A randomized placebo-controlled single center pilot study of the safety and efficacy of Apremilast in subjects with moderate to severe alopecia areata
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HYPOTHESES:

Primary Hypothesis:

1. It is hypothesized that apremilast is superior to placebo for induction of hair regrowth in patients with moderate to severe alopecia areata (AA).

OBJECTIVES and ENDPOINTS:

Primary objective:

1) To study the efficacy of apremilast for induction of hair regrowth in patients with moderate to severe AA.

Secondary objectives:

1) To study the safety of apremilast in patients with moderate to severe alopecia areata.
2) To study the mechanism of action of apremilast in patients with moderate to severe alopecia areata.

Primary endpoints:

1) Percentage of patients achieving 50% or greater improvement in their Severity of Alopecia Tool (SALT) score ($SALT_{50}$) at Week 24 compared to Baseline.

Secondary endpoints:

1) Percentage change in SALT score at Week 24, 48.
2) Proportion of subjects achieving an alopecia areata Physician's Global Assessment (aaPGA) score of 3 or above at Weeks 24 and 48 (0, no regrowth; 1, <25% of regrowth; 2, 25%-49% of regrowth; 3, 50%-74% of regrowth; 4, 75%-99% of re- growth; 5, 100% of regrowth).
3) Percentage change from Baseline in the Alopecia Areata Symptom Impact Scale (AASIS) at Weeks 24 and 48.
4) Percentage change from baseline in the Alopecia Areata Quality of Life questionnaire (AA-QoL) at Weeks 24 and 48
5) Semiquantitative 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) at week 24 and 48.

Mechanistic (exploratory) endpoint:

1) Change from baseline in cellular, and molecular markers in skin biopsies after treatment.

INTRODUCTION:

Alopecia areata (AA) is an auto-immune disease characterized by non-scarring hair loss that can sometimes progress to total loss of all scalp and body hair.[1] The life-time risk of AA has been reported to be 1.7%.[2] AA can cause tremendous emotional and psychosocial distress in affected patients and their families. There is a lack of effective treatments for patients with alopecia areata. Topical treatments are usually not very effective, probably because of limited penetration. Intralesional injections of corticosteroids are effective but can only be considered for patients with limited involvement. Immunotherapy with diphencyprone or squaric acid dibutyl is effective in some patients but is usually limited to treatment of scalp involvement and can cause significant pruritus and dermatitis. None of these therapies are targeted to the underlying mechanism for disease pathogenesis. There is a need for new treatment options for patients with alopecia areata especially based on the recently evolving understanding of the disorder.

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. While the etiology of AA remains unknown, studies have suggested infiltration of CD4+ and CD8+ T cells and a predominant Th1 cytokine profile in the etiology of this disease, leading to destruction of the hair follicle immune privilege site.

During the last decade, interleukin (IL)-23 has been recognized as essential in the development of multiple autoimmune diseases, including psoriatic disease, vitiligo, inflammatory bowel disease, and autoimmune encephalomyelitis.[3, 4]
The pathogenesis of alopecia areata has also been linked to IL-17, which is produced by Th17 cells. In particular, studies have shown the IL-17 RA gene polymorphism to be associated with increased susceptibility to alopecia areata in the Korean population.[5]
Early study findings have demonstrated markedly elevated IL-17 levels in blood from patients with alopecia areata (unpublished but presented at the EADV in 2013). IL-23 is known to regulate IL-17 production and to promote the expansion of Th17 cells.[6]
Increased levels of the cytokine IL-12 have been sown in both serum and lesional skin of AA patients.[7] Furthermore, preliminary analyses from our laboratory (Fig. 1) showed an elevation of IL-12 mRNA expression by RT PCR. Our analyses have further shown that p40, IL-13, IFN-γ, IL-12RB1, IL-22, JAK3 are all significantly induced in AA ($p<0.05$) in a similar fashion as in AD when comparing lesional with nonlesional skin.

