# A PHASE 4 RANDOMIZED, PLACEBO CONTROLLED TRIAL OF 3 DOSES OF INTRALESIONAL TRIAMCINOLONE (KENALOG®) IN THE TREATMENT OF MILD TO MODERATE PATCH TYPE ALOPECIA AREATA

[IL TAC in AA]

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## GOALS

Alopecia areata (AA) is a major cause of morbidity and is ***the most prevalent autoimmune disease*** in the US, affecting approximately 4.6 million people, including males and females across all ethnic groups, with a lifetime risk of 1.7% (1). Additionally, AA represents the second most common form of human hair loss, second only to androgenetic alopecia, and causes significant disfigurement and psychological distress to affected individuals (Figure 1). AA affects more individuals than most other autoimmune diseases *combined,* including lupus erythematosus (LE), type 1 diabetes (T1D), psoriasis, multiple sclerosis (MS) and rheumatoid arthritis (RA). **In stark contrast to these other conditions, research into the pathogenesis and the development of innovative therapies in AA has lagged far behind.** This may be due in part to the perception that AA is merely a cosmetic disorder. **In reality, AA carries one of the highest burdens among any skin diseases, particularly among children and adolescents whose self-image is so closely linked to their appearance (2).**

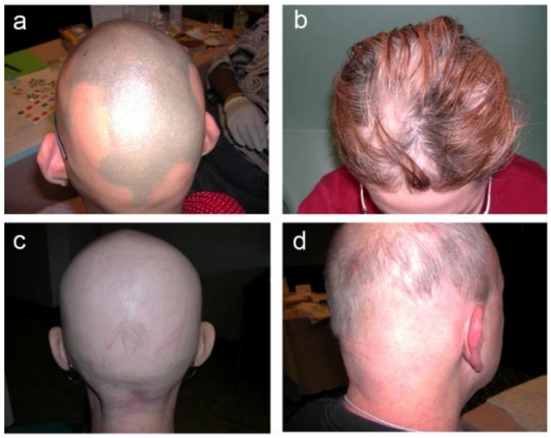


**Figure 1.** Clinical manifestations of AA. In the upper panels (a and b), patients with patch type AA. In b, the patient is in a spontaneous regrowth phase. For patients with alopecia universalis, there is a complete lack of body hair and scalp hair (c), while patients with alopecia totalis lack scalp hair but not body hair. In d, the randomness of hair regrowth is observed, with regrowth in the parietal region but not in either the occipital or temporal regions.

Despite its high prevalence, there are ***no*** evidence-based treatments for AA. A comprehensive Cochrane analysis assessment of seventeen randomized clinical trials (RCTs) involving a total of 540 participants found no treatment with proven efficacy for AA (3). Each trial included from 6 to 85 participants and assessed a range of interventions that included topical and oral corticosteroids, topical cyclosporine, photodynamic therapy and topical minoxidil. Overall, ***none*** of the interventions showed significant treatment benefit in terms of hair growth when compared with placebo. It was concluded that the effectiveness of few (if any) treatments for AA are proven. No RCTs on the use of immune modulators such as diphencyprone, dinitrochlorobenzene, intralesional corticosteroids or dithranol were found, although these drugs are commonly used for the treatment of AA. Similarly, although topical corticosteroids and minoxidil are widely prescribed and appear to be safe, there is no convincing evidence that they are beneficial in the long-term. Most trials have been poorly reported and/or are so small that any important clinical benefits are inconclusive.

Intralesional corticosteroids - triamcinolone acetonide (Kenalog) (IL TAC) are arguably the most commonly used treatments for alopecia areata, and certainly the most commonly used modality for patients with less than 50% hairloss in AA(4). Most practitioners would agree that many patients benefit from this treatment however there is no well controlled evidence to support this belief. Despite the fact that intralesional corticosteroids have been used to treat AA for over 50 years(5, 6), there are no adequately powered, randomized controlled clinical trials examining the efficacy of this treatment(3). In addition, the dosage or strength used varies from practitioner to practitioner. The relative efficacy, safety and duration of effect of alternate doses of IL TAC has never been examined in a well designed randomized clinical trial. A well designed randomized controlled study would provide immediately useful

information that could dramatically impact the way AA patients are currently treated. In addition, quantitative biomarkers for AA are a crucial step toward translational research aimed at clinical trials in AA. Toward this goal, we propose:

**Specific Aim 1.** To evaluate the efficacy of treating patients with mild to moderate patch type alopecia areata for six months with IL TAC at a strength of 2.5mg/ml versus treatment with IL TAC 5mg/ml versus IL TAC 10mg/ml versus intralesional saline as placebo.

**Specific Aim 2.** To identify a clinical correlation between treatment outcome and down modulation of key AA-associated immunohistopathological markers and biomarkers in the treated skin; including NKG2DL expression in the hair follicle, immune infiltration ($CD8^+NKG2D^+$ cells) and expression of interferon response genes.

## *RATIONALE*

### Development of an Alopecia Areata Clinical Trials Center at CUMC

Alopecia areata has been poorly studied clinically in part because molecular mechanisms of pathogenesis have been poorly understood. Work here at CUMC has changed this landscape: GWAS studies published last year (Petukhova et al, Nature 2010) by Angela Christiano in our Department identified for the first time specific genes responsible for inherited risk and identified potential therapeutic targets. This has provided the basis for the initiation of a suite of new clinical research programs in alopecia areata at CUMC using rationally targeted therapies aimed at these genetically identified pathways. Two such innovative programs have been submitted for external research funding including 1) use of a topical JAK inhibitor to inhibit interferon signaling in localized alopecia areata and 2) use of systemic anti-CTLA-4-Ig to block costimulation in severe alopecia. Further, based on these genetic findings, immunopathological findings and transcriptional profiling of alopecic skin, a biomarker platform to evaluate therapeutic responses is being developed using human blood and skin biosamples. In this proposal we seek to use and refine this biomarker platform to evaluate the current “standard of care” intervention, topical steroids, to examine for the first time alopecia biomarkers during treatment. This will provide important experience in the development of this platform and complete a comprehensive clinical program in alopecia evaluating side-by-side both novel therapeutics and current clinical practice (IL steroids) in alopecia areata.

### Clinical experience with IL TAC for AA

One of the earliest studies of intralesional steroid was published by Richard Heffernan in 1964. He injected patients with a variety of dermatologic disorders with TAC in varying doses (2.5mg/ml, 5mg/ml, 10mg/ml) or saline in a “controlled, double-blind study”. Single discrete lesions were divided into 4 quadrants and equivalent volumes of the four coded suspensions were injected intralesionally, each suspension into a different quadrant. A single treatment was given after which patients were evaluated at varying intervals. For the single AA patient treated in this manner, “an equivalent degree of hair regrowth was noted in the three sites injected with active drug, and no regrowth of hair in the site injected [with] diluent”. Hair regrowth was not present at 17 days, but was present at 34 days and 8 months(7).

Subsequently, Abell and Munro treated 84 patients with varying types and degrees (single lesion to alopecia universalis) of AA using IL TAC 5mg/ml. Injections were given at weekly to bi-weekly intervals on 3 occasions, followed by examinations at 6 and 12 weeks to determine efficacy. Fifteen patients were injected with isotonic sterile normal saline

"coincidentally with the triamcinolone therapy". No mention is made of randomization or blinding. At follow-up satisfactory regrowth was defined as "the presence of predominantly pigmented terminal hair at the injection sites in significant quantity". "Significant quantity" was not further described. Regrowth was noted to occur in tufts corresponding to the injection sites within 4 to 6 weeks, and was often accompanied by evidence of minimal, transient skin atrophy. When divided into limited alopecia vs alopecia totalis/universalis, 71% of patients with limited AA had regrowth at 12 weeks vs 28% of patients with alopecia totalis/universalis. Seven percent of patients treated with saline had regrowth at 12 weeks, however the authors attributed the incidence of spontaneous recovery over the period of observation to be 20%. Of note, the clinical photographs showing regrowth indicate relatively limited regrowth limited to small patches at injection sites. Side effects noted were minimal and included: hemorrhage at injection sites, easily controlled with pressure, minimal atrophy, and no incidence of "sepsis". In one patient, plasma cortisol levels were monitored by 9am samples. The pretreatment level of 14.8µg/100ml was reduced to 2.7µg/100ml the day following a dosage of 22.5mg of triamcinolone acetonide. The resting level was regained by day 3 and remained normal over the next 10 days (8).

In general practice, a concentration of 5mg/ml of TAC with a maximum volume of 6ml (30mg total) or 10mg/ml of TAC with a maximum volume of 3ml can be used on the scalp in one visit. Multiple intradermal injections using a 30 gauge needle are administered approximately 1 cm apart with injection of approximately 0.1ml per site. Initial regrowth may be seen in 4 to 8 weeks (9), (10). Side effects include mild, transient skin atrophy, lasting 2 to 8 months especially if too great a volume is injected or if the injection is too superficial or too deep(11). Transient pain is an additional side effect which limits the use of this modality in some patients, especially children(4). Ironically, younger patients (children) have been reported to have comparatively better hair growth in response to intralesional steroids(9). Topical anesthetics may be used to decrease discomfort. If there is no regrowth after 6 months of treatment, therapy with intralesional steroids should be stopped(10).

**Mechanism of action of intralesional steroids**

Despite the years of clinical experience of steroids in autoimmunity, the specific anti-inflammatory mechanisms are still incompletely understood.

