Defining reversal of alopecia areata (AA) phenotype with dupilumab in patients with and without associated atopic dermatitis (AD)

Principal Investigator: Emma Guttman-Yassky, MD/PhD

Co-Investigators: James G. Krueger, MD/PhD
Mark Lebwohl, MD

Study Sites: Icahn School of Medicine at Mount Sinai
Departments of Dermatology and the Immunology Institute
5 East 98 Street, 5th Floor, Box 1048
New York, NY 10029

The Laboratory for Investigative Dermatology
The Rockefeller University
1230 York Avenue, Box 178
New York, NY 10065

Expert Consultant: Dr. David Norris, MD
Professor and Chair, Department of Dermatology
University of Colorado School of Medicine
1665 Aurora Court
Anschutz Cancer Pavilion Building, 3rd Floor
Aurora, CO 80045

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Table of Contents:

BACKGROUND:

Alopecia areata (AA) is a common, complex immune-mediated disease characterized by non-scarring hair loss [1]. The clinical presentation of hair loss is variable. The most common presentation is AA with one or more bald circumscribed patches on the scalp. Hair loss can sometimes progress to total loss of all scalp hair (alopecia totalis, AT) and all scalp and body hair (alopecia universalis, AU). A few patients experience a diffuse type of AA or preferential loss of pigmented hair. Nail changes are also seen in 20% of AA patients. Typically, the affected skin has no sign of inflammation, although pruritus has been occasionally reported in AA patients. Short fragile hairs (so-called exclamation point) are often seen in the periphery of lesions [1-3]. The histologic hallmark of AA is perifollicular inflammation and a peribulbar infiltrate of predominantly lymphocytes around anagen hair follicles, known as a “swarm of bees.” This feature is typically seen in patients with active disease, and may not be present in chronic cases [1].

The lifetime risk of AA in the United States has been estimated to be approximately 2%, and the severe forms of AA (AT and AU) occur in 14 - 25% of all AA cases [5]. The peak incidence occurs in young adults and 66% of patients debut before 30 years of age [1]. In general, there is no gender predilection, although a few studies have indicated a slightly higher prevalence in young males [4, 6]. Patients with disease onset before age 30 have positive family history in 37 % of cases [7], and progeny of AA patients have a 3-fold higher lifetime risk of developing AA [8, 9]. In addition, personal or family history of atopy predispose to AA [10]. Rapid disease progression and debut at young age are poor prognostic factors [1, 11]. Other commonly associated diseases include thyroid disease and autoimmune diseases such as thyroiditis and vitiligo [2]. While spontaneous regrowth is common in patients with minimal scalp involvement with AA [3], there is a minimal rate of spontaneous regrowth in patients with ≥50% hair loss, with only 8% of these patients re-growing at least 25% of the hair on placebo treatment. Relapses are common in AA patients, and only one-third of all cases achieve long-lasting remission that lasts 10 to 15 years [13, 14]. These data suggest a high unmet need for effective treatments for patients with extensive hair loss [15].

AA can cause tremendous emotional and psychosocial distress in affected patients and their families [16]. There is a lack of effective treatments for patients with AA, with no universally proven therapy that induces and sustains remission and there is no cure [3]. Topical treatments are minimally effective, probably because of limited penetration. Nor is there robust evidence for efficacy of topical tacrolimus, cryotherapy or ultraviolet light A combined with oral psoralens (PUVA). Intralesional injections of corticosteroids are effective but can only be considered for patients with limited involvement. For more extensive alopecia forms, including AT or AU, for which spontaneous regrowth is rare, systemic immunosuppressants (systemic corticosteroids, cyclosporine A, mycophenolate mofetil, azathioprine, and Janus kinase inhibitors) have shown some efficacy but are associated with side effects that preclude long-term use [5, 17, 18]. Furthermore, hair loss recurs usually after treatment cessation [19]. Despite the multiple side effects associated with these treatments, these are still widely used due to the absence of other treatment options [3, 6].

TREATMENT RATIONALE:

The current systemic treatments for severe AA are broad acting, and therefore bear many adverse effects. Moreover, none of these therapies show effective, long lasting results [20]. Many patients, especially those with extensive involvement such as AT and AU, are willing to risk significant adverse drug effects and attempt off-label use of various treatments, including JAK kinase inhibitors which carry a black box warning due to potential serious infections and increased malignancies [18, 21]. There is an unmet need for targeted therapeutic strategies for patients with severe AA, similarly to the new effective treatment approaches that are now used or tested for other immune mediated diseases such as psoriasis and atopic dermatitis (AD) [22-25]. New biological treatments approved or in development for inflammatory skin conditions, such as AD, rely on targeting specific activated cytokine pathways. This revolutionary approach leads to a change in treatment paradigms for these diseases, with development of highly efficacious and safer therapeutics.

The immunopathogenesis of AA is poorly understood. While previous studies have primarily focused on the role of Th1 in AA [26], there is increasing evidence for the role of the Th2 axis in this disease. First, AD is the strongest risk factor for AA, and the two diseases commonly co-occur in patients [27]. In a study of more than 3500 AA patients, atopy (e.g. allergic rhinitis, asthma, and/or eczema) was the most common comorbidity, affecting more than 38.2% of AA patients [28]. Second, several studies have reported high levels of Th2 cytokines (e.g. IL-4, IL-5, IL-10) in the skin [27] and the serum of AA patients [29], as well as eosinophilia and elevated IgE levels in the blood [30-32]. Finally, genetic studies in AA have identified polymorphisms in filaggrin, IL-4, and IL-13 [28, 33]. Filaggrin mutations are associated with a more severe AA phenotype [33], and IL-13 is a known susceptibility locus for other autoimmune conditions, including atopy [34]. These findings provide strong biologic and genetic evidence for the observed relationship between AA and AD, and the possible pathogenic role of the Th2 axis in AA.

Our recent data from a cohort of 27 AA patients ranging from mild to severe (total body hair loss) suggest that similar to psoriasis and AD, AA is a highly inflammatory disease with robust activation of Th1, Th2, and IL-23 (both p19 and p40 subunits) cytokine pathways (**Figure 1**) [35]. Using RT-PCR, we showed that the IL-13 cytokine showed the highest increases in gene expression in lesional AA scalp compared to non-lesional AA scalp. Significant down-regulation of hair-associated keratins was also observed and is a hallmark of AA (**Figure 2**). Finally, we found that lesional scalp of patients with extensive AA involvement is associated with greater immune and keratin dysregulation compared with that of patients with limited disease. Importantly, patients with and without concurrent AD were shown to have a similar AA scalp phenotype, and similar activation of the Th2 pathway [35].

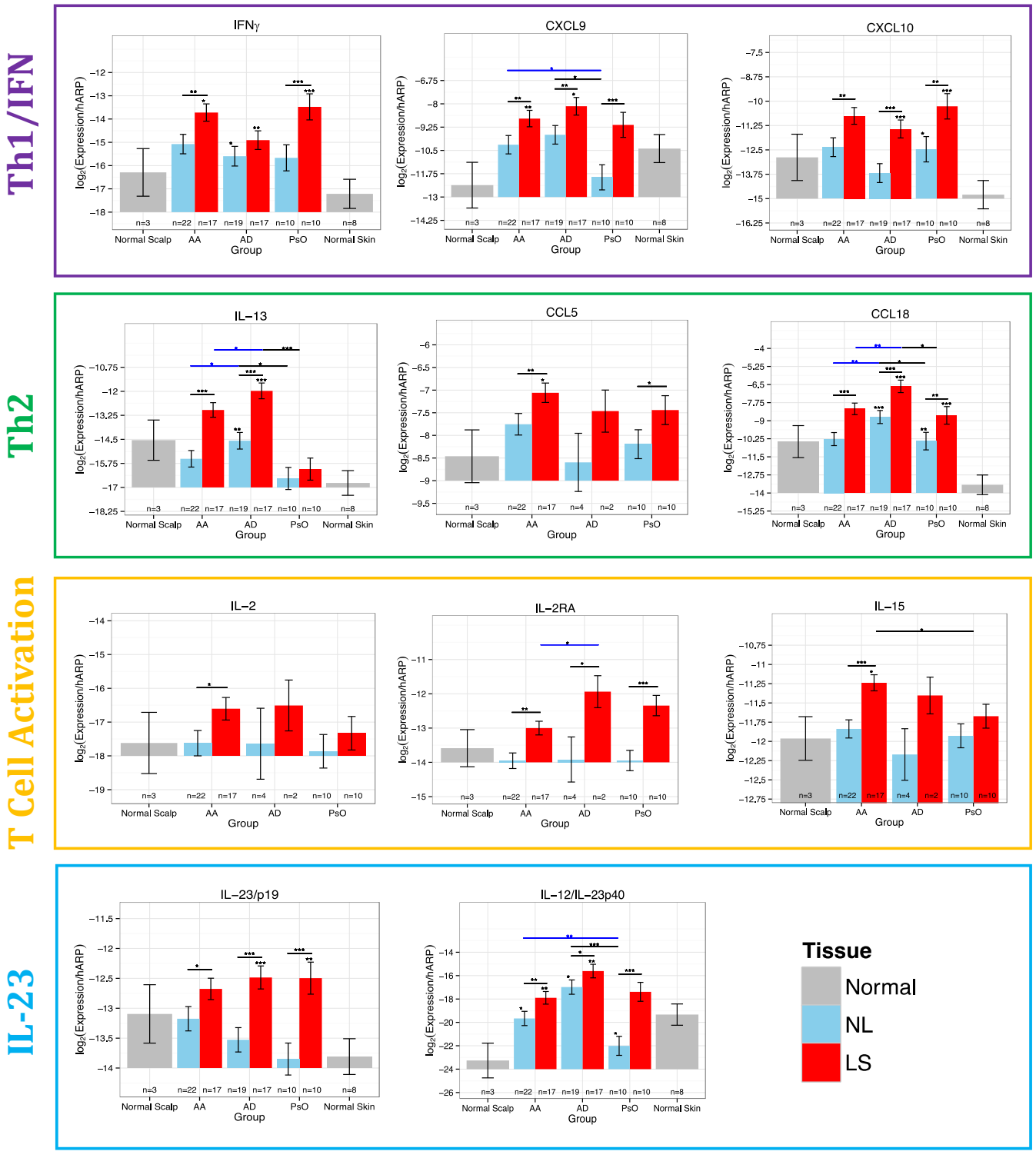
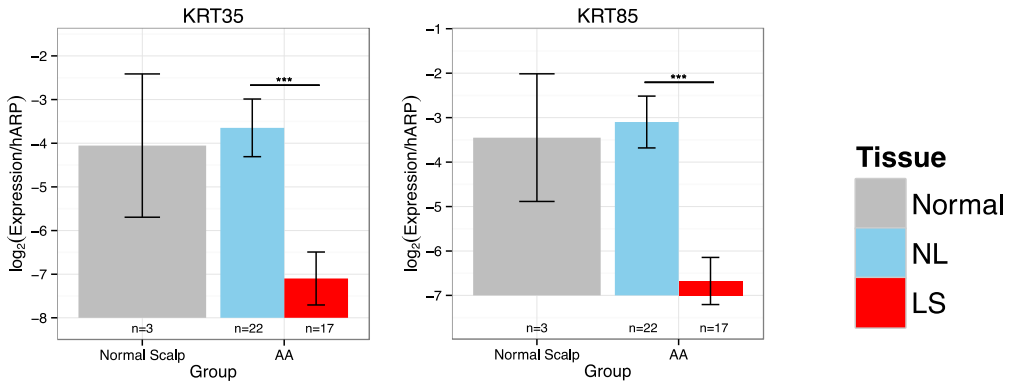


