to physical examinations prior to stem cell graft and 6 months post-procedure, laboratory tests and imaging results serve as evidence of repair process. Internationally recognized lab tests for monitoring alopecia include:

- Complete blood count
- Androgen biomarkers
- Liver enzymes
- Cardiovascular biomarkers
- Adrenal hormone levels
- Thyroid autoantibodies
- Thyroid hormones: thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH)
- Treponema Pallidum hemagglutination (TPHA) test
- Iron and Serum Ferritin and Total Iron Binding Capacity (TIBC)

**B. Objective:** To provide the patient with a treatment that stimulates his / her immune system, promote cellular regeneration and improve symptoms associated with alopecia. 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.

PRELIMINARIES

**Background:** Hair undergoes a regular cycle of growth. Each cycle consists of a long growing phase (anagen), a brief transitional apoptotic phase (catagen), and a short resting phase (telogen). At the end of the resting phase, the hair falls out (exogen) and a new hair starts growing in the follicle, beginning the cycle again. Each day, about 100 scalp hairs reach the end of resting phase and fall out. If more than 100 hairs/day go into resting phase, telogen effluvium (clinical hair loss) may occur. A disruption of the growing phase causing abnormal loss of anagen hairs is called an anagen effluvium.

Alopecia, or loss of hair, has many causes and patterns. Alopecia areata is a non-scarring condition, most commonly appearing on the scalp. This condition presents as sharply defined non-inflamed bald patches, usually on the scalp[1]. During the active stage of hair loss pathognomonic, ‘exclamation mark’ hairs are seen (broken-off hairs of 3-4 mm long, which taper off towards the scalp). The condition may also affect the eyebrows, eyelashes and beard. Patient’s hair usually re-grows spontaneously in small bald patches.

Alopecia areata is associated with hypothyroidism, autoimmune disorders, atopy and Down's syndrome. Topical or intra-lesional steroids are first line treatments. There are three main types of Alopecia: Alopecia Areata, Alopecia Areata Totalis, and Alopecia AreataUniversalis [1].

**Alopecia Areata:** is the most common type of the autoimmune disease [1]
- Most patients have small, localized patches, and some have widespread involvement. This condition has a risk for chronicity, in which factors include extensive skin involvement, onset before adolescence, and ophiasis (involvement of the peripheral scalp).
- Androgenic alopecia is the most common type of hair loss. Male-pattern baldness is physiological in men over 20 years old, though rarely it may be extensive and develop at an alarming pace in the late teens. It also occurs in females, usually post-menopause. The well-known distribution (bitemporal recession and then crown involvement) is described as 'male-pattern' but this type of hair loss in females is often diffuse.

**Alopecia AreataTotalis:** is characterized as the total loss of hair on the scalp [1]

**Alopecia AreataUniversalis:** is the rarest form and is characterized as the loss of hair over the entire scalp and body [1]
B. Causes of Alopecia: Administration of chemotherapeutic agents, medications (valproic acid), endocrine / autoimmune disorders, infection, nutritional deficiencies, mineral deficiencies (Zinc), excessive Vitamin A, heavy metal poisoning, hair shaft abnormalities, and rare dermatologic conditions.

C. Treatment Options: Successful treatment of alopecia is difficult and management of these patients includes support and reassurance. Any underlying condition should be treated.

**Triamcinolone (10 mg/ml):** Alopecia areata sometimes responds to topical or intralesional steroids.

**2% Minoxidil solution (topical):** Is most effective for vertex alopecia in androgenic alopecia. Only about 40% of patients experience significant hair growth and it is generally not effective or indicated for other causes of hair loss except possibly alopecia areata. Hair re-growth can take up to 12 months. Treatment is continued indefinitely, once treatment is stopped hair loss resumes. Frequent adverse effects are mild scalp irritation, allergic contact dermatitis, and increased facial hair.