It is thought that intralesional steroids act in alopecia areata by inhibition of the T cell mediated attack on the hair follicle. "Glucocorticoids exert their effects by binding to the glucocorticoid receptor (GR), a transcription factor capable of regulating many genes in a positive and or negative way"(12). The immunomodulatory action of glucocorticoids is thought to be due to its inhibitory role on the activity of transcription factors, such as activator protein-1 (AP-1) and nuclear factor κ β (NFκβ), that are involved in the activation of proinflammatory genes(13). Consequently, glucocorticoids suppress the production and effects of humoral factors involved in inflammatory responses and inhibit leukocytes' migration to the inflammation sites"(14). While many patients benefit from intralesional steroids in AA, there is a group of patients that demonstrate a diminished response or resistance to this treatment modality.

The decreased expression of thioredoxin reductase 1 in the outer root sheath may be the cause for glucocorticoid resistance in some AA patients (15), (14). Specifically, Sawaya and Hordinsky reported that glucocorticoid receptor is inactive and cannot bind its ligand in cases of glucocorticoid resistant AA and further, that the activity of thioredoxin reductase 1 is significantly lower in glucocorticoid resistant AA. These findings indicate a possible role for thioredoxin reductase 1 in maintaining glucocorticoid sensitivity.

## ***INNOVATION***

Historically, the routine diagnosis of AA has relied upon clinical observation and histopathological findings of a peribulbar infiltrate around the hair follicle, both of which are inherently subjective, non-quantitative and lack sufficient resolution to identify molecular subtypes of disease. Serum biomarkers (Benoit, S.*J Invest Dermatol* 121:933-935. Gregoriou, S., *Mediators Inflamm* 2010:928030. Kuwano, Y., 2007. *Br J Dermatol* 157:466-473. Barahmani, N., 2009. *Clin Exp Dermatol*.) and scalp skin gene expression (Subramanya, R.D. 2010. *Genomics* 96:146-153. Ghoreishi, *Br J Dermatol* 163:57-62) have been studied in small series of patients (n=10 patients), and only one study from whole blood [n=9 patients (Coda, A.B., 2010. *Genes Immun* 11:531-541)] has been published, many of which have been underpowered for statistical significance. **Quantitative biomarkers for AA are a crucial addition to clinical trials in AA.** Drs. Christiano and Clynes have developed a biomarker instrument that can be deployed in clinical trials or monitoring of AA patients over time. This innovative approach will allow us to robustly and quantitatively assess the disease status of AA patients and set the stage for wider validation of these markers in larger AA populations in the future. **As part of this study, we will apply a set of novel biomarkers in AA that provide a translational platform to monitor novel treatments for AA, and may provide insight for other autoimmune diseases.**

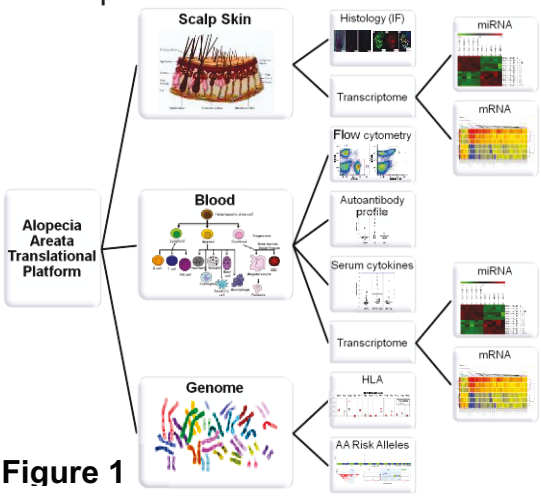


**Figure 1**

## ***METHODS***

**Specific Aim 1: To evaluate the efficacy of treating 68 patients with mild to moderate patch type alopecia areata for six months with intralesional triamcinolone acetonide (IL TAC) at a strength of 2.5mg/ml versus treatment with intralesional triamcinolone acetonide 5mg/ml versus treatment with intralesional triamcinolone acetonide 10mg/ml versus intralesional saline placebo and to monitor the duration of therapy for 6 months following treatment.**

The primary efficacy endpoint of this intention to treat trial will be the proportion of responders in the treated groups compared to the control group after 6 months of treatment, with responders being defined as subjects achieving 50% or greater hair re-growth from baseline as assessed by SALT score at week 24. This is a relatively strict definition for defining responders and non-responders and was chosen to minimize responses in the “vehicle” arm,

in which fewer than 10% are expected to achieve this magnitude of hair regrowth spontaneously. In contrast, clinical experience would suggest that approximately 50% of subjects treated with either dose of IL TAC may achieve >/= 50% improvement in SALT score after 6 months of treatment. The goal of this study is to validate or refute this clinical impression and to objectively and precisely quantify the treatment effect of 2.5mg/ml, 5mg/ml or 10mg/ml of IL TAC in AA.

Subjects will be randomized 1:1:1:1 to the placebo or treatment arms, and will receive 6 months of treatment with the study medication or placebo. IL TAC 2.5mg/ml, 5mg/ml, 10mg/ml or placebo will be administered at baseline, then every 4 weeks thereafter for 5 cycles (weeks 4, 8, 12, 16, 20) for a total treatment period of 6 months. Percent hair regrowth from baseline will be determined by SALT measurements following 4, 8, 12, 20 and 24 weeks of treatment. Punch biopsies and peripheral blood will be obtained at baseline prior to treatment, and then once again at a time point between weeks 4 and 20, to be determined by the investigator as indicated by clinical considerations such as variation in the timeline to regrowth from one subject to another, for immunomonitoring/biomarker studies. Additional, optional scalp biopsies and blood draws may be taken at other key time points in order to monitor response to therapy at the discretion of the investigator and with the consent of the study subject. Efficacy will be measured by change in hair re-growth as determined by physical exam and photography, as well as by patient and physician global evaluation scores. Physician and patient global evaluation scores will be determined based on the use of similar 100mm Visual Analogue scales with 0 labeled as no scalp hair loss and 100 labeled as complete scalp hair loss. Patients and physicians will place a vertical mark along the horizontal line depending on their assessment of the degree of hair loss present at the time of assessment.

As secondary endpoints, efficacy will be measured by changes in hair re-growth as a continuous variable as determined by physical exam, SALT score and Canfield photography, as well as patient and physician global evaluation scores.

To assess the durability of responses, patients who achieved 50% regrowth from baseline (a 50% reduction in baseline SALT score) during the first 6 months, will continue to be followed for an additional 6 months off treatment or until it is determined that relapse has occurred. Percent hair regrowth from baseline will be determined by SALT measurements during the observational phase at weeks 28, 32, 36, 40, 44 and 48. Relapse will be defined as any recurrence of hair loss in responders (subjects achieving greater than 50% decrease in SALT score from baseline during the first 6 months of the study) who had achieved stable regrowth without continued loss for at least 2 months; and loss of >/= 25% of regrowth in patients who had achieved 50% regrowth but still had chronic low grade hair loss.

Nonresponders to 6 months study treatment (defined as failure to achieve 50% improvement in SALT score at week 24 compared to baseline) will not be required to participate in the 6mth follow-up period but will be given the option of receiving monthly IL TAC 2.5mg/ml, 5mg/ml or 10mg/ml injections for 6 months if deemed appropriate by the investigator. The dosage to be utilized (2.5mg/ml vs. 5mg/ml vs. 10mg/ml) will be based on clinical considerations, unless study data is available to support preference of one dose versus the

other. Patients identified by unblinded study personnel as having previously been treated with active drug or with obvious signs of atrophy or other indications suggesting they have already been treated with active drug for 6 months will not be given the option of continuing IL TAC as it is unlikely that they will respond if they have not responded by 6 months unless available evidence suggests that utilization of a different dosage is more likely to be efficacious and safe.

**Human Subjects Involvement, Characteristics, and Design**

The use of human subjects is proposed in this application. Informed consent will be obtained from each subject prior to any study related procedures. After informed consent has been obtained, potential subjects will be screened to ensure they meet diagnostic and safety criteria for inclusion in the study. Subjects will then be randomly assigned via a randomization list to one of three doses (2.5mg/ml versus 5mg/ml versus 10mg/ml) of IL TAC or placebo (intralesional saline) in a 1:1:1:1 ratio. Bacteriostatic saline is chosen as a diluent and placebo instead of xylocaine as the injection stings less. The concentration, frequency of injection of the study medication and diluents used are determined based on current standard of care in clinical practice for the treatment of patients with alopecia areata. Therefore, patients being treated with IL TAC 2.5mg/ml will have up to a maximum of 12ml of drug injected, those treated with IL TAC 5mg/ml will have up to a maximum of 6ml of drug injected, while patients being treated with IL TAC 10mg/ml will receive a maximum of up to 3ml of drug injected. In an attempt to maintain the blinding of the evaluating investigator, patients receiving saline placebo, will have either 3ml, 6ml, or 12ml drawn up drawn up as appropriate for the extent of hair loss present and at the discretion of the unblinded study staff member preparing the medication. The entire syringe will be covered. After injections are completed, the unblended study team member will collect the used and unused syringes and calculate the total volume injected. This amount will be recorded and concealed to maintain integrity. During the course of the study, subjects will be closely monitored for efficacy and the occurrence of adverse events. Determination of immunologic outcome measures will require the collection of blood samples (peripheral blood draws) as well as skin biopsies from the scalp. The subject population will consist of 68 patients with a diagnosis of patchy AA of recent onset (duration of at least 3 months) who have consented to participation in the trial and have met all inclusion and exclusion criteria. Patients will be 18 to 75 years old, and be deemed to be in good general health. We do not intend to enroll patients from vulnerable populations.