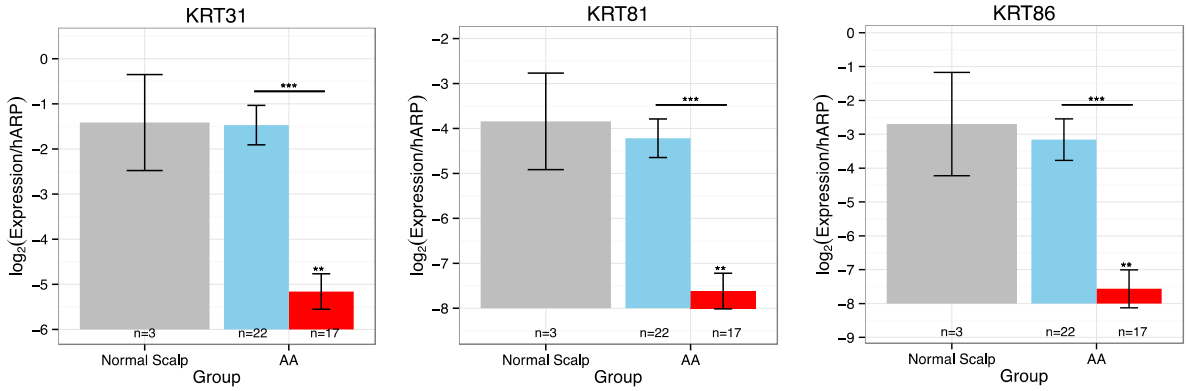
Figure 1. Alopecia areata (AA) scalp lesions are characterized by up-regulation of Th1 (IFNγ, CXCL9, CXCL10), Th2 (IL-13, CCL5, CCL18), and T cell activation (IL-2, IL-2RA, IL-15) related products as well as IL-23 (p19 and p40) cytokine. Real-time (RT)-PCR comparisons of inflammatory markers in lesional (LS), non-lesional (NL), and healthy scalp and skin samples from patients with alopecia areata (AA), atopic dermatitis (AD), or psoriasis (Pso) and normal skin from

healthy subjects. Expressions are normalized to hARP (Human acidic ribosomal protein). Mean (log_2 expression/hARP) ± SEM. *P < .05, **P < .01, and ***P < .001.

Early Keratins:



Middle Keratins:



Late Keratins:

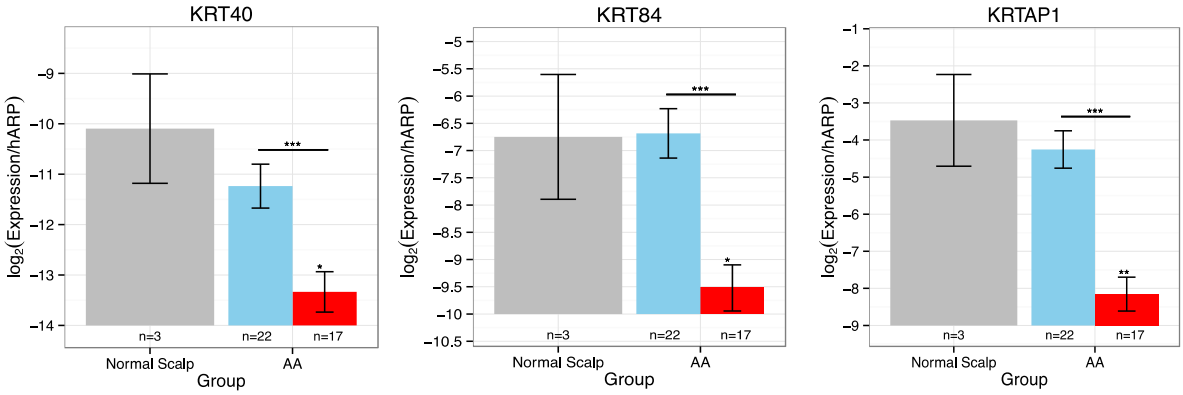


Figure 2. Early, mid, and late hair keratins in normal scalp as compared to lesional and non-lesional scalp of alopecia areata (AA) patients. The mRNA expressions of hair keratins in LS and NL AA scalp were compared to normal scalp by RT-PCR. hARP/human acidic ribosomal protein. Mean (log_2 expression/hARP) ± SEM. *p-value <0.05; **p<0.01; ***p<0.001. LS/Lesional, NL/non-lesional.

We also recently treated 3 patients with moderate-to-severe AA with ustekinumab, an anti-IL12/IL-23p40 inhibitor, and followed clinical responses with mechanistic studies in pre- and post-treatment scalp biopsies after 16 weeks of therapy [36]. Interestingly, although ustekinumab blocks the IL-12/IL-23 cytokines, the highest and most significant reductions coupled with successful hair regrowth were seen in the Th2 cytokines, including CCL13, CCL18, and CCL26, that are also highly up-regulated in AD and go down with dupilumab according to prior studies [37]. Interestingly, the suppression of the Th2 axis with ustekinumab was more consistent and significant than that of interferon/Th1 genes. We also observed suppression of T-cell and dendritic cell genes. This series is the first report to link targeted immune antagonism to clinical and molecular AA reversal, including suppression of immune markers and up-regulation of hair keratin genes.

In another recent report, in which we identified biomarkers of hair regrowth in AA patients using intra-lesional steroid injections, we also observed significant modulation of IL-13 cytokine in post treatment lesions, as well as up-regulation of hair keratins [38]. Further, Wang *et al.* (J Invest Dermatol 2016) isolated PBMCs from AA patients with no prior history of atopy and showed increased expression of IL-13 and chemokine ligand 5 (CCL5 or RANTES) following stimulation with known AA autoantigens, thus providing further evidence for the role of Th2 T-cell populations in the pathogenesis of AA.

Taken together, these studies suggest that perhaps AA may provide a similar model to AD in which cytokines inhibit hair keratinocytes (instead of inhibiting terminal differentiation as in AD), and provide a strong rationale for studying the efficacy of Th2 targeted therapies such as dupilumab for AA patients. We are aware of 3 patients treated with dupilumab for AD who also experienced improvement in their concurrent AA: 2 from our AD trial at Mount Sinai who experienced significant improvement in hair regrowth during dupilumab treatment—an African American AD patient that also had patchy AA (**Figure 3A-D**), as well as another European American male patient treated at MSSM with dupilumab that accompanied Dr Guttman to Sanofi and talked about his AA that resolved with dupilumab (he also included it in his PPT, but pre-treatment pictures are unavailable to us). The third patient was treated at another site, and shared her experience and pictures with Dr Guttman during a Sanofi-organized meeting in France, giving permission to share them in this protocol.

Therefore, we hypothesize that dupilumab will be able to clinically and mechanistically reverse AA and induce hair regrowth in AA patients, regardless of concurrent AD.

A large trial with dupilumab in AA patients might not only change the treatment paradigm for AA patients and provide a safe systemic treatment option, but will also expand the mechanistic understanding of the AA phenotype in patients with and without AD, ultimately leading to rapid therapeutic developments, similar to those now occurring in AD.

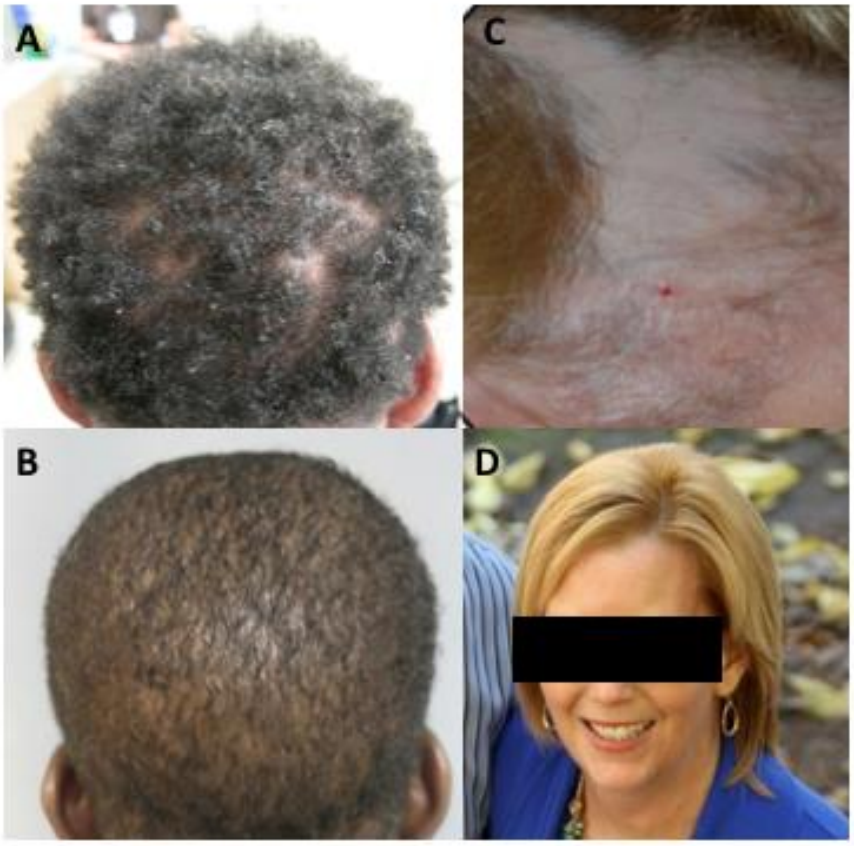


Figure 3. Two of 3 patients we are aware of, with concomitant AA and AD who experienced full hair regrowth of their AA during dupilumab therapy for their moderate-to-severe AD. A) Photo of a patient from the dupilumab study at Mount Sinai Hospital prior to dupilumab treatment, showing patchy AA areas and **B**) after 16 weeks of dupilumab therapy (patient is in the long-term extension study). Of note, patient shaved her hair due to the AA lesions, and new hair growth clearly shows no AA patches as seen in the upper picture. **C**) Photo of a second patient treated with dupilumab at another site who sent us photos of her hair before (showing a large AA patch in frontal scalp) and **D**) after dupilumab therapy with full hair regrowth. The latter patient also shared the story of her AD and AA reversal during the Sanofi meeting in France that EG attended.