**Finasteride (Propecia):** Finasteride (Propecia): Androgenic alopecia (male pattern hair loss) is caused by androgen-dependent miniaturization of scalp hair follicles, with scalp dihydrotestosterone (DHT) implicated as a contributing cause. Finasteride, an inhibitor of type II 5 alpha-reductase, decreases serum and scalp DHT by inhibiting conversion of testosterone to DHT.

Treatment for at least 3 to 6 months is necessary to see hair growth or to prevent further hair loss. Continued use is necessary to sustain benefit. Reported side effects include gynaecomastia, decreased libido, ejaculation disorders and erectile dysfunction, which resolve in most men who remain on therapy and in all men who discontinue use of Finasteride. When testing prostate specific antigen (PSA) for cancer screening, note use of Finasteride since there may be a decrease in PSA levels in older men. (Finasteride is also used as treatment for benign prostatic hyperplasia.) There are no data to support the use of finasteride in females with androgenic alopecia. Pregnant women should not be exposed to Finasteride either by use or handling of tablets because of teratogenic risk.

**Spironolactone:** Used in treating female androgenic hair loss, is a competitive inhibitor of aldosterone and also competes with DHT for androgen receptors in target tissues. It also reduces 17-alpha-hydroxylase activity lowering plasma levels of testosterone and androstenedione.

**Cyproterone acetate:** Is one of the last resort treatments for treating female androgenic hair loss, because of its toxicity and long term side effects. Cyproterone acetate exerts its effects by blocking the binding of DHT to its receptors.

**Other hormone modulators:** such as oral contraceptives may also be useful in treating female androgenic hair loss.

**Scalp surgery and autologous hair transplants:** may also be considered by patients with androgenic alopecia.

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.

**Harvesting of adipose tissue:** Adipose tissue acquisition can be summarized as three step process:

**Application of anesthetic / injection of tumescent solution**

**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 60 to 120 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:** The stem cells obtained from the adipose tissue sample and the PRP are applied to the patient using appropriate protocol for their condition. Autologous Ad-SVF containing adult stem cells are infused locally by multiple local injections, to affected areas of the scalp.

**Procedure for application of local scalp injections:** Prepare PRP mixture and sample of Ad-SVF Containing Adult Stem Cells. Using aseptic technique, a 1 cc syringe, and a 25 G needle, administer 0.2 cc of cell mixture via subcutaneous injections to affected areas of scalp, each approximately a half inch apart.
B. Risks: There are possibilities for unwanted effects related to 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 any results, excellent results can be attained. The medical professional 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
- Nausea
- Allergic reaction
- Vomiting
- Dizziness

C. Benefit: Mesenchymal Stem Cells express immunomodulatory and anti-inflammatory properties to help mitigate the impairment to the hair follicles. Clinical response may include increased activity of hair growth and a higher number of active hair follicles. Increased activity of follicles and changes to scalp will provide evidence of repair after stem cell graft.

D. Treatment Timeline: Recommend dosing every 2 months with cells (with or without PRP) initially until desired results then maintaining as needed; typically every six months.

ALOPECIA – SUPPORTING STUDIES


EPITHELIAL STEM CELLS: A FOLLICULOCENTRIC VIEW.

Cotsarelis G.

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Abstract: Putative epithelial stem cells were identified in the hair follicle bulge as quiescent "label retaining cells". The study of these cells was hindered until the identification of bulge cell molecular markers, such as CD34 expression and K15 promoter activity. This allowed for the isolation and characterization of bulge cells from mouse follicles. Bulge cells possess stem cell characteristics, including multipotency, high proliferative potential, and their cardinal feature of quiescence. Lineage analysis demonstrated that all epithelial layers within the adult follicle and hair originated from bulge cells. Bulge cells only contribute to the epidermis during wound healing, but after isolation, when combined with neonatal dermal cells, they regenerate new hair follicles, epidermis, and sebaceous glands. Bulge cells maintain their stem cell characteristics after propagation in vitro, thus ultimately they may be useful for tissue engineering applications. Understanding the signals important for directing movement and differentiation of bulge cells into different lineages will be important for developing treatments based on stem cells as well as clarifying their role in skin disease.