*Inclusion criteria*

- Patients 18 to 75 years of age
- Patients with a diagnosis of patch type alopecia areata
- Patients will have up to 50% total scalp hair loss at baseline as measured by the Severity of Alopecia Tool (SALT) (2) score.
- Duration of hair loss greater than 3 months without an upper limit of duration as long as there is reason to believe that regrowth is possible in the opinion of the investigator.
- No evidence of active, ongoing regrowth present at baseline.
- Patients with alopecia totalis/universalis can be included as long as the current episode of hair loss meets the criteria of up to 50% total hair loss (i.e. they may have had a

previous episode of AT or AU which demonstrated regrowth of hair, and they are not currently AT or AU), and as long

- Patients with more than 50% total scalp hair loss at baseline can be included as long as the specified area to be treated is limited to less than 50% of total scalp area

*Exclusion criteria*

- Patients with a history of or existing skin diseases affecting the scalp such as psoriasis or seborrheic dermatitis and patients with evidence of infection or skin cancer in the treated areas
- Patients in whom the diagnosis of alopecia areata is questionable
- Patients in whom regrowth is present/evident at baseline in the areas to be treated
- Patients with active medical conditions or malignancies (except adequately treated basal or squamous cell carcinoma of the skin) which in the opinion of the investigator would increase the risks associated with study participation, including patients with a history of recurrent infections
- Women of childbearing potential who are unable or unwilling to use two forms of birth control for the study duration or women who are pregnant or nursing
- Patients known to be HIV or hepatitis B or C positive or otherwise immunocompromised
- Patients with evidence of adrenal cortex abnormality or previous significant adverse reaction to intralesional steroids
- Patients unwilling or unable to discontinue treatments known to affect hair regrowth in alopecia areata
- Patients who have been treated with intralesional steroids, systemic steroids, anthralin, squaric acid, DPCP (diphenylcycloprophenone), protopic, minoxidil or other medication which in the opinion of the investigator may affect hair regrowth, within one month of the baseline visit.

*Study procedures*

*Screening*: A complete medical history will be taken. Patients will be specifically asked about renal, hepatic or adrenal abnormalities and pregnancy (women of child bearing potential only). In keeping with current standard of care, no routine blood work will be done, unless it is deemed necessary by the investigator. Patients will be randomly assigned to IL TAC 2.5mg/ml, IL TAC 5mg/ml or IL TAC 10mg/ml or intralesional saline in a 1:1:1:1 ratio based on a computer generated randomization list. The list will be generated prior to study initiation. Based on the randomization list, patients will be assigned to treatment vs. placebo based on the order in which they are enrolled. The assignment will be made by study personnel not involved in making clinical assessments or evaluations. The patient and the assessing physicians will remain blinded to the treatment assignment for the duration of the study. Baseline assessments of disease severity will be done, including SALT, Physician global assessment (PGA), patient global assessment and patient quality of life assessment. Patients will have study medication injected once per month by the study physician in accordance with current standard of care clinical practice. Per current clinical practice, attempts will be made to treat all areas of hair loss due to AA, however (also in accordance with current standard of care in clinical practice) the dose of intralesional triamcinolone injected will be limited to a maximum of 30mg per month(16, 4). Therefore, patients being

treated with IL TAC 2.5mg/ml will have up to a maximum of 12ml of drug injected, those treated with IL TAC 5mg/ml will have up to a maximum of 6ml of drug injected, while patients being treated with IL TAC 10mg/ml will receive a maximum of up to 3ml of drug injected. In an attempt to maintain the blinding of the evaluating investigator, patients receiving saline placebo, will have either 3ml, 6ml, or 12ml drawn up at the discretion of the unblinded study staff member preparing the medication. When possible, an unblinded physician investigator will perform study medication injections. If the blinded evaluating investigator must perform injections, the syringes will be covered such that the solution contained within is not visible. The entire syringe will be covered. After injections are completed, the unblended study team member will collect the used and unused syringes and calculate the total volume injected. This amount will be recorded and concealed to maintain integrity.

*Baseline to Week* 48: Patients will receive intradermal injection of study medication once per month to all, or as many as possible, areas of hair loss up to the maximum dose of 30mg IL TAC per month, for a total of 6 months. Injections will be performed at baseline, weeks 4, 8, 12, 16 and 20. Injection areas will be chosen based on the investigator's clinical assessment of factors including but not limited to, areas of most active shedding, areas of maximum medical and cosmetic benefit, avoidance of any areas of pre-existing atrophy, etc. Patients will be seen at weeks 28, 32, 36, 40, 44 and 48 after end of treatment in order to assess the frequency and timing of relapse. Nonresponders to 6 months study treatment (defined as failure to achieve 50% improvement in SALT score at week 24 compared to baseline) will not be required to participate in the 6 month follow-up period but will be given the option of receiving monthly IL TAC 2.5mg/ml, 5mg/ml or 10mg/ml injections for 6 months if deemed appropriate by the investigator. The dosage to be utilized (2.5mg/ml vs. 5mg/ml vs. 10mg/ml) will be based on clinical considerations, unless study data is available to support preference of one dose versus the other. Patients identified by unblinded study personnel as having received TAC or with obvious signs of atrophy or other indications suggesting they have already been treated with active drug for 6 months will not be given the option of continuing IL TAC as it is unlikely that they will respond if they have not responded by 6 months unless available evidence suggests that utilization of a different dosage is more likely to be efficacious and safe.

Efficacy evaluations: SALT, PGA, and Patient global assessment will be performed at every visit. PGA and patient evaluations will be based on similar 100mm visual analog scales. Patient quality of life assessments, performed at baseline, weeks 12, 24, 36, and 48, will be based on changes in the Dermatology Life Quality Index DLQI – adapted for hair loss, or the Dermatology Quality of Life Scales. Blood draws for safety assessments and immunology will be drawn at weeks 4, 12, 24 and 28(if needed for safety labs). Scalp biopsies for immunologic parameters will be obtained at baseline, and at one time point between weeks 4 and 20. Additional biopsies and blood draws may be obtained as clinically indicated. Since the timeline to regrowth may vary from subject to subject, unscheduled biopsies and blood draws will insure that tissue and blood samples are obtained at optimal timepoints to evaluate the changes occurring at the onset of growth or in the event of any unexpected clinical change. In addition, areas that are resistant to treatment may be biopsied to determine the cellular and immunologic differences of those areas compared to areas that are responding to treatment.

The patient will be given the option to agree to, or decline, the additional biopsies and blood draws.

Standardized photographs of the subject's scalp will be taken at Baseline, Week 4, 8, 12, 16, 20, 24, 36 and 48 and will be used to support determination of percent hair regrowth. Photography will consist of a high resolution digital camera mounted on a stereotactic arm. Patients will be positioned using a fixed guide after which photographs of the entire scalp will be taking at standardized locations and at a fixed distance. At a minimum, photographs of the crown, right and left lateral scalp and posterior scalp (views consistent with those used in the SALT measurement) will be taken.

Visit Schedule and Assessments
Screening assessments and all scheduled study visits are outlined in the Table of Study Assessments (please see below).

Informed consent
Written informed consent will be obtained from all patients for this study by the principal investigator or his/her designee prior to any protocol-specific procedures. The study will be conducted in accordance with the Food and Drug Administration (FDA) approved revision of the Declaration of Helsinki, current FDA regulations, and International Conference on Harmonization (ICH) guidelines.

Procedures Performed Prior to Treatment Screening (Day -28 to -1):
The following screening evaluations will be performed within 4 weeks prior to enrollment:

- Informed consent, inclusion/exclusion criteria, prior concomitant medications
- Medical history, physical examination, including a dermatological exam
- Vital signs, body weight
- Assessments of disease severity of scalp, body hair and nails will be conducted including SALT (Severity of Alopecia Tool)
- Physician's global assessment (PGA), patient global assessment, patient quality of life assessment
- Clinical laboratory evaluation (complete blood count, basic metabolic profile, hepatic panel and urinalysis) am cortisol, urine pregnancy test and urine analysis or other, only if deemed necessary by the investigator.

The results of all assessments/tests listed above must be reviewed prior to enrolling to ensure that the patient meets entry criteria and that no exclusion criteria are present.

Baseline (Day 0) Procedures Performed Prior to treatment

- Review of inclusion/exclusion criteria
- Vital signs, body weight
- Adverse events reporting, concomitant medication review
- Photography
- Physician evaluation utilizing the PGA and SALT scale

- Patient global assessment and quality of life assessment
- Urine pregnancy testing
- Blood collection for immunological studies
- Scalp biopsy (4mm punch biopsy)
- Non-lesional scalp biopsy may be obtained at this visit or a subsequent visit
- Injection of study medication

Weeks 4, 8, 12, 16, 20 - Visit days will have an acceptable window of ±7 days.