HYPOTHESIS:

Primary Hypothesis

- Dupilumab is superior to placebo for induction of hair regrowth in patients with moderate to severe alopecia areata.

OBJECTIVES:

Primary Objective

- To study the efficacy of dupilumab for induction of hair regrowth in patients with moderate to severe alopecia areata.

Secondary Objectives

- Investigate the mechanism of action for dupilumab in patients with moderate to severe alopecia areata.

- To study the safety of dupilumab in patients with moderate to severe alopecia areata.

ENDPOINTS:

Primary Endpoint

- Determine change from baseline in the Severity of Alopecia Tool (SALT) score at Week 24 (baseline minus Week 24 value) The SALT is a validated instrument for measuring the amount of scalp hair loss at a single point in time (see details regarding SALT score in **Appendix 1**).

Secondary Endpoints

- Determine the change from week 24 in the SALT score at Week 48 (week 24 minus week 48 value).

- Determine the change from baseline in the SALT score at week 48 (baseline minus week 48 value).

- The proportion of patients achieving at least 50% improvement in Severity of Alopecia Tool (SALT) score (SALT-50) at Weeks 24 and 48 compared to Baseline.

- The proportion of patients achieving at least 50% improvement in Severity of Alopecia Tool (SALT) score (SALT-50) at Week 48 compared to week 24.

- The proportion of patients achieving at least 75% improvement in Severity of Alopecia Tool (SALT) score (SALT-75) at Weeks 24 and 48 compared to baseline.

- The proportion of patients achieving at least 90% improvement in Severity of Alopecia Tool (SALT) score (SALT-90) at Weeks 24, 48 compared to Baseline.

- Change in the Alopecia Areata Symptom Impact Scale (AASIS) at Weeks 24 and 48 compared to baseline.

- Change in the Alopecia Areata Quality of Life questionnaire (AA-QoL) at Weeks 24 and 48 compared to baseline.

- Proportion of AA patients with Alopecia Areata Physician's Global Assessment (aaPGA) = 0-1 at Weeks 12, 24, 36, and 48 (see details in **Appendix 1**).

- Change in eyelash and eyebrow scores (see details in **Appendix 1**) at Weeks 12, 24, 36, and 48 compared to baseline.

- Change in eyelash and eyebrow scores (see details in **Appendix 1**) at Week48 compared to Week24.

- Change from baseline in Eczema Area and Severity Index (EASI) at Weeks 12, 24, 36, and 48 (see details in **Appendix 1**).

- Change from week 24 in EASI at weeks 36 and 48 (see details in **Appendix 1**).

- Proportion of patients with Eczema Area and Severity Index (EASI)-50 (≥50% reduction from baseline in EASI scores) at Weeks 12, 24, 36, and 48.

- Proportion of patients with Eczema Area and Severity Index (EASI)-75 (≥75% reduction from baseline in EASI scores) at Weeks 12, 24, 36, and 48.

- Proportion of patients with Eczema Area and Severity Index (EASI)-90 (≥90% reduction from baseline in EASI scores) at Weeks 12, 24, 36, and 48 (see details in **Appendix 1**).

- Semi-quantitative score using SALT subclasses (0 = no hair loss; 1 = <25% hair loss; 2 = 25-49% hair loss; 3 = 50-74% hair loss; 4 = 75-99% hair loss; 5 = 100% hair loss).

- Safety profile of dupilumab in subjects with AA by reported adverse effects, physical examinations and laboratory parameters.

Mechanistic (Exploratory) Endpoints

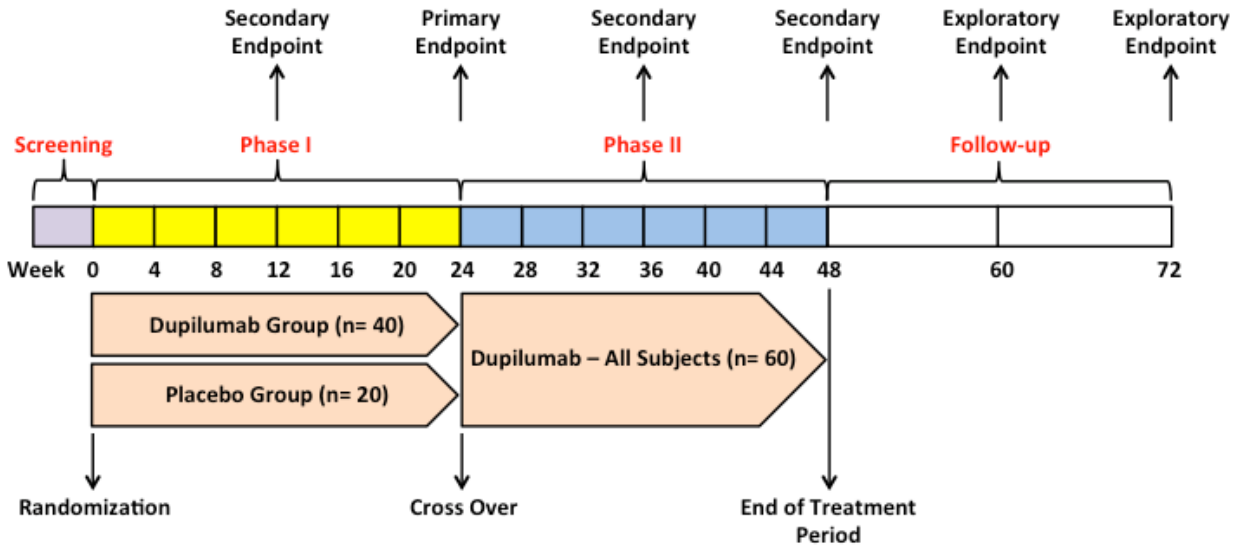
- Determine the effect of dupilumab therapy on the immune cell populations (e.g. T cells, dendritic cells, eosinophils, and mast cells) and related cytokines, chemokines, and inflammatory cells found in the blood and skin of study subjects. RT-PCR will be used to determine these effects.

STUDY DESIGN OVERVIEW:

This is a prospective, randomized, double blind, placebo-controlled clinical trial. The study will take place at two sites: Icahn School of Medicine at Mount Sinai, and Rockefeller University. This trial will include a total of 60 patients with moderate to severe AA (affecting more than 30% of the scalp) at the time of screening. AA subject must have evidence of hair regrowth within 10 years of their last episode of hair loss (see Exclusion Criteria below). Approximately one-third of subjects (20 out of 60 subjects) will also have concomitant AD (i.e. active AD skin lesions *or* a history of atopic dermatitis at the Screening and Baseline visits). Additionally, subjects with a history of alopecia totalis (AT) or alopecia universalis (AU) will be limited to no more than 50% of the total subjects (30 out of 60 subjects). Once the required number of subjects in each of these sub-categories is met, additional subjects in those categories will not be enrolled. Power calculation rationale is detailed below under "Statistical Considerations."

In **Phase I**, subjects will be randomized (2:1) to either receive dupilumab or placebo for 24 weeks (Weeks 0 to 24).

At Week 24 (Primary Endpoint)**,** both groups will enter **Phase II** of the study in which all subjects receive active drug (dupilumab) for an additional 24 weeks (Weeks 24 to 48). All subjects will return for monthly visits between Weeks 24 and 48. The treatment period will conclude at Week 48 (a secondary endpoint), and subjects will be asked to follow up at Weeks 60 and 72 (exploratory endpoints) as shown below for continued observation of skin and hair changes.



After providing consent, subjects will be assessed for study eligibility during the screening period (within 4 weeks of Baseline), which includes a review of past and current medical conditions, detailed review of past and current medications, a physical examination, clinical assessments (SALT, and eyebrow/eyelash assessment score), and additional skin assessments if he/she concurrently has atopic dermatitis (EASI, IGA, SCORAD). The following laboratory tests will be performed on all patients: complete blood count (CBC) with differential, complete metabolic panel (CMP), lactate dehydrogenase (LDH),

C-reactive protein (CRP), Total serum IgE, HIV, hepatitis B surface antigen (HbsAg) and hepatitis C virus antibodies, and serum pregnancy (where applicable).

Subjects who meet inclusion and exclusion criteria for eligibility will undergo Baseline assessments at Week 0, including hair examination, clinical assessments (SALT and eyebrow/eyelash scores), additional skin assessments if he/she concurrently has atopic dermatitis (EASI, IGA, SCORAD), review of medications, standardized clinical photography (optional), questionnaires (AASIS and AA-QLI), and urine pregnancy testing (where applicable). Blood samples and skin biopsies will be collected for mechanistic studies (described below), as well as DNA, RNA, and protein analyses. Two 4.5 mm scalp biopsies will be performed at Baseline: one from an area with active AA (i.e. the peripheral edge of a scalp lesion with active hair loss) and one from an adjacent uninvolved area of the scalp. If the subject has 100% hair loss at Baseline exam, a single biopsy of the posterior scalp will be collected. For subjects with concomitant AD, an additional lesional skin biopsy will be collected from an active AD lesion as outlined in the schedule of events. At this Baseline visit, subjects will undergo randomization and be given their first injection of the study drug (dupilumab or placebo).

The first dose will be a loading dose of 600mg (given as two injections of dupilumab or placebo), provided in the clinic (including a 30-minute post-injection follow-up), followed by the patient's weekly self-administration of 300 mg (one injection of dupilumab or placebo) at home (assuming the patient and the care team feel that the patients is ready to self-inject at home).

After sufficient training and when the subject feels comfortable to self-inject, the subject will be provided with the study drug (dupilumab or placebo), and will receive weekly dosing through Week 24.

