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Department of Dermatology
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Title of Study

Safety and Efficacy of Combined Restylane® and Triamcinolone Acetonide 10 mg/cc Intralesional Injections for the Treatment of Alopecia Areata

Purpose

The purpose of this pilot research study is to determine the safety and efficacy of combined hyaluronic acid, Restylane®, and intralesional Triamcinolone acetonide 10 mg/cc in the management of alopecia areata. Dermal injections of Restylane® and triamcinolone acetonide 10 mg/cc will be performed at areas of hair loss on study participants.

Background

AA is an autoimmune organ specific T-cell mediated disease which targets hair follicles and causes non-scarring hair loss[5]. Hairs that are shed are either in telogen or dystrophic anagen[6]. AA can present as patches of alopecia on the scalp, complete loss of hair on the scalp (AA totalis) or loss of hair from the entire body (AA universalis)[1].

This fairly common disorder can affect patients of all ages[8]. Lifetime risk has been estimated to be 1.7%. A significant number of these cases, approximately 7%, may eventually evolve into chronic and severe hair loss[8]. Because the pathogenesis of the disease and the factors contributing to development are not fully understood, predicting the course of the disease is extremely difficult[1]. The psychological affects on patients with AA can be devastating. Increased risk of psychiatric disorders such as anxiety disorders (39%) and major depression (39%) have been found in patients affected by the disease[3].

With regards to the management of AA, use of IL corticosteroids has become a first line therapy. Synthetic corticosteroids are utilized in

both an anti-inflammatory and immunosuppressive manner, likely controlling the infiltration of T-cells in the perfollicular area by induction of lipocortins[14]. Lipocortins antagonize phospholipase A_2 (PLA_2), the enzyme that leads to lysosomal breakdown and release of arachidonic acid in the inflammatory pathway toward the production of inflammatory substances such as prostaglandins, kinins, histamine, liposomal enzymes and complement[15]. As a result of this decrease in the amount of inflammatory mediators, inhibition of chemotaxis by vasoconstriction and decreased vascular endothelial permeability results[14]. Additionally, these compounds inhibit macrophage and major histocompatibility complexes human leukocyte antigens (MHC HLA) antigen presenting cells[15].

Administration of IL injections of corticosteroids employs injection of triamcinolone acetonide, 3 mg/ml-10 mg/ml typically, administered every 4 to 6 weeks[12,13]. This method typically involves injection with a 0.5-inch-long 30-gauge needle into the skin at approximately the level of the hair bulb[12,13]. Regrowth is seen in 4 to 8 weeks and IL corticosteroid injections are then repeated every 4 to 6 weeks. Draw backs and limiting factors of this treatment modality includes atrophy at sites of injections, discomfort and the need for recurrent treatments every 4-6 weeks in patients who cannot sustain hair growth without ongoing therapy. Atrophy of the scalp may also lead to transient cosmetic defects.

Interest in the administration of IL triamcinolone acetonide with hyaluronic acid has developed because of the ability of hyaluronic acid to act as a dermal filler and its reparative properties. Restylane®, hyaluronic acid, is a high-molecular weight polysaccharide glycosaminoglycan that enhances fibroblast movement and metabolism during wound healing, increasing collagen fibers forming granulation tissue[2]. Hyaluronic acid is known to be important in repair processes such as providing a hydrated, unresisting matrix in which cells are able to migrate[9].

We hypothesize that Restylane® could serve as a repair matrix which also maintains IL triamcinolone acetonide concentrations at higher levels for a longer period of time in the skin, giving a more sustained local anti-inflammatory effect and thus, arresting the AA process and promoting hair regrowth. Furthermore, the combination of Restylane®

with IL triamcinolone acetonide injections may prevent a common side, namely, atrophy. With the prevention of scalp atrophy and the preservation of higher concentrations of triamcinolone acetonide for longer periods of time, patient quality of life will be improved, and the expense of medical visits and medications will be reduced as fewer IL corticosteroid injections would be needed each year.