- Vital signs, body weight
- Adverse events reporting, concomitant medication review
- Photography
- Physician evaluation utilizing the PGA and SALT scale
- Patient global assessment
- Patient quality of life assessment (only week 12)
- Urine pregnancy testing
- Clinical laboratory evaluation (only weeks 4 and 12)
- Scalp biopsy (4mm punch biopsy) and blood collection for immunological studies; one scalp biopsy to be obtained during this time period, at a time point determined by the investigator as indicated by clinical considerations such as variation in the timeline to regrowth from one subject to another
- Additional scalp biopsies and blood collection optional; patient will be given the option to agree to, or decline, additional scalp biopsies and blood draws.
- Injection of study medication

Week 24 +/- 7 days – End of treatment also end of study for nonresponders not continuing on to open label treatment and beginning of open label treatment for nonresponders continuing to open label treatment

- Vital signs, body weight
- Physical and dermatological examination
- Adverse events reporting, concomitant medication review
- Photography
- Physician evaluation utilizing the PGA and SALT scale
- Patient global assessment and quality of life assessment
- Urine pregnancy testing
- Clinical laboratory evaluation
- Optional scalp biopsy (4mm punch biopsy) and blood collection for immunological studies
- IL TAC injection for qualified nonresponders continuing to open label treatment

Weeks 28, 32, 36, 40, 44 and 48 +/- 7 days– Observational Period for responders or IL TAC Injection period for nonresponders deemed appropriate for open label injection

- Vital signs, body weight

- Physical and dermatological examination (only week 48)
- Adverse events reporting, concomitant medication review
- Photography (weeks 36 and 48)
- Physician evaluation utilizing the PGA and SALT scale
- Patient global assessment
- Patient quality of life assessment (only weeks 36 and 48)
- Optional Scalp biopsy (4mm punch biopsy) at additional timepoints at the discretion of the principal investigator and with the consent of the subject, based upon perceived utility in context of individual patient response to therapy and durability of that response.
- Optional Blood collection for immunological studies at additional timepoints at the discretion of the principal investigator and with the consent of the subject.
- IL TAC injection for qualified nonresponders.

Early Termination

- Vital signs, body weight
- Physical and dermatological examination
- Adverse events reporting, concomitant medication review
- Photography
- Physician evaluation utilizing the PGA and SALT scale
- Patient global assessment and quality of life assessment
- Clinical laboratory evaluation
- Scalp biopsy (4mm punch biopsy) – if indicated and if patient agreeable to biopsy and 2 week f/u for suture removal if deemed necessary.
- Blood collection for immunological studies

Unscheduled Visits

An unscheduled visit can occur at any time during the study. A source document must be maintained for these unscheduled visits. The date for the visit and any data generated must be recorded on the appropriate CRF. At treatment discontinuation/early termination subjects will undergo off study evaluations per the Schedule of Assessments.

Telephone contact/retention

Patients will be contacted on a biweekly basis throughout the duration of the study to ascertain the occurrence of adverse events as well as to assess the status of hair growth or loss. Any concerns or questions the patient may have will also be addressed.

Assessment of efficacy

The primary efficacy endpoint of this intention to treat trial in AAP will be the proportion of responders in the treated compared to the control group after 6 months of treatment, with response defined as 50% or greater hair re-growth from baseline as assessed by SALT (Severity of Alopecia Tool) (Olsen et al. 2004) score at week 24. This is a relatively strict definition for defining responders and non-responders and was chosen based on consensus of clinicians and AA experts that 50% reduction in SALT score represents clinically

meaningful response and to minimize responses in the "vehicle" arm, in which fewer than 10% are expected to achieve this magnitude of hair regrowth spontaneously. This outcome was also used in two recent RCT trials (Strober et al. 2009 and Price et al. 2008) evaluating therapies for AA.

As secondary endpoints, efficacy will be measured by changes in hair re-growth as a continuous variable as determined by physical exam and Canfield photography, as well as patient and physician global evaluation scores. To assess the durability of responses, patients who achieve 50% reduction in baseline SALT score will continue to be followed for an additional 6 months off treatment.

Secondary efficacy outcomes in detail:
1. Percent hair regrowth from baseline determined by SALT measurements following 4, 8, 12, 20 and 24 weeks of treatment and during the observational phase at weeks 28, 32, 36, 40, 44 and 48.
2. Comparison of the proportion of responders in each group, with response defined as 50% change in SALT score (50% regrowth) from baseline, at weeks 12, 24, 36 and 48.
3. Comparison of the proportion of subjects in each group attaining global overall improvement SALT score of A5 (100% coverage) supported by </= SALT 25 at weeks 12, 24, 36 and 48.
4. Change in PGA (Physician Global Assessment - (100mm Visual analogue scale)) based on evaluation of hair loss on clinical exam and evaluation of standardized photographs between baseline, week 12, 24, 36 and 48.
5. Change in patient global assessment (100mm Visual analogue scale) between baseline, Week 12, 24, 36 and 48.
6. Change in patient quality of life assessment from baseline to weeks 12, 24, 36, and 48 (Finlay and Khan 1994).
6. Frequency of occurrence and timing of relapse (as defined above) in responders followed for 6 months off therapy.

PGA and patient evaluations will be based on similar 100mm visual analog scales with 0 labeled as no scalp hair loss and 100 labeled as complete scalp hair loss (Yamada et al. 2002). Patient quality of life assessments will be based on changes in the Dermatology Life Quality Index DLQI (Olsen et al. 2004) – adapted for hair loss, or the Dermatology Quality of Life Scales (Finlay and Khan 1994).

<u>Photography</u>
Photography will be performed using a high-resolution digital camera with Intelliflash system from Canfield Scientific, Inc., mounted on a stereotactic arm. Patients will be positioned using a fixed guide, after which photographs of the entire scalp will be taken at standardized locations and at a fixed distance. These standardized positions will include at least four views of the scalp (top, both sides and back) and will be consistent with those used in the SALT score. The hair will be combed prior to photography to ensure maximum visualization of the scalp and facilitate comparisons of sequential photographs. Close-up photographs may also

be taken using an epiluminescent attachment in order to assess for the presence of early regrowth.

Biopsy

All punch biopsies will be performed by the study doctor according to the schedule of study assessments. 4mm punch biopsies will be performed using standard of care techniques. The site chosen will be the leading edge (active or involved edge of a patch with some hairs still present) of an alopeic patch or other appropriate areas of the alopeic patch or scalp as determined by the investigator. One additional scalp biopsy may be obtained from a non-lesional area. The area will be selected at the discretion of the investigator. This non-lesional biopsy may be obtained during any visit. All risks and possible adverse effects from the procedure will be discussed in detail with the subject prior to the procedure. The biopsy site will be anesthetized with an injection of 1% lidocaine with epinephrine. After approximately 1 minute, the physician applies pressure to the biopsy site using a 4 mm skin punch (a sterile cylindrical tube with a sharp edge). The punch is twisted until the blade of the skin punch has pierced the epidermis and dermis of the skin and enters the subcutaneous fat. Depending on the thickness of the skin in the area being biopsied, the cylindrical blade may be buried to the hub (approximately 6mm in depth). After the blade has sufficiently cored or carved out a cylinder of skin, the skin punch is removed. Nontraumatic forceps are used to gently grasp the cored skin, pulling upward to remove the core and reveal the subcutaneous fat. Scissors are used to cut the cored tissue free from the underlying subcutaneous fat. The specimen is placed immediately into the appropriate media. Once the specimen has been removed, pressure is applied to the biopsy site with a sterile gauze. The biopsy site is then closed with several simple interrupted sutures. Either an absorbable or nonabsorbable suture may be used at the investigators discretion. Antibiotic ointment is applied and the area is covered with a standard BandAid or sterile gauze and paper tape. When necessary, a small pressure dressing may be applied. Subjects will be instructed in wound care and will be advised to call the research unit if they have any concerning signs or symptoms during healing.

SALT – Severity of Alopecia Tool

A. The proportion of scalp involvement is determined by dividing the scalp into 4 quadrants and estimating the percentage of the scalp surface that all the alopecic areas would occupy if placed
together. The following groups will be used:

S: Scalp hair loss

_____S0 = No hair loss
_____S1 = less than or equal to 25% hair loss
_____S2 = 26%-50% hair loss
_____S3 = 51%-75% hair loss
_____ S4 = 76%-99% hair loss
_____ a = 76%-95% hair loss
_____ b = 96%-99% hair loss
_____ S5 = 100% hair loss

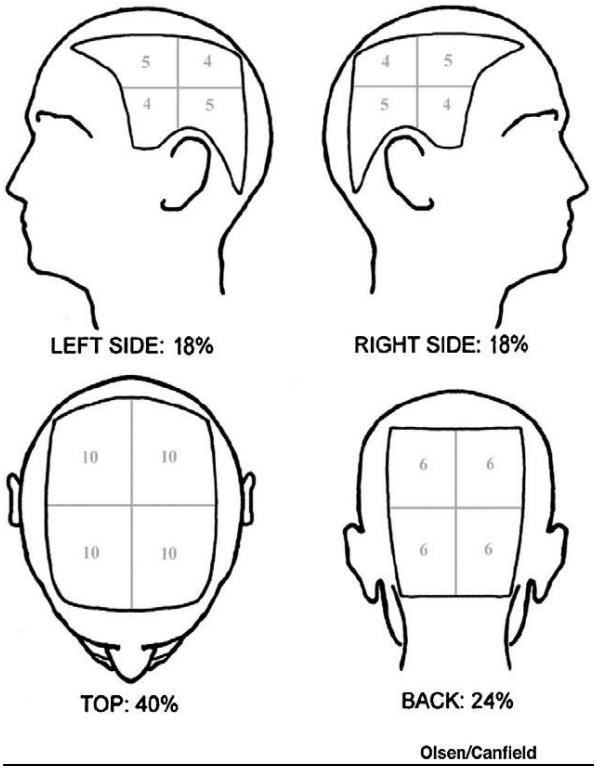


**FIG. 2. Olsen/Canfield tool for determination of %scalp hair loss. Percentages represent scalp surface area (reprinted with permission).**