During Phase I and II of the study, subjects will return for visits every four weeks for repeat clinical assessments, medication reviews, and monitoring for adverse events. Female subjects will undergo a urine pregnancy test (where applicable) at each of these visits.

At Week 48, if subjects want to seek other treatment options (including commercially available dupilumab) for AA, they may do so through their own dermatologist/physician. Any treatments will be documented as concomitant medications.

INDEPENDENT EXPERT ASSESSMENT:

Dr. David Norris, Professor and Chairman of the Department of Dermatology-University of Colorado School of Medicine, has agreed to serve as a hair expert and provide full support and expertise throughout the entire study period. Dr. Norris will also review study progress for all participants every 3-6 months. We will arrange monthly teleconference calls every 3-6 months to discuss progress and biannual visits to New York for both sites to jointly look at collected data and analyses.

MECHANISTIC ANALYSES:

In order to identify the molecular and cellular immune profiles of AA at baseline and identify treatment response biomarkers with dupilumab (including immune and hair keratin biomarkers).

Based on biomarkers recently identified in AA lesions [35-36, 38-39], we plan to perform one LS and one NL 4.5 mm punch biopsy at Baseline, and one LS biopsy at Weeks 12 and 24 (from the same area previously biopsied, but at least 1 cm away from the previous scar). If the subject has 100% hair loss at the Baseline exam, a single biopsy of the involved posterior scalp will be collected. An optional scalp biopsy at 48 weeks will also be performed. From patients that have both AD and AA, we will also perform a LS skin biopsy from a representative lesion at Baseline, and also after 24 weeks of treatment. This will serve to characterize similar response biomarkers to AD and AA in the same patients.

Gene expression studies (RT-PCR and gene arrays) will also be performed. The expression levels of markers of the different immune pathways, including: Th1 (IFN-gamma, CXCL9, CXCL10, STAT1), Th2 (IL-13, IL-5, IL-10, CCL5, CCL13, CCL17, CCL18, CCL26), Th17 (IL-17A, IL23p19, IL23p40, CCL20, CXCL1, elafin/PI3), Th9/IL-9, Treg/FOXP3, Th22/IL-22, and the IL-17/IL-22 regulated S100A7 gene will be evaluated. We will also assess for modulation of inflammatory markers (MMP12, S100A12) and an innate immune genes (IL-1B, IL-8). We will also assess for other AA biomarkers such as IL-16, IL-15, IL-2, IL-32, PDE4, JAK-1, JAK-3, STAT-3, that we have recently associated with AA activity. IL-4R, IL-12R, IL-23R, IL-15R and IL-2RA will also be evaluated. We will also assess for hair keratins (keratin 35, 40, 75, 83, 84, 85, 86) and keratin-associated proteins (KRTAP1) that are good biomarkers of AA and serve as good treatment response markers of hair regrowth with treatment. Gene arrays (Affymetrix U133A Plus 2 gene array platform) will also be performed.

Blood Analyses

OLINK proteomic platform will be used to assess for biomarkers in blood. This platform is able to evaluate ~200 analytes, including all the inflammatory markers that are analyzed in skin, as well as cardiovascular markers, and neuro-immunological markers, including IL-13 and all the above Th2 chemokines, IFN gamma and the Th1 chemokines, as well as IL-17A, IL-22, PDE-related markers, and many other markers. Blood assessments will be performed on serum obtained at baseline, and Weeks 12, 24, 36, and 48.

A complete list of the biomarkers we will assess via OLINK can be found at:
http://www.olink.com/proseek-multiplex/complete-biomarker-list/

INCLUSION CRITERIA:

1) Male of female subjects who are at least 18 years old at the time of informed consent.

2) Subject is able to understand and voluntarily sign an informed consent document prior to participation in any study assessments or procedures.

3) Subject is able to adhere to the study visit schedule and other protocol requirements.

4) Females of childbearing potential (FCBP) must have a negative pregnancy test at Screening and Baseline. While on investigational product and for at least 28 days after taking the last dose of investigational product (IP), FCBP who engage in activity in which conception is possible must use one of the approved contraceptive options described below:

   a. **Option 1:** Any one of the following highly effective methods: hormonal contraception (oral, injection, implant, transdermal patch, vaginal ring); intrauterine device (IUD); tubal ligation; or partner's vasectomy;

      OR

   b. **Option 2:** Male or female condom (latex condom or non-latex condom NOT made out of natural [animal] membrane [for example, polyurethane]); PLUS one additional barrier method: (a) diaphragm with spermicide; (b) cervical cap with spermicide; or (c) contraceptive sponge with spermicide.

5) If subject is a female of non-childbearing potential, she must have documented history of infertility, be in a menopausal state for one year, or had a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy.

6) Subject has a history of at least 6 months of moderate to severe AA (≥ 30% scalp involvement) as measured using the SALT score; OR subject has ≥ 95% loss of scalp hair for enrollment as AA totalis (AT) or universalis (AU) subtypes.
   a. AT/AU will be limited to no more than 50% of the total subjects enrolled.
   b. Approximately one-third of subjects will have active AD skin or a concomitant history of AD at the time of the Screening and Baseline visits.

7) Subject has a negative Tuberculin purified protein derivative (PPD) or QuantiFERON TB-Gold test (QFT) prior to baseline. Subjects with a positive or indeterminable PPD or QFT result must have a documented negative workup for tuberculosis and/or completed standard tuberculosis therapy.

8) Subjects must meet the following laboratory criteria:

   a. White blood cell count ≥ 3000/mm^3 (≥ 3.0 x 10^9/L) and < 14,000/mm^3 (≤ 14 x 10^9/L).
   b. Platelet count ≥ 100,000/µL (≥ 100 x 10^9/L).
   c. Serum creatinine ≤ 1.5 mg/dL (≤ 132.6 µmol/L).
   d. AST (SGOT) and ALT (SGPT) ≤ 2 x upper limit of normal (ULN). If the initial test shows ALT or AST > 2 times the ULN, one repeat test is allowed during the Screening Phase.
   e. Total bilirubin ≤ 2 mg/dL (34 µmol/L). If the initial test shows total bilirubin > 2 mg/dL (34 µmol/L), one repeat test is allowed during the Screening Phase.

f. Hemoglobin ≥ 10 g/dL (≥ 6.2 mmol/L).

9) Subject is judged to be in otherwise good overall health following a detailed medical and medication history, physical examination, and laboratory testing.

EXCLUSION CRITERIA:

The presence of any of the following will exclude a subject from enrollment:

1) Subject is pregnant or breastfeeding.

2) Subject's cause of hair loss is indeterminable and/or they have concomitant causes of alopecia, such traction, cicatricial, pregnancy-related, drug-induced, telogen effluvium, or advanced androgenetic alopecia (i.e. Ludwig Type III or Norwood-Hamilton Stage ≥ V).

3) Subject has a history of AA with no evidence of hair regrowth for ≥ 10 years since their last episode of hair loss.

4) Subject has an active bacterial, viral, or helminth parasitic infections; OR a history of ongoing, recurrent severe infections requiring systemic antibiotics

5) Subject with a known or suspected underlying immunodeficiency or immune-compromised state as determined by the investigator.

6) Subject has a concurrent or recent history of severe, progressive, or uncontrolled renal, hepatic, hematological, intestinal, metabolic, endocrine, pulmonary, cardiovascular, or neurological disease.

7) Active hepatitis B, hepatitis C, human immunodeficiency virus (HIV), or positive HIV serology at the time of screening for subjects determined by the investigators to be at high-risk for this disease.

8) Subject has a suspected or active lymphoproliferative disorder or malignancy; OR a history of malignancy within 5 years before the Baseline assessment, except for completely treated in situ non-melanoma skin and cervical cancers without evidence of metastasis.

9) Subject has received a live attenuated vaccine ≤ 30 days prior to study randomization.

10) Subject has any uncertain or clinically significant laboratory abnormalities that may affect interpretation of study data or endpoints.

11) Subject has any other medical or psychological condition that, in the opinion of the investigator, may present additional unreasonable risks as a result of their participation in the study and/or interfere with clinic visits and necessary study assessments.

12) History of adverse systemic or allergic reactions to any component of the study drug.

13) Severe, untreated asthma or a history of life-threatening asthma exacerbations while on appropriate anti-asthmatic mediations.

14) Use of systemic immunosuppressive medications, including, but not limited to, cyclosporine, systemic or intralesional corticosteroids, mycophenolate mofetil, azathioprine, methotrexate, tacrolimus, or ultraviolet (UV) phototherapy with/without Psoralen Ultraviolet A (PUVA) therapy within 4 weeks prior to randomization.

15) Use of an oral JAK inhibitor (tofacitinib, ruxolitinib) within 12 weeks prior to the Baseline visit.

16) Subject has used topical corticosteroids, and/or tacrolimus, and/or pimecrolimus within 1 week before the Baseline visit.

17) Subject has been previously treated with dupiliumab.

18) Subject currently uses or plans to use anti-retroviral therapy at any time during the study.

POTENTIAL RISKS OF DUPILUMAB:

Dupilumab showed good safety based on early and late phases in AD and asthma [37, 39-42]. Based on the nature of dupilumab, its mechanism of action, data from human and animal studies, and what is known about other similar drugs, possible risks include:

- Infections, including those caused by parasites (e.g. intestinal worms).
- Injection site reactions: Injection site reactions observed in dupilumab clinical trials have been generally mild to moderate and resolved without requiring any treatment. Occasionally, such reaction may be severe and last more than 24 hours.
- Hypersensitivity/allergic reactions.
- Adverse consequences of forming antibodies to dupilumab
- Cancer: A potential risk for medications interfering with the immune system.
- Potential for interactions with other medications: It is not known if dupilumab may increase or decrease the effect of other medications.
- Inflammation of the eye: a potential risk of conjunctivitis has been reported.

PRIOR TREATMENT:

All relevant treatment received by the subject within 30 days before screening will be recorded. Over-the-counter drugs (e.g. vitamins, acetaminophen) taken by the subject within 14 days before screening will also be recorded.

CONCOMITANT TREATMENT:

Concomitant medications are permitted for the treatment of stable, chronic illness. Use of such concomitant medication or other medications that may be required throughout the study will be recorded (including the reason for treatment and name, dose, unit, route, and the date of treatment as appropriate) until week 72 or Early Termination.

At Week 48, if subjects want to seek other treatment options (including commercially available dupilumab) for AA, they may do so through their own dermatologist/physician. If a subject initiates a new treatment or obtains commercially available dupilumab at Week 48, then that treatment must be documented as a concomitant medication.