Recent studies investigating the role of peripheral nerves in the pathogenesis of AA have indicated that peripheral nervous system dysfunction exists in the scalp of patients with alopecia areata when compared to normal controls[18]. Another mechanism that may account for hair regrowth in AA patients receiving IL hyaluronic acid is that it may provide a regenerative environment for the peripheral nerves of the scalp. Previous studies with hyaluronic acid have demonstrated its ability to facilitate cellular and axonal growth during peripheral nerve regeneration[11].

Preclinical Data

The rodent model for the study of AA has not yet been standardized for use in clinical research. Per the President and CEO of the National Alopecia Areata Foundation (NAAF), Victoria Kalabokes, the work is still in progress to validate the model for clinical testing. Intradermal injections performed in mice often results in subcutaneous injections. Although unpublished, it has been reported to NAAF that several of the rodent models died from unknown reasons during clinical studies.

Preclinical testing in the Dundee experimental bald rat (DEBR) of AA with intralesional injections has proven to be difficult to conduct due to their extremely thin skin.

Clinical Data to Date

Resytlane® is FDA approved as a medical device in United States for mid to deep dermal implantation for the correction of moderate to severe facial wrinkles and folds such as nasolabial folds. There is no available clinical research data to date investigating the treatment of AA with IL Resytlane® or its use in the scalp.

In a recent study, eleven healthy human volunteers with photodamaged forearm skin were injected with a hyaluronic acid filler and isotonic

sodium chloride (a vehicle) and skin biopsy specimens were obtained at 4 and 13 weeks[15]. It was found that injection of cross-linked hyaluronic acid partially restores dermal matrix components via stimulation of collagen production that had been lost in photodamaged skin. This finding implies that hyaluronic acid has the potential to benefit atrophic skin conditions[15]. Thus, AA patients may benefit from both stimulation of therapeutic collagen production in the dermis and collagen production induced by Restylane® to prevent scalp atrophy due to AA corticosteroid injections of the scalp[15].

In an ongoing clinical study at the University of Minnesota titled, "Adrenal Function and Use of Intralesional Triamcinolone Acetonide 10mg/ml in Patients with Alopecia Areata," the efficacy of intralesional corticosteroids on hair regrowth in moderate to severe alopecia areata is being evaluated. Subjects undergo intralesional triamcinolone acetonide (10mg/cc) injections every 6 weeks for a period of 6 months, followed by a 6 week, injection-free, safety follow-up visit. Up to 40 mg of triacinolone acetonie are injected intralesionally at each visit.

Preliminary results from 12 alopecia areata subjects, age ranges 20 to 68, are available. The effects of IL triamcinolone acetonide 10 mg/cc on the hypothalamic-pituitary-adrenal axis in AA patients were recorded. No statistically significant difference in cortisol levels were found from time 0 to 30 minutes following stimulation with triamcinolone acetonide between weeks 0 and 24 (mean difference=0.13). A linear mixed model was also fitted to repeatedly measured cortisol levels (at week 0, 6, 12, 18, and 24) to examine the trend over time. The type III test of coefficient of time variable demonstrated a p-value of 0.99, indicating that cortisol levels from time 0 to 30 minutes following stimulation did not change over time. This data suggests that IL triamcinolone acetonide 10 mg/cc of the scalp does not effect or pose risk to the hypothalamic-pituitary-adrenal axis in AA patients.

In a secondary analysis the alopecia areata severity score (SALT score) was compared between week 0 and week 24 to evaluate hair regrowth using a paired t-test. The mean difference between week 0 and week 24 is 20.4 with standard deviation of 26.5 and is statistically different from zero (p value is 0.03). This indicates that the SALT

score was significantly different between week 0 and week 24, proving that statistically significant hair re-growth was observed as result of treatment with intralesional triamcinolone acetonide 10mg/ml in AA patients.

Methods

Ten subjects (sex ratio not predetermined) ages 18 and older who have extensive alopecia areata of less than 2 years duration will be recruited to participate in this study. Consenting and qualified (meeting the inclusion and exclusion criteria) subjects will participate in this study. This will be a cohort study. The subjects will have three scheduled visits. The total duration of the study for each volunteer is expected to be approximately 12 weeks.