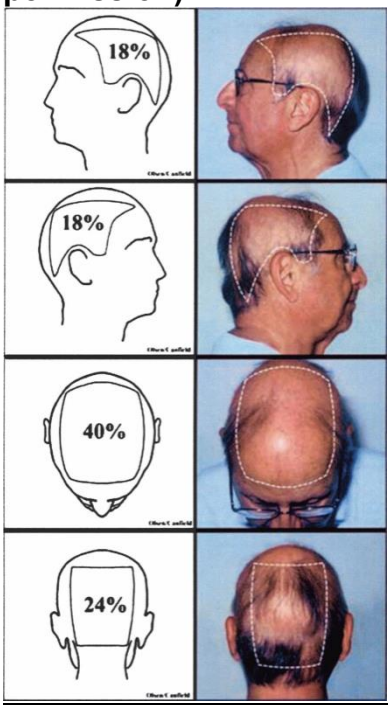


**Figure 9. SALT score.**

The percentage of hair loss in any one of the four views (areas) of the scalp = the percentage hair loss x percent surface area of the scalp in that area. The SALT score then equals the sum of the scalp hair loss in each area.
(a) Top (left side view) = 95% x .18 = 17.1
(b) Second (right side view) = 90% x .18 = 16.2
(c) Third (top of scalp) = 95% x .40 = 38 (realizing that most of hair loss is probably male pattern hair loss)
(d) Bottom (back of scalp) = 55% x .24 = 13.2
a+b+c+d = 17.1 + 38 + 16.2 + 13.2 = 84.5% hair loss or SALT 84.5

Body hair loss
B0 = No body hair loss
B1 = some body hair loss
B2 = total (100%) body hair loss

Nail involvement
N0 = No nail involvement
N1 = some nail dystrophy
N1a = 20 nail dystrophy

Percentage change from baseline (% regrowth) =
[(SALT BL – SALT F/U)/ SALT BL] x 100% = % change from baseline

Absolute regrowth = SALT BL – SALT F/U = absolute change from BL

Study outcomes
*Primary efficacy outcome*:
1. Comparison of the proportion of responders in each group, with response defined as 50% change (% change NOT absolute change) in SALT score from baseline (50% regrowth at week 24).

This is a relatively strict definition for defining responders and non-responders and was chosen based on consensus of clinicians and AA experts that 50% reduction in SALT score represents clinically meaningful response and to minimize responses in the “vehicle” arm, in which fewer than 10% are expected to achieve this magnitude of hair regrowth spontaneously. This outcome was also used in two recent RCT trials (Strober et al. 2009 and Price et al. 2008) evaluating therapies for AA.

As secondary endpoints, efficacy will be measured by changes in hair re-growth as a continuous variable as determined by physical exam and Canfield photography, as well as patient and physician global evaluation scores. To assess the durability of responses, patients who achieve 50% reduction in baseline SALT score will continue to be followed for an additional 6 months off treatment.

*Secondary efficacy outcomes will include*:

1. Percent hair regrowth from baseline determined by SALT measurements following 12, 24 weeks of treatment and at weeks 36 and 48 following discontinuation of treatment (Price et al Efalizumab study)
2. Comparison of the proportion of responders in each group, with response defined as 50% change in SALT score (50% regrowth) from baseline, at weeks 12, 36 and 48.
3. Comparison of the proportion of subjects in each group attaining global overall improvement SALT score of A5 (100% coverage) supported by </= SALT 25 at weeks 12, 24, 36 and 48.
4. Change in PGA (Physician global assessment) based on evaluation of live evaluations and standardized photographs between baseline, week 12, 24, 36 and 48
5. Change in patient global assessment (100mm Visual analogue scale) between baseline, Week 12, 24, 36 and 48
6. Change in patient quality of life assessment (DLQI or DQLS) from baseline to weeks 12, 24, 36 and 48. (Finlay and Khan 1994).
7. Frequency of occurrence and timing of relapse (as defined above) in responders followed for 6 months off therapy.

*Safety and tolerability measures*:
Incidence and severity of adverse effects (AEs) including the presence and degree of skin atrophy.
Incidence of treatment-emergent laboratory abnormalities.

Sample size
Power analysis is based on a chi-squared test of proportions of responders in the 4 study groups, where response is defined as 50% reduction in SALT score. Effect size is quantified in terms of Cohen's *w*, a function of the response rates in the fourgroups (15). A minimal treatment effect size of 40% (Cohen's *w* = 0.4) i.e. a 50% response rate for active drug (IL TAC 2.5mg/ml, 5mg/ml or 10mg/ml) versus a presumed spontaneous remission response rate of 10% for patients receiving inactive IL saline, would be considered clinically meaningful in this study. To achieve 80% power to detect the desired effect size of 40% with a usual 5% level of significance (alpha), we would require approximately 17 subjects per arm (68 subjects in total). With this sample size, an effect size of 50% (Cohen's *w* = 0.5, "large" effect) would ensure approximately 95% power. Our postulated effect size for IL TAC is based on impressions due to clinical use of IL TAC in AA, however an explicit aim of this pilot study is to objectively and more precisely quantify the actual treatment effect of the various doses of IL TAC versus saline placebo.

Data Analysis Plan
The primary analysis will be a comparison of response rates in the 4 study groups, using a chi-squared test of proportions. If the 4-group comparison is significant, we will compare the efficacy and safety profiles of the three doses of intralesional triamcinolone head to head. For secondary outcomes that are binary (response/non-response), we will follow the same strategy. For secondary outcomes that are continuous (e.g. change in SALT measurement or PGA), we will conduct 4-group ANOVA tests, followed by pairwise

comparisons, if necessary. In both cases, Bonferroni correction will be made to adjust for multiple testing. To assess safety, we will summarize and test the incidence rates of adverse events across groups.

If possible, we will attempt to stratify the patients based on baseline degree of hair loss e.g. 0-25% vs. 26-50% hair loss in order to assess if response is correlated with degree of hair loss present at baseline. (Olsen E. Investigative guidelines for alopecia areata. Dermatologic therapy 24, 2011 311-319

All outcomes will be analyzed on the intent to treat (ITT) basis.
A 3-month interim analysis of the primary and secondary outcomes will be performed by an independent group in order to determine if statistical significance has been attained. A Haybittle-Peto rule for alpha spending (Haybittle 1971; Peto et al. 1976) will be followed for the interim analysis. If statistical evidence of efficacy is attained at 3 months and if the safety profile is acceptable, all patients will be offered the option of receiving open label active drug for the remaining duration of the trial.

To compare the frequency of occurrence of relapse in responders (followed for 6 months off therapy) across the four groups, we will employ a chi-squared test for comparing multiple proportions.
Furthermore, to compare the timing of relapse in responders (followed for 6 months off therapy) across the four groups, we will use tools from survival analysis (or, time-to-event analysis, with relapse as the event of interest). More specifically, first, we will conduct a descriptive analysis by graphically comparing the Kaplan-Meier survival (time-to-relapse) curves for the four groups. Next, we will use the log-rank test to formally test the null hypothesis of no difference between the survival functions of the four groups.

**Specific Aim 2.** To identify a clinical correlation between treatment outcome and down modulation of key AA-associated immunohistopathological markers and biomarkers in the treated skin; including NKG2DL expression in the hair follicle, immune infiltration ($CD8^+NKG2D^+$ cells) and expression of interferon response genes.

**Rationale** Establishing the genetic basis of AA marks the beginning of rationally-designed mechanism-driven clinical research, but it is just the beginning. Very little is known about the immunopathogenesis of AA in humans. Rigorous clinical investigation of AA cannot be satisfied by mere clinical response evaluation. Indeed, our study will include longitudinal collection of AA biospecimens that will enable temporal assessment of biomarkers of patients (controls and treated) providing a unique resource to characterize the natural history of this disease. Given the budget constraints, our mechanistic studies in this Aim are focused on correlating treatment and disease status with; 1) histological improvement; 2) reduced IFN responses; 3) reduced HF NKG2DL expression; and 4) declines in circulating and peribulbar $CD8^+NKG2D^+$ infiltration. Previous studies in AA have reported elevated NKG2D expression in circulating CD8 T cells and NK cells[39], supporting the feasibility of our approach. Here we will be able to correlate the numbers of $CD8^+NKG2D^+$ cells in the blood with those infiltrating the target organ of patients with each other and as a function of disease status.

**Procedures**

*i. Flow Cytometry Studies.* The presence of $CD8^+NKG2D^+$ cells will be tracked in the blood and in the skin during treatment with IL TAC or vehicle. By assessing $CD8^+NKG2D^+$ involvement in longitudinally obtained skin biopsies we can correlate disease severity and clinical response (progression or regression) with immunopathological evidence of resolution of total cellular infiltration (by H&E) or loss of specific $CD8^+NKG2D^+$ cells in the skin (by IF) or in the blood (flow studies). $CD8^+NKG2D^+$ frequency in the peripheral circulation will be quantified using freshly isolated PBMCs obtained from 30 mls of blood obtained upon entry into the trial and at 4 wks, 12 wks and 24 wks during treatment. A portion of the PBMCs $2x10^6$ cells will be stained with anti-CD8, anti-CD25 and anti-NKG2D antibodies, stimulated with PMA/ionomycin in the presence of brefeldin and stained with anti-IFN-$\gamma$ Abs. The remaining PBMCs will be viably frozen for future ancillary studies. We will seek evidence of a comparable pathogenic T cell subset in the human that we observed in C3H mice, namely an activated $CD25^+$ IFN-$\gamma$ producing $CD8^+NKG2D^+$ T cell.