PROHIBITED TREATMENT:

All treatments prohibited during the screening period are also prohibited throughout the course of the study. No live attenuated vaccinations are permitted through the study. All subjects treated previously with dupilumab will be excluded from the study as above.

PROCEDURES:

Monitoring of AEs will begin at signing of the informed consent form and will be continued throughout the study. All study procedures will be completed at designated times during the study (see **Table 1**).

Trained assessors will perform clinical assessments. It is suggested that the same assessor/s perform these assessments for a subject.

Visit 1 – Screening (within 4 weeks of Baseline)*****

- Sign and date an IRB-approved consent and HIPPA agreement
- Review Inclusion and Exclusion Criteria
- Record gender, race, ethnicity, and medical history (including personal and family history of AA, atopic dermatitis, asthma, allergies, and autoimmune diseases)
- Complete physical exam and vitals, including: height (cm), weight (kg), blood pressure, and heart rate
- Record all concomitant medications as well as all those received within the past month prior to screening
- Record all prior systemic and topical therapies/treatments, including phototherapy, used for AA
- PPD placement or Quantiferon blood test as outlined in Inclusion/Exclusion criteria
- Serum HCG for all female subjects of child-bearing potential to confirm subject is not pregnant
- HIV, HBV, and HCV serology
- Complete blood count (CBC) and Comprehensive metabolic panel (CMP)
- Total serum IgE, Lactate dehydrogenase (LDH), and C-reactive protein (CRP)
- AA Clinical assessment (SALT, , and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)

*If more than 4 weeks has lapsed between screening and Baseline/Visit 2, then all procedures should be repeated except for TB screening and HIV/HBV/HCV serologies.

Visit 2 – Baseline (Week 0)

- Confirm all inclusion and exclusion criteria have been met
- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Randomize subject
- Obtain vital signs
- AA Clinical assessments (SALT and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Questionnaires (AASIS and AA-QLI)
- Collect blood sample for mechanistic endpoints
- Standardized clinical photography
- Perform biopsies of lesional and non-lesional skin of the scalp, or a single lesional biopsy for subjects with AT or AU
- Perform a biopsy of lesional skin for subjects with a concomitant history of AD and active AD lesions
- Record concomitant medications and adverse events
- Dispense/administer study drug

Visit 3 – Week 4

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Dispense/administer study drug
- Record concomitant medications and adverse events

Visit 4 – Week 8

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Dispense/administer study drug
- Record concomitant medications and adverse events

Visit 5 – Week 12

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Questionnaires (AASIS and AA-QLI)
- Standardized photographs
- Obtain CBC, CMP, LDH, CRP, and Total serum IgE
- Collect blood sample for mechanistic endpoints
- Perform biopsy of lesional skin of the scalp
- Dispense/administer study drug
- Record concomitant medications and adverse events

Visit 6 – Week 16

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Standardized photographs
- Dispense/administer study drug
- Record concomitant medications and adverse events

Visit 7 – Week 20

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Standardized photographs
- Dispense/administer study drug
- Record concomitant medications and adverse events

Visit 8 – Study Cross Over (Week 24)

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Questionnaires (AASIS and AA-QLI)
- Standardized photographs
- Obtain CBC, CMP, LDH, CRP, and Total serum IgE
- Collect blood sample for mechanistic endpoints
- Perform biopsy of lesional skin of the scalp

- Perform a biopsy of lesional skin for subjects with a concomitant history of atopic dermatitis and active AD lesions if present; if no active AD lesions, biopsy of skin will be collected near biopsy performed at Baseline
- Dispense/administer study drug (subjects previously on dupilumab will receive 1 injection of 300 mg dupilumab and 1 injection of placebo to maintain the blind of the study; subjects previously on placebo will receive 2 injections of 300 mg dupilumab). All subjects should be observed for 30 minutes following the injection to preserve the blind.
- Record concomitant medications and adverse events

Visit 9 – Week 28

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Dispense/administer study drug
- Record concomitant medications and adverse events

Visit 10 – Week 32

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Dispense/administer study drug
- Record concomitant medications and adverse events

Visit 11 – Week 36

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Questionnaires (AASIS and AA-QLI)
- Standardized photographs
- Obtain CBC, CMP, LDH, CRP, and Total serum IgE
- Collect blood sample for mechanistic endpoints
- Dispense/administer study drug
- Record concomitant medications and adverse events

Visit 12 – Week 40

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs

- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Standardized photographs
- Dispense/administer study drug
- Record concomitant medications and adverse events

Visit 13 – Week 44

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Standardized photographs
- Dispense/administer study drug
- Record concomitant medications and adverse events

Visit 14 – Week 48

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Questionnaires (AASIS and AA-QLI)
- Standardized photographs
- Obtain CBC, CMP, LDH, CRP, and Total serum IgE
- Collect blood sample for mechanistic endpoints
- Record concomitant medications and adverse events
- Obtain **optional** biopsy of lesional scalp
- Obtain **optional** biopsy of lesional skin for subjects with a concomitant history of atopic dermatitis

Visit 15 – Week 60

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Record concomitant medications and adverse events

Visit 16 – Week 72

- Obtain vital signs
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)

- Questionnaires (AASIS and AA-QLI)
- Standardized photographs
- Record concomitant medications and adverse events

LABORATORY TESTING:

Each site may utilize their own local lab for CBC, CMP, LDH, CRP, IgE, HIV, HBV, HCV, Quantiferon Gold, and pregnancy testing. Lab certifications for any lab used should be kept in the study binder. RU will have to transport biopsy samples as well as blood samples for mechanistic testing (and potentially IgE testing) to Mount Sinai via proper and documented means.

SAFETY MONITORING:

The study will be conducted in accordance with our department's Standard Operating Procedures, which are based on US FDA Title 21 Code of Federal Regulations and ICH Good Clinical Practice guidelines. An investigator will review all laboratory results and assess for adverse events. The principal investigator will be informed of all adverse events. In the event that a subject's safety is compromised, the investigator will discontinue the subject immediately.

STUDY WITHDRAWAL:

In the event a study subject wants to withdraw early from the study or the subject is being discontinued from the study for any reason, the following procedures should be followed:

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs
- Obtain CBC, CMP, LDH, CRP, and Total serum IgE
- Collect blood sample for mechanistic endpoints
- AA Clinical assessments (SALT, aaPGA, and eyebrow/eyelash)
- AD Clinical assessments, if applicable (EASI, IGA, SCORAD)
- Questionnaires (AASIS and AA-QLI)
- Standardized Photographs
- Perform biopsy of lesional skin of the scalp
- Perform a biopsy of lesional skin for subjects with a concomitant history of atopic dermatitis and an active AD lesion if present
- Record all changes in medications and adverse events

Considering there is no current treatment that is very effective in treating alopecia areata, these subjects are highly motivated to participate in a study that provides a treatment option.

TOTAL VOLUME OF BLOOD COLLECTED DURING THE STUDY:

	Blood volume (mL)					
Visit	**1**	**2**	**5**	**8**	**11**	**14**
Week	**Screen**	**0**	**12**	**24**	**36**	**48**
Serum HCG (where applicable)	10	n/a	n/a	n/a	n/a	n/a
HIV	10	n/a	n/a	n/a	n/a	n/a
HBV, HCV	10	n/a	n/a	n/a	n/a	n/a
CBC with diff	4	4	4	4	4	4
CMP, CRP, LDH	10	10	10	10	10	10
Total Serum IgE	10	10	10	10	10	10
Mechanistic studies	n/a	10	10	10	10	10
Total volume	**54**	**34**	**34**	**34**	**34**	**34**

The total estimated blood volume is 224 mL. Additional volumes may be required for repeat testing or as part of unscheduled visits.

DISCONTINUATION OF TREATMENT AND WITHDRAWAL OF SUBJECTS:

The reasons why a subject may discontinue or be withdrawn from the study include, but are not limited to the following: subject request, protocol violation, loss to follow up, subject non-compliance, study termination by investigators, and a confirmed grade 3 or higher adverse event, which is suspected to be related to test article administration. Subjects that elect to withdraw from the trial due to lack of efficacy and/or exacerbation of disease will undergo the following: vitals; urine pregnancy test (if applicable); lesional biopsy of the scalp (if withdrawing before the Week 24 visit); lesional biopsy of the AD lesion (if applicable, and if withdrawing prior to the Week 24 visit); blood evaluations (CBC, CMP, LDH, CRP, Total serum IgE, and blood sample for mechanistic endpoints); clinical evaluation (SALT, SCORAD, EASI, aaPGA, and eyebrow/eyelash scores); subject evaluations (AASIS and AAQLI); and photographs.

Stopping Rules

Patients will be permanently discontinued from study treatment in the event of:

- Anaphylactic reaction or other severe systemic reaction to study drug injection
- Diagnosis of a malignancy during study, excluding carcinoma in situ of the cervix, or squamous or basal cell carcinoma of the skin
- Evidence of pregnancy
- Any infection that is opportunistic, such as active TB and other infections whose nature or course may suggest an immuno-compromised status
- Severe laboratory abnormalities:
    - Neutrophil count ≤0.5 x 10^3/µL
    - Platelet count ≤50 x 10^3/µL

    - Alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) values >3x upper limit of normal (ULN) with total bilirubin >2x ULN (unless elevated bilirubin is related to confirmed Gilbert's Syndrome)
    - Confirmed AST and/or ALT >5 x ULN (for more than 2 weeks)
- Treatment with any prohibited concomitant medication or procedure
- Other reasons that may lead to the permanent discontinuation of study drug include certain AEs deemed related to the study drug.

Study drug dosing may be <u>temporarily</u> suspended in the event of:

- Clinically important laboratory abnormalities, such as:
    - Neutrophil count ≤1.0 x 10^3/μL but >0.5 x 10^3/μL
    - Platelet count ≤100 x 10^3/μL but >50 x 10^3/μL
    - Creatine phosphokinase (CPK) >10x ULN
- Other intercurrent illnesses or major surgery
- An infection that requires systemic treatment with antibiotic, antifungal, antiviral, anti-parasitic, or anti-protozoal agents or requires oral treatment with such agents for longer than 2 weeks
- Treatment with systemic corticosteroids or non-steroidal immunosuppressive/ immunomodulating medications (eg, cyclosporine, methotrexate, azathioprine, mycophenalate-mofetil, Janus kinase inhibitors, biologic agents, etc).

Duration of study drug suspension should be reviewed on a case by case basis and can be discussed among the PIs.