Visti 1.
At the first visit, patients will receive dermal injections of Restylane® followed by IL of triamcinolone acetonide, 10 mg/cc, to approximately 20 sites on one half of the scalp. To assess baseline hair growth, photographs and a modified version of the SALT score will be obtained for each side of the scalp. Degree of scalp atrophy, if any, will be recorded. The scalp will also be examined for erythema, scale, folliculitis. Pull tests will be done.

Restylane® 0.02-0.04 mL will be injected to the mid dermis, at the level of the hair bulb, in diseased areas on one half of the scalp. Injections will be placed approximately 1 cm apart and will be repeated for up to 3mL of Restylane®.. When injections have been completed, the injected areas will be massaged.

Using a fine 1 cc syringe and 30 gauge needle, triamincinolone acetonide 10mg/cc will be administered intralesionally to the entire scalp. Injections will be placed approximately 1 cm apart, injecting 0.1 mL Kenalog-10 into the skin at approximately the level of the hair bulb. Injections will be repeated as needed for up to 4 mL Kenalog-10.

Subjects will remain in clinic 20 minutes following the procedure for recording of adverse reactions.

Visit 2.
Subjects will be asked to return for a second visit 5-6 weeks after injections and the visit 1 procedures will be repeated. The SALT score, alopecia areata half head severity score (AAHHSS score), photography and extent of scalp atrophy will be obtained.

Visit 3.
At the third visit, no injections will be given. The SALT score, AAHHSS score, photography, and extent of scalp atrophy will again be obtained.

Adverse experiences will be recorded at each visit.

Schedule of Study Procedures	**Visit 1 (Baseline)**	**Visit 2 (Weeks 5-6)**	**Visit 3 (Weeks 11-12)**
Signed Informed Consent / Assent and HIPAA Authorization (as required)	X		
Demographic Information	X		
Medical History/Review Medications	X	X	X
Assess Inc/Exc Criteria/Consent	X		
Scalp Physical Examination	X	X	X
Record Concomitant Meds	X	X	X
SALT score	X	X	X
AAHHSS	X	X	X
Photography	X	X	X
Assessment of scalp atrophy	X	X	X
Administer Restylane® and triamcinolone acetonide 10 mg/cc	X	X	
Record Adverse Experiences	X	X	X

Inclusion criteria

1. Men and women ages 18 and greater.
2. Alopecia areata diagnosis in the last two years with extensive scalp involvement – between 74% and 99%. . Alopecia areata must involve the left and right hemispheres of the scalp.
3. Willing to abstain from use of over the counter products and prescription products other than triamcinolone acetonide 10mg/cc which may promote hair growth.
4. Willing to abstain from the use of non-steroidal anti-inflammatory medications, aspirin, St. Johns Wart, and high doses of Vitamin E supplementation.
5. Subjects must be capable of giving informed consent.
6. Willing to adhere to protocol, including scalp examinations and photography.

The gender of the subjects will not determine enrollment. There is no requirement of male to female ratio for this study. The age range of the subjects will be 18 and greater. No specific racial or ethnic restrictions will be present for this study.

Exclusion criteria

1. Allergy or intolerance to Restylane® or hyaluronate preparations
2. Allergy or intolerance to triamcinolone acetonide, 10 mg/cc.
3. Underlying disease that might be adversely affected by Restylane® or triamcinolone (ex. patients with bleeding disorders).
4. Immunosuppressed patients (history of transplantation, cancer, chemotherapy, splenectomy, HIV).
5. Pregnant or lactating female.
6. Application of topical immunomodulatory or immunosuppressive agent in the preceding 6 weeks.
7. Systemic administration of corticosteroid or other systemic treatment (i.e. prednisone) that has immunomodulatory or other immunosuppressive mechanism of action, in the preceding 8 weeks.
8. Clinical evidence of secondary skin infection (i.e., folliculitis).