*ii. Histology and Immunofluorescence Analysis.* As shown in previous studies[4,39] in active AA NKG2D ligands are upregulated on hair follicles in association with peribulbar NKG2D-bearing $CD8^+$ T-cells and natural killer cells. We will seek evidence of resolution of total cellular infiltration (by H & E) and loss of specific $CD8^+NKG2D^+$ cells (by IF) and NKG2D ligands by comparing baseline biopsies with biopsies obtained on treatment after 4, 12, and 24 weeks. Frozen sections will be stained with anti-CD4, anti-CD8, anti-CD56 anti-NKG2D and anti-IFN-$\gamma$ antibodies to visualize the total number of leukocytes, cytotoxic T-cells and NK cells present in the drug treated and placebo treated skin, respectively. Immunostainng with anti-ULBP3, anti-MICA antibodies and recombinant NKG2D soluble receptor (NKG2D-Ig, detects all NKG2D ligands, R & D systems) will determine whether NKG2DL "danger/stress" signals are down modulated with Jak1/Jak2 inhibition. Punch biopsies from patient skin before and after treatment will be embedded in OCT and 7-8 micron frozen sections will be cut. The sections will be fixed in 4% paraformaldehyde and stained overnight with primary antibodies for NKG2D ligands, receptor and immune markers. The following day, sections will be treated for 1 hr with fluorescence labeled antibody, counter-stained and mounted with DAPI containing media. Frozen sections will also be used to for H&E staining. The total number of immune cells, as well as NKG2D bearing cells, will be quantified by counting stained cells per magnification field using the NIH imaging software ImageJ and a student T-test will be carried out to determine significant changes in the number of infiltrating, NKG2D bearing T cells to correlate the simultaneous presence or absence of these cells in lesional skin and in circulation (by flow) during active disease or in remission.

*iii. Analysis of Gene Expression by Quantitative RT-PCR*
We will analyze the transcriptional expression signature of IFN-response genes in the skin and PMBC of drug-treated AA patients using qPCR as an indirect measure of efficacy. Signature genes have been selected based on their differential expression in affected vs. unaffected C3H mice, and on published studies on human AA patients vs. controls[40]. The genes in the list represent mediators and effectors of the IFN response, which we found to be instrumental in disease pathology. Briefly, biopsies and peripheral blood from placebo control and treated patients will be collected at 0, 4, 12, and 24 week time points. Additional, optional scalp biopsies and peripheral blood may be taken at other time points in order to monitor response to therapy at the discretion of the investigator and with the consent of the study subject. RNA will be extracted from the tissues, and Sybr green based qPCR assays will be performed. Differential gene expression resulting from inhibitor treatment will be normalized to the expression of a housekeeping gene. We expect many of the genes on this list to revert to a basal level of expression, as compared in unaffected skin of an AA patient, or skin from a healthy control.

## ***FUTURE PLANS AND INTEGRATION***

Establishing the genetic basis of AA marks the beginning of rationally-designed mechanism-driven clinical research, but it is just the beginning. Very little is known about the immunopathogenesis of AA in humans. For example, can we identify clonal antigen specific T cells in the skin and blood of AA individuals? What antigens are the killer T cells recognizing in the hair follicle? Are there fundamental differences in the blood and skin between patients with mild or severe disease that would suggest different treatment approaches? These questions require analysis of patient materials (blood and skin) to isolate and investigate the immune cells responsible. **Thus, rigorous clinical investigation of AA cannot be satisfied by mere clinical response evaluation. Indeed our study will include, for the first time, longitudinal collection of AA biospecimens that will enable temporal assessment of biomarkers of patients (controls and treated) providing a unique resource to characterize potential biomarkers of the natural history of this disease.** In collaboration with colleagues at other institutions, we plan to utilize the clinical and immunological findings of this study to facilitate the conduct and analysis of future clinical trials of therapies in alopecia areata. The findings will guide us in the selection of appropriate immunomodulators and will allow us to objectively quantify the immunologic response to therapy. We have received feedback from the NIH indicating that they consider the development of biomarkers of disease progression to be a crucial step in facilitating future trials and further that the absence of attempts to identify biomarkers will adversely affect the scoring of future studies submitted for funding. In addition, this study utilizes agreed upon parameters proposed by experts in the field of AA for standardization of study design including patient selection, inclusion and exclusion criteria and evaluation of clinically meaningful outcomes and will help to validate and refine these parameters for future studies of therapies in AA.

Recruitment

Each year the Dermatology Department at CUMC sees 600 AA visits each year.Of these we expect roughly 20 to 30% to be eligible for this study of mild to moderate patch type alopecia. We also have a database of almost 400 patients evaluated at CUMC as part of the alopecia areata registry who have expressed an interest in being contacted for clinical trials in alopecia areata. In addition, we hope to have information regarding the study posted on the website for the National Alopecia Areata Foundation (NAAF) the largest AA patient advocacy organization**.** We expect to complete accrual of 68 patients within the 1 to 2 year study period (38 patients at CUMC and 30 at the University of Minnesota). This time frame will enable 6 months on treatment and 6 months follow-up off drug (for patients who qualify) and completion of the clinical correlative portion of the study

Assessment of Safety

Safety precautions such as the use of highly experienced investigators, frequent visits, and close monitoring of clinical and laboratory findings have been implemented in the protocol and are specified in the Schedule of Assessments. Hematology and chemistry measurements, including WBC count, platelet and absolute neutrophil counts, urinalysis and hepatic panel may be obtained at screening and at other time points if deemed necessary by the investigator.

All physical examination findings, vital sign abnormalities, and clinical laboratory abnormalities, will be captured as AEs when deemed medically significant by the investigator. A qualified physician associated with the study will be available to assess clinical signs and symptoms that may be indicative of an adverse event. Safety will be assessed by

summarizing the incidence and type of AEs. The proportion of patients who discontinued treatment will be summarized.

Methods and Timing of Safety Assessments

*Adverse Event Reporting*

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a study medication(s), whether or not related to the study medication(s). AEs that occur after randomization should be recorded on the AE page of the CRF. Recording should be done in a concise manner using standard, acceptable medical terms. The AE recorded should not be a procedure or a clinical measurement (i.e., a vital sign), but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement. Any clinically significant changes in pre-existing conditions (based on medical history/physical examination results) will be recorded on the CRF AE pages. The intensity of all AEs should be classified as follows:

Mild: A mild AE is defined as an event characterized by "awareness of symptoms which are easily tolerated."

Moderate: A moderate AE is defined as an event in which "sufficient discomfort is present to cause interference with usual activity."

Severe: A severe AE is defined as an event characterized by "extreme distress causing significant impairment of function or incapacitation."

A serious adverse event (SAE) is any AE occurring at any dose that results in:

- Death
- A life-threatening event
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect.

In addition, an important medical event that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the patient and/or may require medical or surgical intervention to prevent one of the outcomes listed above.

An unexpected AE is any AE of which the specificity or severity is not consistent with the current Investigator Brochure/Package Insert. A reasonably related AE is one that is possibly, probably, or definitely related to study medication (as determined by the study investigator).

Immediate Reporting by Investigator

In the event of serious adverse events that fulfill a reason for expedited reporting as outlined above, the investigator or designee should inform the Columbia University Medical Center Institutional Review Board (IRB) of any **serious, unlisted/unexpected, and associated events** within 24 hours of being aware of the event. This will be documented on a FDA 3500 or MedWATCH form or the manner preferred by the IRB. This form must be completed within 24 hours/1 business day or at the latest on the following working day. The initial report must

be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event received after the report) must be documented on a follow-up MedWATCH, or otherwise appropriate, form.

Non-Expedited Reporting
For the 2 other types of serious events not requiring expedited reporting (serious, listed and associated; and serious, unlisted and not associated), the investigator should notify the Columbia University Medical Center Institutional Review Board (IRB) within 7 business days. If at any time an adverse event noted in this category changes is upgraded, the investigator should notify the IRB accordingly as noted in section above.

Please note, this study has been deemed exempt from the need for an IND by the Columbia University Clinical Trials office.

Institutional Review Board
The investigator will notify the IRB of any serious, life-threatening, or unexpected adverse experiences, including any death, regardless of the relationship to the study medication.

Protocol Amendments and Deviation
The investigator will not deviate from the protocol without the prior written approval of IRB except in medical emergencies. In medical emergencies, prior approval for protocol deviations will not be required, but the IRB must be notified as soon as possible (within 24 hours). All other protocol deviations require prior written approval from the IRB. The IRB/Ethics Committees (EC) will be informed of all protocol changes and violations by the investigator in accordance with the procedures established by the IRB/EC. No deviations from the protocol of any type will be made without complying with all of the procedures established by the IRB/EC.

Quality Control and Assurance
Analyses and Reporting
Investigator responsibilities are set out in the ICH guideline for the Good Clinical Practice (GCP) and in the US Code of Federal Regulations.