Unblinding

In case of an emergency, when knowledge of the test article assignment is required for the medical management of an individual subject, the subject will be unblinded. The code should be broken only in the event of a medical emergency, when knowing the treatment assignment is absolutely necessary. The investigators will notify the IRB, FDA and any other regulatory group, within 24 hours after determining that it is necessary to unblind the treatment assignment. The investigator must also indicate in source documents and in the CRF that the blind was broken and provide the date, time, and reason for breaking the blind. Any AE or SAE associated with breaking the blind must be recorded and reported as specified in this protocol.

A clinical data manager will assemble all the patient data by groups (treatment versus placebo) and the biostatistician may request the unblinding of subject treatment assignment for analyzing safety data, upon the appearance of unequal adverse events.

Safety Evaluation

This is a phase II study. Safety and tolerability will be evaluated from the AEs, physical examinations, vital sign measurements, and clinical laboratory test results. More frequent safety evaluations may be performed if clinically indicated or at the discretion of the investigator.

Aggregated AEs will be evaluated monthly by the PI/research teams.

Safety data will be reviewed by the investigators, study teams, biostatisticians, who will monitor the study on a regular basis and meet regularly (approximately every 3 months, depending on the number of subjects enrolled). The local IRBs and FDA will be notified of adverse events (as required by each institution).

Physical Examination/Vital Signs
A licensed physician, physician assistant, nurse practitioner, or registered nurse will perform physical examinations at the time of screening. Body weight (kg), height (cm), blood pressure, and heart rate will be measured at screening. At all other visits, only blood pressure and heart rate will be measured.

Physical examination consists of assessments of general appearance; skin; head, eyes, ears, nose, and throat (HEENT); heart; lungs; abdomen; extremities; neurological function; and lymph nodes.

Safety Laboratory Determinations
Laboratory evaluations will be performed according to the flowchart on page by a CLIA certified laboratory at the study site. All laboratory tests with clinically important abnormal results identified after test article administration will be repeated until the values return to normal, baseline or non-clinically significant, as appropriate. Each abnormal lab will be indicated as clinically significant or not clinical significant.

Biopsy Site Wound Infections
If a wound infection occurs at the site of the biopsy, the site will perform a bacterial culture as part of standard of care procedures.

MEASURES TO MINIMIZE/AVOID BIAS:

Subject Identification
Subjects are numbered sequentially. Each subject will be assigned a unique number and will keep this number for the duration of the study. Subject numbers will not be reassigned or reused for any reason. Subjects who discontinue or withdraw from the study before receiving a treatment assignment code, who re-enroll at a later time must be assigned a new subject number. Subjects should be identified only by their assigned identified code number. The investigator must maintain a subject master log linking the subject number to the subject's name. The investigator must follow all applicable privacy laws in order to protect a subject's privacy and confidentiality.

Randomization and Blinding
A subject who meets all of the inclusion and exclusion criteria will be assigned a randomization number pre-dose on day 0/baseline. The randomization number is distinct from the subject number.

After fulfilling the enrollment criteria, the subjects enrolled will be randomized in a 2:1 ratio of dupilumab to placebo.

The randomization for both sites will be performed by the designated Research Pharmacist/personnel at the Icahn School of Medicine utilizing the Web site http://www.randomization.com (or similar). The method uses random block sizes and randomly permuted blocks.

Approximately 60 subjects will be enrolled in the study in order to have at least 54 subjects complete (taking into account a 10% drop out rate). Subjects will be assigned a randomization number on day 0/Baseline. The randomization schema will be kept confidentially after generation.

INVESTIGATIONAL DRUG SUPPLY AND BLINDING:

Regeneron Pharmaceuticals will provide dupilumab 300 mg for 40 subjects in Phase I and for all 60 subjects during Phase II (a total of 25 doses in Phase I to those 40 subjects randomized to receive dupilumab, followed by another 24 doses in Phase II; a total of 24 doses in Phase II to the 20 subjects who received placebo in Phase I).

Eligible patients will receive a 600 mg SC dupilumab loading dose (300 mg initial dose, followed by a 300 mg loading dose) on day 0/Baseline, and then continue with 300 mg SC dupilumab once weekly starting on day 8. At Week 24, subjects previously on dupilumab will receive 1 injection of 300 mg dupilumab and 1 injection of placebo to maintain the blind of the study; subjects previously on placebo will receive 2 injections of 300 mg dupilumab. All subjects should be observed for 30 minutes following the injections at Baseline and at Week 24.

Matching placebo prefilled syringes (that will appear identical to the dupilumab syringes) will be provided by the company to the study sites. The subjects and the staff will remain unaware of the individual treatment assignment during the study. All study medication will be stored in a secure cool area between 2-8˚C. Study drug will be administered to subjects at each study visit, with subsequent weekly injections performed by the patients at home. Patients (and/or caregivers) who are willing and able to administer dupilumab outside of the clinic will be trained on injecting study drug during the initial clinic visit.

We will query the subjects during each study visit on any missed injections and document this information. The investigative sites will account for all study drug dispensed and stored during the study.

STATISTICAL CONSIDERATIONS:

Sample Size Calculation

The primary outcome for this study is the SALT change at week 24. For patients with similar disease severity, a change in SALT of less 5 units was observed (Guttman et al, 2019). Assuming that dupilumab will induce a change in SALT of at least 25 units with a standard deviation of 10 atweek 24, a sample size of 54 completed patients randomized 2:1 treatment to placebo (i.e. 36 in the treatment arm, 18 in the placebo arm) will provide 97% power to detect differences with Placebo based on a two-sided Student's t-test with a 0.05 type I error. Assuming that 10% of patients drop out of the study, we plan to enroll 60 total patients (i.e. 36 in the treatment arm, 18 in the placebo arm).

Demographics and Other Baseline Characteristics
Descriptive statistics of demographics and other baseline characteristics will be presented for all subjects and tabulated by treatment group. Presentations of age, sex, ethnicity, race and baseline will also be given. Other baseline characteristics include height, weight, and vital signs, duration of the AA and AD, concurrent diagnoses from medical history and indications for concomitant medication, concomitant medication, and previous AA and AD treatments.

Analysis of Primary Endpoint
A Mixed effect Model Repeated Measurement (MMRM) will be used to detect any overall differences in the treatment effect at week 24 compared to baseline.

Analysis of Secondary Endpoints
A Mixed effect Model Repeated Measurement (MMRM) will be used to detect any overall differences in the treatment effect at week 48 compared to baseline, as well as at week 48 compared to week 24. This formulation intrinsically models the within patient correlation structure as in the case of a paired t-test. This approach introduces less bias than restricting the analysis for those patients who completed the study. A similar model will be used to evaluate the change in eyelashes and eyebrows scores, as well as in Eczema Area and Severity Index (EASI) at each visit compared to baseline.

A two-sided Fisher exact test will be used to compare the percentage of patients who achieve 50% improvement in SALT, eyelashes, eyebrows and EASI scores (calculated as 100 x ($SCORE_{baseline}$ - $SCORE_{W24}$)/ $SCORE_{baseline}$) between drug and placebo groups. The same approach will be used for other discrete variables, SALT-75, and SALT-90 at each visit compared to baseline.

Analysis of Exploratory Endpoints
The change from Baseline in molecular markers in skin biopsies after treatment will be analyzed using a mixed effect linear model with treatment and time interaction as a fixed effect and a random effect for each patient. This formulation intrinsically models the within patient correlation structure as in the case of a paired t-test, and introduces less bias than restricting the analysis for those patients who completed the study.

Analysis of Mechanistic Endpoints
For comparison of expression levels of lesional, and non-lesional AA skin, we will use RT-PCR by Taqman Low-density arrays and RNA-sequencing. RT-PCR values will be normalized to the housekeeping gene RPLP0 (validated in our AD and AA studies) and log-2 transformed prior to analysis. RNA-sequening data will be pre-processed using standard pipeline (exhaustively used by our group in a large number of studies) and log-2 voom-transformed expression. Mixed effect modelwill be used and p-values for the moderated t-tests and paired-t-test will be adjusted by Benjamini-Hochberg procedure.

Extensive bioinformatics tools will be employed to gain insights into the results and test hypotheses that are generated in the “data mining stage”. This will include (but will not be limited to) pathway and gene-set enrichment analyses using Ingenuity software, GSEA, the R package GSVA (Gene Set Variation Analysis) and other in-house codes produced

by our group. To define cellular and molecular biomarkers of AA in skin biopsies, biomarkers will be divided into those that show significant group differences and those that do not. Multiple regression models will be used to determine the best set of biomarkers that predict disease activity and response.

Gene expression changes in hair keratins and the immune axes (Th1, Th2, Th17, Th22) will be jointly correlated with clinical responses by multivariate analysis using multivariate u-statistics and R package muStat, as previously reported [43].

Data Transfer and Management
Data including the patient info, demographics, clinical scores and treatment allocation code will be sent to our Biostatistical Team in password-protected files.

Data from the Guttman Lab (where all mechanistic blood and skin studies will be done), including FASTQ files, real-time PCR (RT-PCR), and serum proteomics will be deposited in the Mount Sinai Box and Amazon S3 bucket, having the necessary safeguards for protection of human subjects research. Dr. Guttman lab members will not have access to the treatment code until all lab work has been completed and the data transferred to our Biostatistical Team.

All data management steps taken to reformat and merge the clinical and laboratory data will be carried out using R codes, so all the steps of the processes can be reproduced.

Management of the Analysis
Analysis of RT-PCR and RNA-sequencing will all be conducted using R language and bioconductor packages. Codes will be QC-ed by at least one independent member of the Biostatistical Team, other than the member who coded the analysis.

Analysis will be run in our desktops, with a backed up copy in the Mount Sinai Box. Analysis results will be shared with the study teams of Drs. Guttman, Lebwohl, and Krueger, as well as Dr. Jason Hawkes who holds joint appointments at Rockefeller University and Mount Sinai, and Dr Norris who is our study consultant, via the Mount Sinai Box. Once the analysis is finalized, all data will be deposited in Dr. Guttman's and Dr. Krueger's central file storage system (CFS), which is accessible to all research members. Both Mount Sinai Box and the CFS have backup capabilities and are encrypted and password protected. Access to the Mount Sinai Box specific for the project will be shared with the Rockefeller team and Dr Norris.