9. Other inflammatory or infectious skin disease that might interfere with evaluations during the study.
10. Investigational medications within the past 30 days.
11. Investigational medications within the past 30 days.
12. Patients with susceptibility to keloid formation.
13. Severe allergies manifested by a history of anaphylaxis, or history or presence of multiple severe allergies
14. Patients with allergies to gram positive bacterial proteins
15. Unable to give consent.

During the study period, subjects will be allowed to apply non-medicated shampoos to the scalp.

Statistical Plan

Sample size:
We plan to study 10 subjects. This is a pilot study. The reasoning for sample size selection is to proceed with preliminary studies in a small group. All subjects will be selected based on inclusion and exclusion criteria.

Data Collection:
Data for this study will be entered into a REDCap database, which uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. REDCap includes a complete suite of features to support HIPAA compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL database and the web server will both be housed on secure servers operated by the University of Minnesota Academic Health Center's Information Systems group (AHC-IS). The servers are in a physically secure location on campus and are backed up nightly, with the backups stored in accordance with the AHC-IS retention schedule of daily, weekly, and monthly tapes retained for 1 month, 3 months, and 6 months, respectively. Weekly backup tapes are stored offsite. The AHC-IS servers provide a stable, secure, well-maintained, and high-capacity data storage environment, and both REDCap and MySQL are widely-used, powerful, reliable, well-supported systems. Access to the study's data

in REDCap will be restricted to the members of the study team by username and password.

Statistical Analysis:

Data will be analyzed for safety and efficacy of administration of IL triamcinolone acetonide 10 mg/cc and Restylane® in the management of (alopecia areata) AA.

The primary endpoint of evaluating the efficacy of administration of IL triamcinolone acetonide 10 mg/cc and Restylane® in the management of AA is the alopecia areata half head severity score (AAHHSS) comparing week 12 with baseline hair loss. The primary analysis will be a comparison of means of AAHHSS between two treatments on the two sides of AA patients scalp using paired t-tests.

The secondary analysis will examine the mean trend over time for the AAHHSS and SALT score at weeks 0, 6, and 12 using linear mixed models (specifically, using the MIXED procedure of the SAS system, version 9.2) with time after baseline as the fixed effect and using the repeated-measures option of the MIXED procedure.

Safety will be analyzed through determination of the frequency of side effects and/or adverse events recorded during the study.

Supportive analyses will examine the demographic and baseline characteristics of the study sample. Descriptive statistics including either numerically or graphical representations will be used to summarize the data.

Primary Study Endpoints

To assess the efficacy of IL Restylane® and triamcinolone acetonide 10 mg/cc in the management of AA, baseline hair growth will be recorded via photographs of the scalp and the SALT score and AAHHSS score. Subjects will be asked to return for a second visit at 5-6 weeks for injections. Markers of hair regrowth include the SALT score, the AAHHSS score and photography.

The Severity of Alopecia Tool (SALT) score measures the percentage hair loss at each visit[19]. The SALT score is determined through a visual examination of 4 views of the scalp; the percent of terminal hair loss visible in each area is recorded and then aggregated, with a maximum SALT score of 100 corresponding to 100% scalp hair loss[19]. In this study, the AAHHSS score will be used to determined hair loss on each half of the scalp when the scalp is divided in the sagittal plane. Each half of the scalp may have a maximum score of 50.

The primary efficacy endpoints are as follows, ranked in order of importance:

1. The AAHHSS will be used to compare hair regrowth on each side of the scalp at 0, 6 and 12 weeks.

2. The SALT score will be used to assess hair regrowth throughout the entire scalp at weeks 0, 6, and 12.

Secondary Study Endpoints

To asses the safety of intralesional Restylane® and triamcinolone acetonide 10 mg/cc in the management of alopecia areata scalp examinations will be preformed before treatment, immediately after treatment and at 5-6 weeks follow up. Medication side effects and adverse experiences will be recorded. Additionally, subjects will be given a telephone contact number to report side effects anytime after visit 1.

Degree of scalp atrophy will be assessed by the principal investigator and be recorded for comparison across visits.