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ALOPECIA – SUPPORTING STUDIES

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Multi-layered environmental regulation on the homeostasis of stem cells: the saga of hair growth and alopecia.

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Abstract: Stem cells are fascinating because of their potential in regenerative medicine. Stem cell homeostasis has been thought to be mainly regulated by signals from their adjacent micro-environment named the "stem cell niche". However, recent studies reveal that there can be multiple layers of environmental controls. Here we review these environmental controls using the paradigm of hair stem cells, because to observe and analyze the growth of hair is easier due to their characteristic cyclic regeneration pattern. The length of hair fibers is regulated by the duration of the growth period. In the hair follicles, hair stem cells located in the follicle bulge interact with signals from the dermal papilla. Outside of the follicle, activation of hair stem cells has been shown to be modulated by molecules released from the intra-dermal adipose tissue as well as body hormone status, immune function, neural activities, and aging. The general physiological status of an individual is further influenced by circadian rhythms and changing seasons. The interactive networks of these environmental factors provide new understanding on how stem cell homeostasis is regulated, inspiring new insights for regenerative medicine. Therapies do not necessarily have to be achieved by using stem cells themselves which may constitute a higher risk but by modulating stem cell activity through targeting one or multiple layers of their micro- and macro-environments.

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ALOPECIA – SUPPORTING STUDIES
Treatment of alopecia by transplantation of hair follicle stem cells and dermal papilla cells encapsulated in alginate gels.
Zhao J, Liu LQ, Wang YJ, Yang W, Geng WX, Wei J, Li LW, Chen FL.
Source: Rege Lab of Tissue Engineering, Department of Bioscience, Faculty of Life Science, Northwest University, No. 229 North Taibai Road, Xi'an 710069, PR China.
Abstract: The affected individual of hair loss demands help, because hair is viewed as a sign of youth and good health. Nowadays treatment of alopecia includes drug therapy and hair transplantation. Some drugs may promote hair growth, at least temporarily, but the treatment is effective only in milder alopecia, instead of extensive alopecia. Furthermore, the side effect of long period medication could not be avoided. Hair transplantation involves harvesting small pieces of hair-bearing scalp grafts from a donor site and relocating them to a bald area. This method does not increase the number of existing hairs, but only redistributes them. The operation is sophisticated and time-consuming, thus the patient suffers a lot during the process. The discovery of hair follicle stem cells (FSC) brings gospel to the affected individual of hair loss because of its capacity of generating new hair when they interact with mesenchymal dermal papilla cells (DPC). Besides, both FSC and DPC have strong proliferative capacity and the patient’s own cells could be expanded considerably in vitro. Thus we hypothesize that the microencapsulation of the two kinds of cells in alginate gels could be implanted into the bald scalp of the patient since alginate gels is effective in cell

ALOPECIA – SUPPORTING STUDIES
Up-to-date Clinical Trials of Hair Regeneration using Conditioned Media of Adipose-Derived Stem Cells in Male and Female Pattern Hair Loss.
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Abstract: The primary roles of mesenchymal stem cells (MSCs) are to maintain the stem cell niche, facilitate recovery after injury, and ensure healthy aging and the homeostasis of organ and tissues. MSCs have recently emerged as a new therapeutic option for hair loss. Since adipose-derived stem cells (ADSCs) are the most accessible sources of MSCs, ADSC-based hair regeneration is currently under investigation. Besides replacing degenerated cells in affected organs, ADSCs exhibit their beneficial effects through the paracrine actions of various cytokines and growth factors. Several laboratory experiments and animal studies have shown that ADSC-related proteins can stimulate hair growth. In this paper, we introduce our clinical pilot studies using conditioned media of ADSCs for pattern hair loss in men and women. We also discuss practical therapeutic challenges and the direction of future research.

ALOPECIA – REFERENCES