Data Collection
All subject related information will be collected in the source document. Study related parameter will then be captured in standardized case report forms (CRFs). CRF will be used to collect patient data and assessments that will be used for evaluation of the patient's response to treatment. Other patient data collected will include incidence and severity of adverse events.
Collected data, photographs, blood and tissue specimens will be identified only by an assigned subject number. All study data and specimens will be kept in secure study areas. In addition, the code linking the subject number to individually identifiable private information will be kept under lock and key in a secure study site and will be accessible only to key study personnel.

A study specific database, meeting all regulatory and IRB requirements, will be created in order to capture securely all study data including demographics, outcome data, and adverse events.

Data and Safety Monitoring Plan

The performance of all aspects of the study, including the methods used to obtain informed consent, will be conducted in accordance with all applicable state and federal guidelines as well as the principles for protection of research subjects as outlined in the declaration of Helsinki, ICH Guidelines for Good clinical practice, applicable local health authority regulations and US Title 21 of the Code of Federal Regulations.

Before implementing this study, the protocol, informed consent document and other information for subjects will be reviewed by the Columbia University IRB. During the conduct of the study the principal investigator and her designee will continually monitor the study to ensure that the study is conducted in compliance with the IRB approved study protocol. Safety data will be reviewed by the PI or designee as soon as it is received. All adverse events will be reported to the IRB in accordance with the above requirements. Serious adverse events requiring expedited reporting will be reported to the IRB their preferred methods of communication within 24 hours of study personnel becoming aware of the event. Serious adverse events not requiring expedited reporting will be communicated within 7 business days. The investigator will also notify the IRB of any serious deviations from the protocol, and any new information indicating added risks to subjects.

The clinical research coordinator in the Dermatology Clinical research unit will review 100% of all source documentation of data collected including all safety data. Case report forms will be reviewed to ensure agreement with source documentation and to ensure accurate and complete capture of study data. Database data capture will be double verified and response parameters will be included to minimize the risk of erroneous entries. Data will be verified and cleaned prior to data lock. The regulatory binder will also be reviewed to ensure that it is complete and up to date. The first review will occur after the first 2 patients are enrolled and will continue every eight weeks during the enrollment and treatment period. Any discrepancies will be discussed with the PI and relevant study personnel for clarification and/ or correction. A record will be maintained of all monitoring activities, findings, conclusions and action taken to correct deficiencies.

Annual reports and any interim reports of necessary study modifications will be submitted as required to the IRB and funding agency.

An unblinded member of the study team who is not involved in patient assessment will examine the efficacy and safety data available when all subjects have completed 3 months of study drug. Efficacy and safety parameters will be put in place to determine if efficacy has been established at this time point. If efficacy has been established and the safety profile is acceptable, study participants will be offered the option to continue the study in an open label format for the remaining duration of the study.

Regulatory Considerations

*Institutional Review Board/Independent Ethics Committee Review and Approval*

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki. The review of this protocol by the IRB/IEC and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated in the declaration, as well as ICH Guidelines, applicable local health authority regulations, and US Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards. Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted IRB/IEC.

The investigator(s) will be responsible for preparing documents for submission to the relevant IRB/IEC and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study. The investigator is responsible for notifying the IRB/IEC of any serious deviations from the protocol, or anything else that may involve added risk to subjects.

Informed Consent

The investigator must obtain informed consent of a subject or his/her designee prior to any study related procedures according to GCP as set forth in US 21 CFR Parts 50 and 56, the EU Clinical Trial Directive, local health authorities, and ICH guidelines. Documentation that informed consent occurred prior to the subject's entry into the study and of the informed consent process should be recorded in the subject's source documents. The original consent form signed and dated by the subject and by the person consenting the subject prior to the subject's entry into the study must be maintained in the Investigator's study files and a copy given to the subject. In addition, if a protocol is amended and it impacts on the content of the informed consent, the informed consent must be revised. Subjects participating in the study when the amended protocol is implemented must be re-consented with the revised version of the informed consent. The revised consent form signed and dated by the subject and by the person consenting the subject must be maintained in the Investigator's study files and a copy given to the subject.

| STUDY SCHEDULE OF ASSESSMENTS – IL TAC IN ALOPECIA AREATA | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Visit | 1<br>Screening | 2<br>Baseline | 3<br>Week 4 | 4<br>Week 8 | 5<br>Week 12 | 6<br>Week 16 | 7<br>Week 20 | 8<br>Week 24<br>ET/ES for nonresponders not qualified for open label IL TAC injections | 9<br>Week 28 | 10<br>Week 32 | 11<br>Week 36 | 12<br>Week 40 | 13<br>Week 44 | 14<br>Week 48 |
| Day | -28 to -1 | 0 | 28±7 | 56±7 | 84±7 | 112±7 | 140±7 | 168±7 | 196±7 | 224±7 | 252±7 | 280±7 | 308±7 | 336±7 |
| Informed Consent | X | | | | | | | | | | | | | |
| Inclusion/Exclusion Criteria | X | X | | | | | | | | | | | | |
| Medical History | X | | | | | | | | | | | | | |
| Body Weight | X | X | | | X | | | X | | | X | | | X |
| Physical/Cutaneous Exam | X | | | | | | | X | | | | | | X |
| Vital Signs | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Concomitant Medications | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Adverse events | | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Photography | | X | X | X | X | X | X | X | | X | | X | | X |
| SALT evaluation | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Physician global assessment | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Patient quality of life assessment | X | X | | | X | | | X | | | X | | | X |
| Patient global assessment | X | X | X | X | X | X | X | X | X | X | X | X | X | X |

| Visit | 1<br>Screening | 2<br>Baseline | 3<br>Week 4 | 4<br>Week 8 | 5<br>Week 12 | 6<br>Week 16 | 7<br>Week 20 | 8<br>Week 24<br>ET/ES for nonresponders not qualified for open label IL TAC injections | 9<br>Week 28 | 10<br>Week 32 | 11<br>Week 36 | 12<br>Week 40 | 13<br>Week 44 | 14<br>Week 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | -28 to -1 | 0 | 28±7 | 56±7 | 84±7 | 112±7 | 140±7 | 168±7 | 196±7 | 224±7 | 252±7 | 280±7 | 308±7 | 336±7 |
| Clinical laboratory evaluation[a] | X | | X | | X | | | X | X* | | | | | |
| Hepatitis screening panel | X | | | | | | | | | | | | | |
| Lipid Profile | X | | | | | | | | | | | | | |
| Urine pregnancy test[b] | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Scalp Biopsy[c] | | X | X[c] | X[c] | X[c] | X[c] | X[c] | X[c] | | | | | | |
| Nonlesional biopsy[c] | | X** | | | | | | | | | | | | |
| Phlebotomy for immunological studies | | X | X[c] | X[c] | X[c] | X[c] | X[c] | X[c] | | | | | | |
| Injection of study medication | | X | X | X | X | X | X | X (for nonresponders qualified for open label treatment) | | | | | | |
| Injection of open label IL TAC for qualifying nonresponders | | | | | | | | X | X | X | X | X | X | X |

[a] Clinical laboratory evaluation may consist of CBC, BMP, hepatic panel, and/or urinalysis or other as deemed appropriate by the investigator. Cortisol levels may be checked at baseline and at weeks 24, 28 and 48.
* Week 28 evaluation will only be performed if needed to verify normalization of previously abnormal lab values and to check cortisol levels.
[b] Urine pregnancy test will be continued after week 24 only for patients receiving open label IL TAC.

[c] Additional, optional scalp biopsies and blood draws may be taken at other time points in order to monitor response to therapy at the discretion of the investigator and with the consent of the study subject.

ET = end of treatment. ES = end of study
**Non-lesional biopsy may be obtained at the baseline visit or at subsequent visits. A subject's choice to decline to participate in this biopsy will not affect enrollment.

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Addendum 1

**DERMATOLOGY LIFE QUALITY INDEX (Modified for hair loss)**

**DLQI**

Hospital No: Date:
Name: Score:
Address: Diagnosis:

**The aim of this questionnaire is to measure how much your hair loss has affected your life OVER THE LAST WEEK. Please tick ☑ one box for each question.**

| | | | | |
|---|---|---|---|---|
| **1.** | Over the last week, how **itchy**, **sore**, **painful** or **stinging** has your scalp been? | Very much<br>A lot<br>A little<br>Not at all | ❒<br>❒<br>❒<br>❒ | |
| **2.** | Over the last week, how **embarrassed** or **self conscious** have you been because of your hair loss? | Very much<br>A lot<br>A little<br>Not at all | ❒<br>❒<br>❒<br>❒ | |
| **3.** | Over the last week, how much has your hair loss interfered with you going **shopping** or looking after your **home** or **garden**? | Very much<br>A lot<br>A little<br>Not at all | ❒<br>❒<br>❒<br>❒ | <br><br><br>Not relevant ❒ |
| **4.** | Over the last week, how much has your hair loss influenced the **clothes** you wear? | Very much<br>A lot<br>A little<br>Not at all | ❒<br>❒<br>❒<br>❒ | <br><br><br>Not relevant ❒ |
| **5.** | Over the last week, how much has your hair loss affected any **social** or **leisure** activities? | Very much<br>A lot<br>A little<br>Not at all | ❒<br>❒<br>❒<br>❒ | <br><br><br>Not relevant ❒ |
| **6.** | Over the last week, how much has your hair loss made it difficult for | Very much<br>A lot | ❒<br>❒ | |

| | | | | |
|---|---|---|---|---|
| | you to do any **sport**? | A little | ❒ | |
| | | Not at all | ❒ | Not relevant ❒ |
| **7.** | Over the last week, has your hair loss prevented you from **working** or **studying**? | Yes | ❒ | |
| | | No | ❒ | Not relevant ❒ |
| | If "No", over the last week how much has your hair loss been a problem at **work** or **studying**? | A lot | ❒ | |
| | | A little | ❒ | |
| | | Not at all | ❒ | |
| **8.** | Over the last week, how much has your hair loss created problems with your **partner** or any of your **close friends** or **relatives**? | Very much | ❒ | |
| | | A lot | ❒ | |
| | | A little | ❒ | |
| | | Not at all | ❒ | Not relevant ❒ |
| **9.** | Over the last week, how much has your hair loss caused any **sexual difficulties**? | Very much | ❒ | |
| | | A lot | ❒ | |
| | | A little | ❒ | |
| | | Not at all | ❒ | Not relevant ❒ |
| **10.** | Over the last week, how much of a problem has the **treatment** for your hair loss been, for example by making your home messy, or by taking up time? | Very much | ❒ | |
| | | A lot | ❒ | |
| | | A little | ❒ | |
| | | Not at all | ❒ | Not relevant ❒ |