Medication, Dosing and Dose Rationale

At visit 1, Restylane® will be delivered by IL injection into the dermis of the diseased scalp. The minimum and maximum dose of Restylane® will be 1mL and 3mL, respectively. The dose of Restylane® does not exceed the recommended maximum dose per treatment. Based on clinical U.S. studies on the use of Restylane® as a dermal filler for correction of moderate to severe facial wrinkles, the

recommended maximum dose is 6mL. Restylane® will be injected throughout the diseased area by serial puncture. IL injections will be spaced approximately 1cm apart, administering approximately 0.02-0.04mL at each site.

Triamcinolone acetonide, 10 mg/cc, will be then be delivered by IL injection into the dermis of diseased areas of the scalp, bilaterally. A maximum dose 4 cc of triamcinolone acetonide will be administered. A total of 2mL may be used on each side of the scalp. The dose of Triamcinolone acetonide 10 mg/cc does not exceed the one used in everyday clinical practice at the University of Minnesota. All subjects will be administered IL triamcinolone acetonide 10 mg/cc proportional to their required lesion size. Injections of approximately 0.05-0.1 mL will be injected at each site administering approximately 1 mg triamcinolone acetonide, 10mg/ml per injection site. IL injections will be spaced approximately 1 cm apart.

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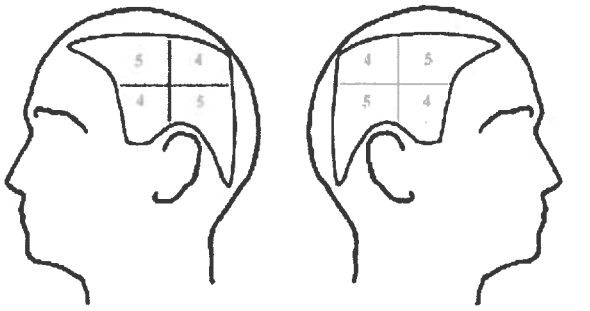
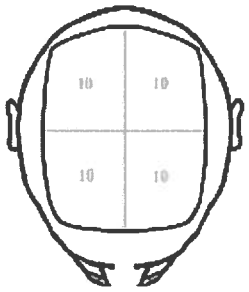
Visual Aid for Determining Modified SALT Score

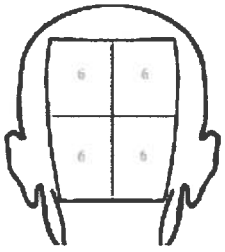


LEFT SIDE: 36%

RIGHT SIDE: 36%



TOP: 40% PER SIDE



BACK: 24 % PER SIDE

Reference: Figure 1. Modified version of, "Alopecia Areata Investigational Assessment Guidelines –Part II," (Olsen, et. al. 2004).

Determining SALT Score (cont)

The subject's SALT score will be determined as follows:

1. For each of the four main scalp surface areas, the percentage of missing hair will be documented in Row 1.
2. The percentages recorded in Row 1 will be multiplied by the percentages in Row 2 of the same column (Areas as % of Total Scalp Area). The results will be entered in the appropriate columns of Row 3.
3. The four results in Row 3 will be summed to get the percentage hair loss (Row 4).

Sample Worksheet for Determining SALT Score

Row	Parameter	Left	Right	Top	Back
1	Enter % Hair loss for Each Scalp Surface Area				
2	Area as % of Total Scalp Surface	X 0.36	X 0.36	X 0.40/side	X 0.24/side
3	Multiply Row 1 x Row 2				
4	Total % Hair Loss (Sum the 4 Products from Row 3)				

Once a subject's SALT score is recorded at baseline (Week 0) and at week Z follow up, the percent scalp hair regrowth based on SALT score using the following formula will be determined:

$$\frac{\text{SALT Score at Baseline (Week 0)} - \text{SALT Score at Weeks Z}}{\text{SALT Score at Baseline (Week 0)}} \times 100$$

Where Week Z = the Week for which % hair regrowth from baseline is calculated

50% scalp hair regrowth based on SALT score can be expressed as $SALT_{50}$
75% scalp hair regrowth based on SALT score can be expressed as $SALT_{75}$