Analysis of Safety and Tolerability
Safety will be evaluated by tabulations of adverse events (AEs) and will be presented with descriptive statistics at each visit. AEs will be coded using the CTCAE, Common Terminology Criteria for Adverse Events, V 4.0. The number and percentage of subjects/lesions experiencing an AE/SAE will be stratified by system organ class, or a preferred term, and/or severity of the adverse event, and recorded and tabulated overall by each sub-strata. Each subject will be counted only once within a system organ class or a preferred term using the adverse events with the highest severity within each category. All information pertaining to adverse events noted during the study will be listed by subject, detailing verbatim given by the investigator, preferred term, system organ

class, date of onset, date of resolution, severity, and relationship to treatment. A tabulation of AEs will be provided by subject. The rates of AEs will be compared between treatment groups. If the rate of AE occurs in ~5% in either treatment group will be compared using the Fisher's exact test.

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Table 1. Schedule of Events

Visit Week	(D -31 to -1)	0	4	8	12	16	20	24	28	32	36	40	44	48	60	72	
Visit #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**Early Termination**
Visit Type	**Screening**	**Baseline**	**Tx**	**Tx**	**Tx**	**Tx**	**Tx**	**Cross-Over**	**Tx**	**Tx**	**Tx**	**Tx**	**Tx**	**Tx**	**F/U**	**F/U**	
Informed Consent	X																
Inc/Exc Criteria	X	X															
Demographics and Medical Hx[1]	X																
Physical Exam	X																
Vitals [2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TB Screening[3]	X																
HIV, HBV, and HCV [4]	X																
CBC, CMP	X				X			X			X			X			X
LDH, CRP	X				X			X			X			X			X
Total Serum IgE	X				X			X			X			X			X
Blood Sample (Mechanistic)		X			X			X			X			X			X
Pregnancy Test[5]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
AA Clinical Assessments (SALT, eyelash/eyebrow)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AA Clinical Assessment (aaPGA)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AD Clinical Assessments (EASI, IGA, SCORAD)[13]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Questionnaires (AASIS and AA-QLI)		X			X			X			X			X		X	X
Standardized Photographs		X			X	X	X	X			X	X	X	X		X	X
Dispense Drug		X[11]	X	X	X	X	X	X[12]	X	X	X	X	X				
Scalp Biopsy		X[6]			X[8]			X[8]						X[10]			X[8]*
Skin Biopsy		X[7]						X[9]						X[10]			X[9]*
Concomitant Medications	X	X	X	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	X	X	X	X

[1]Includes personal and family history of alopecia, atopic dermatitis, asthma, allergies, and autoimmune conditions;
[2]Includes height and weight (only at screening), blood pressure, and pulse;
[3]PPD or QuantiFERON TB-Gold test;
[4]HBS-ag and HCV-ab;
[5]Where applicable; serum HCG at screening and urine pregnancy tests at all other visits;
[6] One lesional and one non-lesional biopsy; or only one lesional in those with AT or AU;
[7] Where applicable, one biopsy of lesional skin for subjects with concomitant AD *and* active lesion;
[8] One biopsy of lesional AA skin close to the lesional biopsy performed at baseline;
[9] For AD pts, one biopsy of lesional skin close to the biopsy performed at baseline. If no biopsy was performed at baseline, then no biopsy required at this visit (regardless of active lesions);
[10] Optional biopsy of lesional scalp and lesional AD skin (if applicable);
[11] Initial dose will be a loading dose of 600mg (two injections of 300 mg dupilumab or placebo), followed by a required 30 minute observation period;
[12] Subjects previously on dupilumab will receive 1 injection of 300 mg dupilumab and 1 injection of placebo to maintain the blind of the study; subjects previously on placebo will receive 2 injections of 300 mg dupilumab. Both groups will be observed for 30 minutes following the injections.
[13] For subjects with active AD lesions at Screening and/or Baseline, these assessments should be done at each visit.
* Performed only if Early Termination visit occurs prior to Week 24.

Appendix 1. Clinical Assessments/Scoring

Alopecia Areata Assessments:

Severity of Alopecia Tool (SALT)
Scalp divided into four areas: vertex (40% of scalp surface area), right profile (18% of scalp surface area), left profile (18% of scalp surface area), and posterior scalp (24% of scalp surface area). Percentage of hair loss in these areas is multiplied by percent surface area of the scalp in that area. SALT score is the sum of percentage of hair loss in all areas.

SALT score:

	Left Side (18%)	**Right Side (18%)**	**Top (40%)**	**Back (24%)**	
Percentage Hair Loss					
Percentage X Area	X 0.18	X 0.18	X 0.4	X 0.24	
Total	**+**	**+**	**+**	**=**	

Eyelash/Eyebrow Assessment Score
0= None
1= Minimal eyelashes/eyebrows
2= Moderate eyelashes/eyebrows
3= Prominent eyelashes/eyebrows
4= Very prominent eyelashes/eyebrows

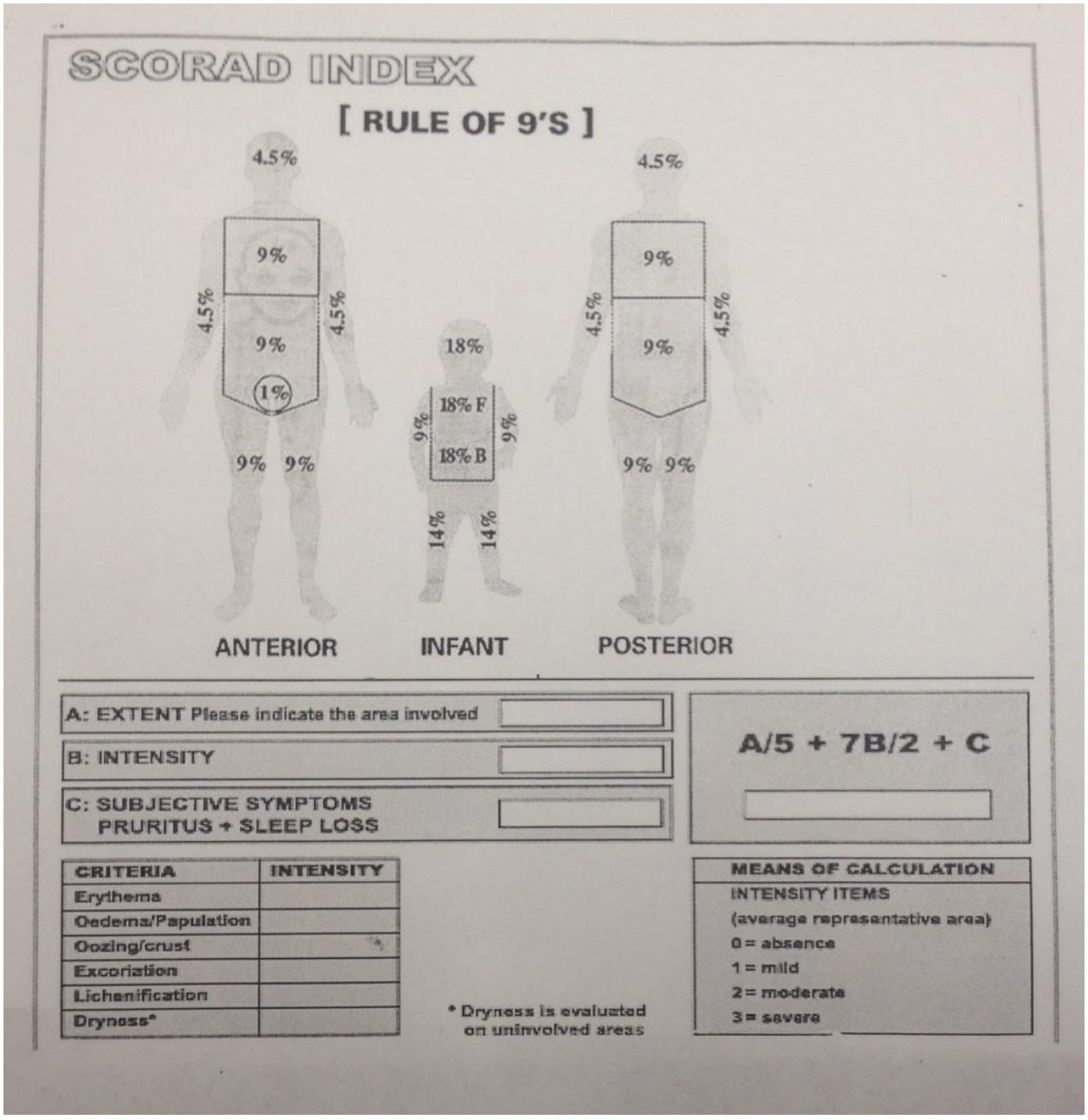
Alopecia Areata Physician's Global Assessment (aaPGA):
The aaPGA is used to assess the clinical response to treatment based on a 6-point scale ranging from 0 (no regrowth) to 5 (100% regrowth):

No regrowth	<25% regrowth	25-49% regrowth	50-74% regrowth	75-99% regrowth	100% regrowth
0	1	2	3	4	5

<u>Atopic Dermatitis Assessments</u>:

Scoring atopic dermatitis (SCORAD)

3 components: A) % body surface area involvement (the rule of 9); B) Intensity of eczema evaluated on representative lesions, rating 5 lesions (scale 0 to 3) for features including erythema, thickness, excoriations, wound/scab formation, lichenification, and xerosis; C) Functional impact based on pruritus (0 to 10) and sleep disturbance (0 to 10). Final score determined by SCORAD = A/5 + (7 x B/2) + C



VAS

Init:_____ ID # _____

Date: _____

Subject VAS:

Draw a vertical line across the present line to represent the average for the last 3 days or nights regarding how much itching and sleep loss you have experienced:

Itching:

Sleep Loss:

Eczema Area and Severity Index (EASI)

The EASI is used to assess the severity and extent of AD. Four AD disease characteristics will be assessed (0 = absent to 3 = severe). Area of AD involvement will be assessed as a percentage by involved body area of the head, trunk, arms, and legs, and converted to a score of 0 to 6.