**Please check you have answered EVERY question. Thank you.**

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Skindex29


# DERMATOLOGY SURVEY

This survey concerns the scalp/hair loss condition which has bothered you the most during the past four weeks.

All answers are confidential. Your name will NOT appear on your survey, be put in your chart or be seen by your doctor. This research is part of a study being conducted by the *San Francisco Veterans Affairs Medical Center.* Thank you for your participation.

These questions concern your feelings over the past 4 weeks about **the scalp/hair loss condition that has bothered you the most**. Check the answer that comes closest to the way you have been feeling.

| HOW OFTEN DURING THE PAST FOUR WEEKS DO THESE STATEMENTS DESCRIBE YOU? | NEVER | RARELY | SOMETIMES | OFTEN | ALL THE TIME |
|---|---|---|---|---|---|
| 1. My scalp/areas of hair loss hurt(s) | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 2. My hair loss affects how well I sleep | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 3. I worry that my hair loss may be serious | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 4. My hair loss makes it hard to work or do hobbies | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 5. My hair loss affects my social life | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 6. My hair loss makes me feel depressed | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 7. My scalp/areas of hair loss burns or stings | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 8. I tend to stay at home because of my hair loss | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 9. I worry about getting scars from my skin condition | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 10. My scalp/areas of hair loss itches | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 11. My hair loss affects how close I can be with those I love | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

| | | | | | |
|---|---|---|---|---|---|
| 12. I am ashamed of my hair loss . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 13. I worry that my hair loss may get worse . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 14. I tend to do things by myself because of my hair loss . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 15. I am angry about my hair loss . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 16. Water bothers my hair loss (bathing, washing hands) . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 17. My hair loss makes showing affection difficult . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 18. I worry about side-effects from scalp/hair loss medications / treatments | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 19. My scalp/areas of hair loss is (are) irritated . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 20. My hair loss affects my interactions with others . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

Please turn to next page

These questions concern your feelings over the past 4 weeks about **the scalp/hair loss condition that has bothered you the most**. Check the answer that comes closest to the way you have been feeling.

| HOW OFTEN DURING THE PAST FOUR WEEKS DO THESE STATEMENTS DESCRIBE YOU? | NEVER | RARELY | SOMETIMES | OFTEN | ALL THE TIME |
|---|---|---|---|---|---|
| 21. I am embarrassed by my hair loss . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

| | | | | | |
|---|---|---|---|---|---|
| 22. My hair loss is a problem for the people I love . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 23. I am frustrated by my hair loss . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 24. My scalp/areas of hair loss is (are) sensitive . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 25. My hair loss affects my desire to be with people . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 26. I am humiliated by my hair loss . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 27. My scalp/areas of hair loss bleed(s) . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 28. I am annoyed by my hair loss . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 29. My hair loss interferes with my sex life . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |
| 30. My hair loss makes me tired . . . . . | ☐1 | ☐2 | ☐3 | ☐4 | ☐5 |

SKINDEX 29 SCORING (note that item #18 is not used in scoring)

| SCALE | ITEMS |
|---|---|
| | |
| Emotion | 3-6-9-12-13-15-21-23-26-28 |
| Symptoms | 1-7-10-16-19-24-27 |
| Functioning | 2-4-5-8-11-14-17-20-22-25-29-30 |

Addendum 3 – DERMATOLOGY QUALITY OF LIFE SCALES

**DERMATOLOGY QUALITY OF LIFE QUESTIONNAIRE – modified for hair loss**

Dermatology Quality of Life Scales (DQOLS comprise 29 patient-generated items, consisting of a 17 item psycho-social scale grouped into 4 sub-scales (embarrassment, despair, irritableness, distress) and 12 item physical activities scale grouped into 4 sub-scales (everyday activities, summer, social and sexual). The psycho-social scale asks patients to indicate, 'the extent to which you generally feel this way' using a 5-point Likert scale (very slightly to extremely). The activities scale similarly requires patients to rate 'how much your skin problem/hair loss generally affects or restricts you in these things.' The DQOLS have a high level of internal consistency (Cronbach's alpha coefficient of 0.92 for the 17 psycho-social items and 0.83 for the 12 activity items), and satisfy requirements for reliability and construct validity.

## Psychosocial scale

This consists of a number of words that describe different feelings and emotions. Please read each item then mark the appropriate answer in the box to indicate to what extent you generally feel this way about your hair loss.

| | very slightly or not at all | a little | moderately | quite a bit | extremely |
|---|---|---|---|---|---|
| feel embarrassed | | | | | |
| feel ashamed | | | | | |
| feel depressed | | | | | |
| worry about appearance | | | | | |
| feel distressed | | | | | |
| feel suicidal | | | | | |
| feel self conscious | | | | | |
| worry about what others think | | | | | |
| feel short tempered | | | | | |
| feel lack of hope | | | | | |
| | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| feel isolated | | | | | |
| feel lack of understanding from others | | | | | |
| feel dependent | | | | | |
| worry about reactions of others | | | | | |
| feel anxious | | | | | |
| feel frustrated | | | | | |
| worry about long-term effects | | | | | |

## Physical activities scale

Please indicate how much your hair loss <u>generally affects or restricts you in these things</u>

| | very slightly or not at all | a little | moderately | quite a bit | extremely | not relevant |
|---|---|---|---|---|---|---|
| going out | | | | | | |
| walking | | | | | | |
| | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| making friends/meeting people | | | | | | |
| choice of clothes | | | | | | |
| summer activities/swimming | | | | | | |
| going out in the sun | | | | | | |
| household tasks | | | | | | |
| sleep | | | | | | |
| work | | | | | | |
| sexual activities | | | | | | |
| bathing | | | | | | |
| sporting activities/exercise | | | | | | |

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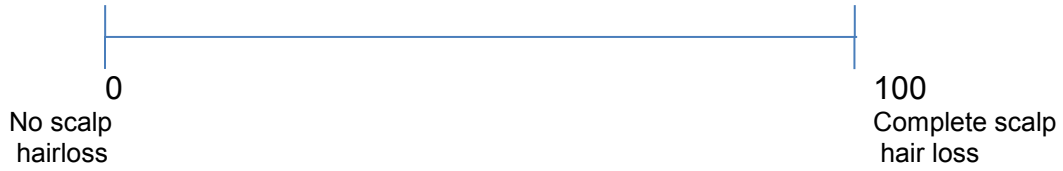
Addendum 4 – VISUAL ANALOGUE SCALE

100mm VISUAL ANALOGUE SCALE




The patient or physician will mark a vertical line somewhere on the horizontal line between 0 to 100 in the location which they feel best reflects the degree of hair loss present at the time of evaluation.

A numerical score (between 0 and 100) will be assigned to the location of the vertical mark based on measurement with a standard 100mm ruler. This score will be recorded in the source document and case report form/database as the Physician's or patient's global assessment of hair loss for that time point/visit.

Instructions for processing of blood and tissue samples for immunologic studies.

Blood samples from study participants are transported at room temperature to the Christiano Lab for processing within 24 hrs of blood draw. Serum will be aliquoted in 0.5 ml aliquots and stored at -80°C for cytokine/chemokine, autoantibody and other biomarker analysis. PBMC are isolated by density centrifugation and frozen in 10% DMSO in aliquots of 5 x $10^6$ cells and stored in liquid $N_2$. These storage conditions keep the cells viable and suitable for all planned functional and phenotypic analyses. Samples (skin biopsies and blood cells) for total RNA, miRNA extraction and histology will be collected in PAXgene tissue system (PreAnalytiX) for tissue stabilization and transported at room temperature to the lab. To minimize variation between samples coming from different sites, all tissue samples for RNA / miRNA extraction will be left in PAXgene at room temperature for the same amount of time (48 hrs) before further processing.

Biopsies for T cell isolation are collected in tissue culture media and transported to the lab at room temperature. The biopsies are processed directly using a protocol developed by Clark and Kupper, who are consultants to the AACORT (J. Invest. Dermatol. 2006) for the efficient isolation of T cells from skin biopsies using 3 dimensional matrices. Isolated T cells will be aliquoted in 1-5 x $10^5$ cells per vial, frozen in 10% DMSO and stored in liquid N2 until phenotypic and functional assays.