Apremilast is an oral small molecule phosphodiesterase-4 (PDE4) inhibitor that has been shown to regulate inflammatory mediators.[8] Apremilast enters cells by passive diffusion and, once intracellular, binds PDE4. PDE-4, the dominant phosphodiesterase expressed in immune cells, degrades cyclic AMP (cAMP) into AMP. PDE4 inhibition thereby elevates intracellular cAMP, which can down-regulate the inflammatory responses such as TNF-α, IFN-γ, interleukins (IL) 2, 12, 17, and 23 through mechanisms such as partially inhibiting expression of inflammatory cytokines and increasing expression of anti-inflammatory mediators such as IL2 and IL10.[8] Apremilast further downregulates target organ MHC class II expression.[9] A recent pilot study demonstrated that apremilast suppresses experimentally induced AA in a humanized mouse model.[10] We therefore hypothesize that apremilast may have beneficial effects in AA. The U.S. Food and Drug Administration (FDA) approved apremilast for the treatment of adult patients with active psoriatic arthritis in March 2014, and for patients with moderate-to-severe plaque psoriasis in September 2014.

Figure 1

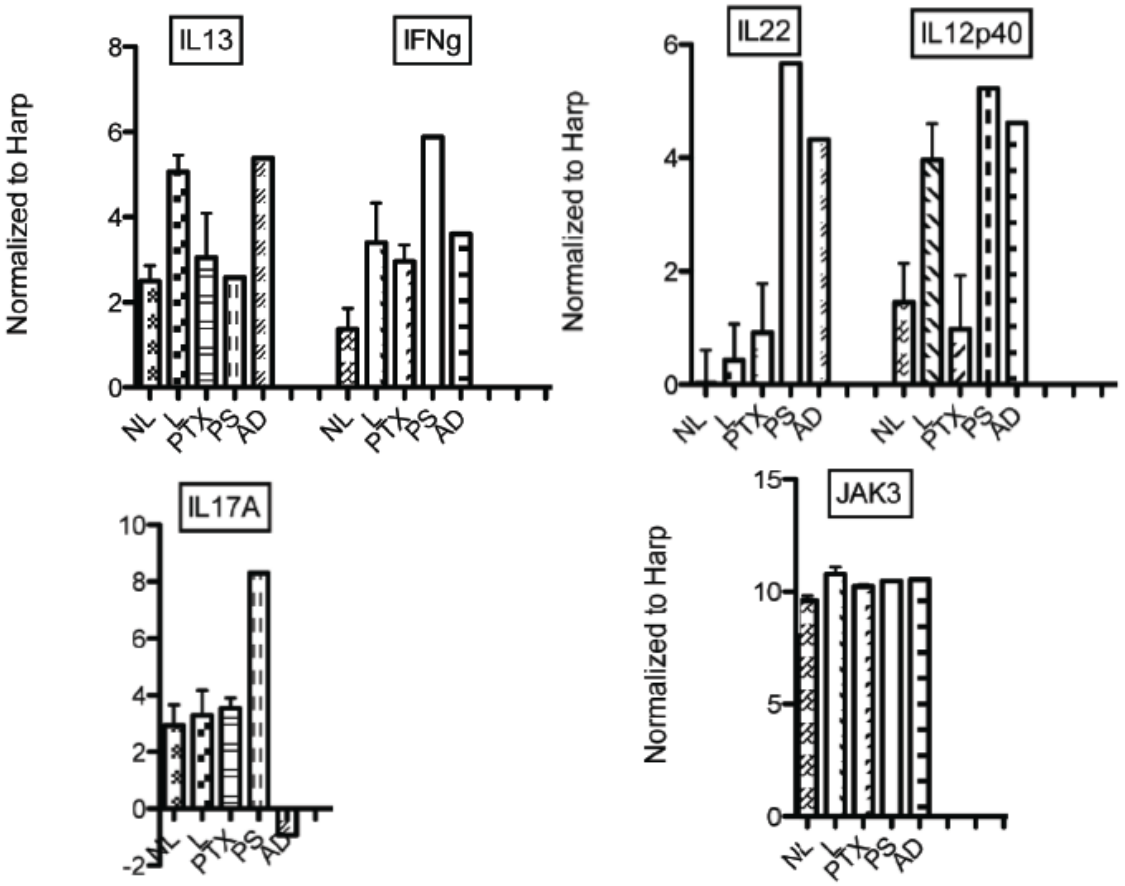

Fig 1. Figure 1 shows comparable cytokine expression of the defining Th2 cytokine/IL-13, the defining Th1 cytokine/IFN gamma, and the Th17/IL-23 cytokines/IL-17A and IL12/23p40 in AA and AD. Lesional psoriasis skin tissues show a significantly higher expression of both IL-17A and IFN gamma, with significantly lower expression of IL-13 as compared with both AA and AD. Significant decreases in mRNA expression of IL-13, and IL12/23p40 are seen after treatment with intralesional corticosteroids, without significant changes in the other cytokines. P value= 0.0002 for the IL-13, and p=0.1 for the IFN gamma lesional vs non-lesional alopecia skin comparisons. JAK3 shows similar activation in AA, AD, and Psoriasis. NL- non lesional skin from scalp of AA patients; L-lesional skin from scalp of AA patients; PTX- post intralesional steroid treatment skin from scalp of AA patients; PS-psoriasis lesional skin; AD- AD lesional skin. N=6 patients for both NL and L, and 4 for PTX.

STUDY DESIGN:

This is a randomized, double-blind, placebo-controlled pilot study consisting of two phases. A total of 30 subjects with moderate to severe alopecia areata (including universalis and totalis) involving 50-100% of the scalp will be enrolled. A possible maximum of 15 patients (approximately 7 patients each) with current episodes of AA totalis / universalis may be included in this study.

In Phase 1, subjects will be randomized (2:1) to either receive apremilast or placebo for 24 weeks.

In Phase 2, eligible subjects will receive apremilast from Week 24 through Week 48. The following subjects will be eligible to enter into Phase 2:

1. Subjects who received placebo in Phase 1 of the study
2. Subjects who received apremilast in Phase 1 of the study, and who achieved any ~~minimum of 50%~~ regrowth ~~($SALT_{50}$)~~ at Week 24, compared to Baseline.

After providing informed consent, subjects will be assessed for study eligibility at the Screening visit (day -28 to day -1), which includes limited physical examination, assessment of regrowth pattern, SALT scoring, review of medical history and concomitant as well as prior medications/treatments, and serum pregnancy test (if applicable). Laboratory tests will be performed for complete Blood Count (CBC) and white blood cell count (WBC) with differentials (basophils, eosinophils, lymphocytes, monocytes, neutrophils), serum chemistry for albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatinine, potassium, sodium and total bilirubin, as well as hepatitis B surface antigen (HbsAg) and hepatitis C antibody.

Subjects who meet eligibility criteria will undergo Baseline / Day 0 assessments. These assessments include hair examination, SALT scoring, aaPGA scoring, review of concomitant medications, clinical photography, questionnaires (AASIS and AA-QoL), and urine pregnancy test (if applicable). Blood samples will be collected for mechanistic studies, DNA analysis and RNA and proteomic analysis. Two biopsies of the scalp will be performed: one from an area affected with alopecia areata and one from an area that is not affected. If the subject has 100% hair loss, then only one biopsy from an affected area will be performed. At this Baseline visit, subjects will undergo randomization, and will receive the first oral dose of study drug (apremilast or placebo).

Subjects will be provided with the study drug, and will receive twice daily (BID) dosing of study drug (apremilast or placebo orally) through Week 24.

Subjects will return for visits every four weeks through Week 24 (Weeks 4, 8, 12, 16, 20 and 24) so that a review of concomitant medications and adverse events can be assessed. Females of childbearing potential will undergo a urine pregnancy test at each of these visits. Additional study drug will be dispensed every 4 weeks. In addition, the following procedures will be collected at various time points:

Week 4: assessment of regrowth pattern, SALT scoring, aaPGA scoring.

Week 8: assessment of regrowth pattern, SALT scoring, aaPGA scoring, and clinical photography.

Week 12: assessment of regrowth pattern, SALT scoring, aaPGA scoring, clinical photography, blood tests (for mechanistic studies, Pax RNA and proteomic analysis), and questionnaires (AASIS and AA-QoL). One optional biopsy of the scalp (close to the involved area that was biopsied at Baseline) may be performed.