EASI	Head & Neck	Upper Extremities	Trunk, Axillae and Genitals	Lower Extremities & Buttocks
Erythema				
Infiltration / Papulation				
Excoriation				
Lichenification				
Sum				
Area Score				
Sum x Area Score				
x	X .1	X .2	X .3	X .4
Total				

Area Score (of the respective body region):
0=No Eruption 1=< 10%, 2= 10%-29%; 3=30%-49%, 4 = 50% - 69%; 5= 70% - 89%, 6= 90% - 100%

Erythema	0 - None	
	1 – Mild	Faintly detectable erythema; very light pink
	2 – Moderate	Dull red, clearly distinguishable
	3 – Severe	Deep / dark red
Infiltration / Papulation	0 - None	
	1 – Mild	Barely perceptible elevation
	2 – Moderate	Clearly perceptible elevation but not extensive
	3 – Severe	Marked and extensive elevation
Excoriations	0 - None	
	1 – Mild	Scant evidence of excoriations with no signs of deeper skin damage (erosion,crust)
	2 – Moderate	Several linear marks of skin with some showing evidence of deeper skin injury (erosion,crust)
	3 – Severe	Many erosive or crusty lesions
Lichenification	0-None	
	1 – Mild	Slight thickening of the skin discernible only by touch and with skin markings minimally exaggerated
	2 – Moderate	Definite thickening of the skin with skin markings exaggerated so that they form a visible criss-cross pattern
	3 – Severe criss-	Thickened indurated skin with skin markings visibly portraying an exaggerated cross pattern

IGA (Investigator's Global Assessment)

IGA Severity	Features
4 – Severe Disease	Severe erythema and severe papulation/infiltration
3 – Moderate Disease	Moderate erythema and moderate papulation/infiltration
2 – Mild Disease	Mild erythema and mild papulation/infiltration
1 - Almost Clear	Just perceptible erythema and just perceptible papulation/infiltration
0 - Clear	No inflammatory signs of AD

Appendix 2. Subject Questionnaires (AASIS and AA-QLI)

ID# _____

Alopecia Areata Symptom Impact Scale (AASIS)

Alopecia areata is a condition that may affect you. Please rate how severe the following symptoms of your alopecia areata have been ***in the past week***. Please select one response from 0 (symptom has not been present) to 10 (the symptom was as bad as you can imagine it could be) for each item.

	Not Present									As bad as you can imagine
Scalp hair loss	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐
Body or eye lashes hair loss	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐
Tingling/numbness of the scalp	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐
Itchy or painful skin	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐
Irritated skin	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐
Feeling anxious or worry	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10
Feeling sad	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐

Your alopecia areata may interfere with your daily functioning. Please rate how the following items were interfered with by alopecia areata ***In the past week***: Please select one response from 0 (did not interfere) to 10 (interfered completely) for each item.

	Did not Interfere									Interfered completely
Work	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐
Enjoyment of life	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐
Interaction with others	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐
Daily activities	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐
Sexual relationships	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐
Quality of life	0☐	1☐	2☐	3☐	4☐	5☐	6☐	7☐	8☐	9☐ 10☐

Alopecia Areata Quality of Life Index (AA-QLI)

	Subjective symptoms		
1.	I feel uncomfortable using a wig	Very much A lot A little Not at all	☐ ☐ ☐ ☐
2.	I tend to hide my scalp with hats or bandages	Very much A lot A little Not at all	☐ ☐ ☐ ☐
3.	I am sad about the appearance of my hair/eyebrows/eyelashes	Very much A lot A little Not at all	☐ ☐ ☐ ☐
4.	I worry about having this hair problem for the rest of my life	Very much A lot A little Not at all	☐ ☐ ☐ ☐
5.	I cannot forget that I have this hair problem	Very much A lot A little Not at all	☐ ☐ ☐ ☐
6.	I worry that it might spread	Very much A lot A little Not at all	☐ ☐ ☐ ☐
7.	I do not take my wig/hat/bandana off in front of my partner/relatives/ friends	Very much A lot A little Not at all	☐ ☐ ☐ ☐
8.	It costs me a lot of money to look after my hair	Very much A lot A little Not at all	☐ ☐ ☐ ☐
9.	I am afraid my children may have alopecia areata	Very much A lot A little Not at all	☐ ☐ ☐ ☐
	Relationship		
10.	I feel that people find it unpleasant to look at me	Very much A lot A little Not at all	☐ ☐ ☐ ☐
11.	I think that other people notice my hair/eyebrows/eyelashes problem	Very much A lot A little Not at all	☐ ☐ ☐ ☐

12.	I am afraid that other people think my hair looks badly cared for	Very much A lot A little Not at all	☐ ☐ ☐ ☐
13.	I am embarrassed when going out to a party	Very much A lot A little Not at all	☐ ☐ ☐ ☐
14.	I have to explain to others what is wrong with my hair/eyebrows/eyelashes	Very much A lot A little Not at all	☐ ☐ ☐ ☐
15.	I feel that others are afraid of catching diseases from me	Very much A lot A little Not at all	☐ ☐ ☐ ☐
16.	I feel I have sexual difficulties because of alopecia areata	Very much A lot A little Not at all	☐ ☐ ☐ ☐
17.	I feel I have difficulties in establishing relationships with friends and/or relatives	Very much A lot A little Not at all	☐ ☐ ☐ ☐
18.	My work/studying has deteriorated because of alopecia areata	Very much A lot A little Not at all	☐ ☐ ☐ ☐
	Objective signs		
19.	My scalp is visible	Very much A lot A little Not at all	☐ ☐ ☐ ☐
20.	I lose tufts of hair when I comb or shampoo	Very much A lot A little Not at all	☐ ☐ ☐ ☐
21.	I feel itchy on my scalp	Very much A lot A little Not at all	☐ ☐ ☐ ☐

Amendment #1 Description

Page 1 – Amendment # 1 January 31, 2018 added

Page 2 – Table of Contents added

Page 7, 3rd paragraph – EG changed to Dr. Guttman and sentence clarified.

Page 10, last paragraph (Study Design Overview) – Added "The study will take place at two sites: Icahn School of Medicine at Mount Sinai, and Rockefeller University." Also "(20 out of 60 subjects)" was added for clarification of the number of subjects allowed to have AA and AD.

Page 11, 1st paragraph – Added "(30 out of 60 subjects)" to clarify number of subjects allowed to have AT or AU.
Added "Once the required number of subjects in each of these sub-categories is met, additional subjects in those categories will not be enrolled" for clarification.

Page 11, 4th and 5th paragraph – Description regarding clinical assessments was split into two types to distinguish between those for all subjects (AA) and those for the subset of subjects with AA + AD: "clinical assessments (SALT, and eyebrow/eyelash assessment score), and additional skin assessments if he/she concurrently has atopic dermatitis (EASI, IGA, SCORAD)".

Page 12, 1st paragraph – Size of biopsy was corrected to 4.5 mm (which matches consent form).

Page 12, 2nd and 3rd paragraph – sections clarified regarding the loading dose and all other doses.

Page 12, 5th paragraph (Independent Expert Assesment) - Dr Norris will not approve screening photos as both PIs are experts in diagnosing AA, thus that statement has been deleted. Dr. Norris will still review photographs of those subjects that consent to them, to assess improvement/progress during the study.

Pages 17-21 - Description regarding clinical assessments was split into two types to distinguish between those for all subjects (AA) and those for the subset of subjects with AA + AD: "clinical assessments (SALT, and eyebrow/eyelash assessment score), and additional skin assessments if he/she concurrently has atopic dermatitis (EASI, IGA, SCORAD)".

Page 17 – Standardized Clinical Photography deleted from Screening visit and added to Baseline visit.

Page 19 Visit 8 – Added for clarification regarding Week 24 injections "(subjects

previously on dupilumab will receive 1 injection of 300 mg dupilumab and 1 injection of placebo to maintain the blind of the study; subjects previously on placebo will receive 2 injections of 300 mg dupilumab). All subjects should be dupilumab). All subjects should be observed for 30 minutes following the injection to preserve the blind.

Page 21 – Added section:

LABORATORY TESTING:

Each site may utilize their own local lab for CBC, CMP, LDH, CRP, IgE, HIV, HBV, HCV, Quantiferon Gold, and pregnancy testing. Lab certifications for any lab used should be kept in the study binder. RU will have to transport biopsy samples as well as blood samples for mechanistic testing (and potentially IgE testing) to Mount Sinai via proper and documented means.

Page 22 – (Study Withdrawal) - Description regarding clinical assessments was split into two types to distinguish between those for all subjects (AA) and those for the subset of subjects with AA + AD: “clinical assessments (SALT, and eyebrow/eyelash assessment score), and additional skin assessments if he/she concurrently has atopic dermatitis (EASI, IGA, SCORAD)”.

Page 24 after first bullet – added “Duration of study drug suspension should be reviewed on a case by case basis and can be discussed among the PIs” in cases of temporary discontinuation of drug.

Page 25 Added (or similar) in reference to the website used to create the randomization code.

Page 26, 1st paragraph – added for clarification: “At Week 24, subjects previously on dupilumab will receive 1 injection of 300 mg dupilumab and 1 injection of placebo to maintain the blind of the study; subjects previously on placebo will receive 2 injections of 300 mg dupilumab. All subjects should be observed for 30 minutes following the injections at Baseline and at Week 24.”

Page 28, 2nd paragraph – Typo corrected as well as clarifications made, and statement added regarding the sharing of photos and results with Dr Norris.

Page 33 and 34 (Schedule of Events) – Clinical assessments split into multiple rows for clarification; corrections as to when specific procedures are done; and clarifications made to the footnotes.

Page 35-38 Additional calculation tables added to descriptive details regarding assessments.

Page 39-41 Added “Appendix # 2 Subject Questionnaires (AASIS and AA-QLI)

Page 42 – Added Amendment # 1 Description.

Amendment #2 Description

Page 24 Unblinding Section Paragraph 2 – "the data safety monitoring board" was deleted as there is no DSMB for this study. It had been originally included in error.

Amendment #3 Description

Page 1 – Jason Hawkes was removed as a co-investigator and Protocol Version was added: January 08, 2020 Amendment # 3

Pages 9, 10 and 11 Endpoints - Statistician reviewed protocol and recommended some revisions to the endpoints. Primary endpoint was revised to only compare Week 24 to Baseline instead of comparing both Week 24 and 48 to Baseline as Week 24 ends the blinded placebo controlled phase. Three secondary endpoints evaluating lower improvements in SALT scores were deleted. Second mechanistic endpoint analyzing relapse was removed.

Page 27 and 28 Statistical Consideratons – The statisitical analyses sections were updated to correlate with the updated Primary and Secondary Endpoints. The sections defining Analysis of Mechanistic and Exploratory Endpoints have been expanded upon and updated with the most recent information available to date.

Page 13 Study Design Overview – The option for subjects to seek other treatments (or obtain commercially available dupilumab) at Week 48 was added.

Page 17 – Concomitant Treatment - If subjects seek other treatments (or obtain commercially available dupilumab) at Week 48, then these treatments must be documented as concomitant medications.