Weeks 16 and 20: assessment of regrowth pattern, SALT scoring, aaPGA scoring.

Week 24: limited physical examination, assessment of regrowth pattern, SALT scoring, aaPGA scoring, clinical photography, questionnaires (AASIS and AA-QoL), blood tests (for mechanistic studies, RNA and proteomic analysis), and one scalp biopsy will be performed (in the vicinity of the involved area biopsied at Baseline).

At the Week 24 study visit, eligible subjects will be allowed to enter into Phase 2 of the study, in which all eligible subjects will receive apremilast. Subjects who received placebo in Phase 1 of the study, will start apremilast as per appropriate titration dose. Subjects who received apremilast in Phase 1, and who achieved any regrowth compared to Baseline will enter into Phase 2 and will continue their current dose of apremilast (30 mg twice daily). Subjects who received apremilast in Phase 1 and showed no regrowth will be discontinued from the study.

Subjects continuing in Phase 2 will return to the site every 4 weeks through Week 52 (Weeks 28, 32, 36, 40, 44, 48 and 52) so that a review of concomitant medications and adverse events can be assessed. Females of childbearing potential will undergo a urine pregnancy test at each of these visits (except Week 52). In addition, the following procedures will be collected at various time points:

Weeks 28 and 32: assessment of regrowth pattern, SALT scoring, aaPGA scoring and clinical photography.

Week 36: assessment of regrowth pattern, SALT scoring, aaPGA scoring, clinical photography, and questionnaires (AASIS and AA-QoL).

Weeks 40 and 44: assessment of regrowth pattern, SALT scoring, aaPGA scoring and clinical photography.

Week 48: limited physical examination, assessment of regrowth pattern, SALT scoring, aaPGA scoring, clinical photography, and questionnaires (AASIS and AA-QoL).

Subjects will end treatment with apremilast at Week 48 and will be asked to return for a follow-up visit at Week 52. At this visit, subjects will undergo assessment of regrowth pattern, SALT scoring, aaPGA scoring, and assessment of any adverse events and change in concomitant medications.

If a subject terminates early or chooses to discontinue the study, he/she will be asked to

return for a final visit, which would include: limited physical examination, examination of hair, SALT scoring, aaPGA scoring, clinical photography and questionnaires (AASIS and AA-QoL). If the subject terminates or discontinues prior to Week 24, then the Early Termination Visit will also include blood tests (for mechanistic studies, RNA and proteomic analysis) and one scalp biopsy (in the vicinity of the involved area biopsied at Baseline).

Subjects will not receive any other active treatment for alopecia areata (outside of the study drug) while participating in the study.

Apremilast will be provided as 10-, 20-, or 30-mg tablets. Apremilast will be taken orally twice daily, approximately 12 hours apart. Apremilast can be administered without regard to meals, and tablets should not be crushed, split, or chewed.
When subjects first start to take apremilast, the dose will be titrated in 10-mg/day increments. Following the 5-day titration, the recommended maintenance dosage is 30 mg twice daily taken orally starting on Day 6. This titration is intended to reduce the gastrointestinal symptoms associated with initial therapy.

Day 1: 10 mg in morning
Day 2: 10 mg in morning and 10 mg in evening
Day 3: 10 mg in morning and 20 mg in evening
Day 4: 20 mg in morning and 20 mg in evening
Day 5: 20 mg in morning and 30 mg in evening
Day 6 and thereafter: 30 mg twice daily

Safety will be assessed by evaluation of adverse events, physical examinations and laboratory parameters. Safety, laboratory, and clinical assessments will be performed at specified clinic visits. A serum pregnancy test will be performed at Screening and urine pregnancy tests will be performed at Baseline and every 4 weeks thereafter, in females of childbearing potential.

Efficacy will be evaluated using the Severity Alopecia Tool (SALT)[11] score, and the alopecia areata Physician Global Assessment (aaPGA). The SALT score is a mathematical approach to the determination of hair loss and hair regrowth.[12] The percentage of scalp hair loss in each of the sides, back and top of the scalp are determined independently, and are multiplied by the percentage scalp covered in that area of the scalp and the products of each section summed for a final total percentage hair loss, designated as the SALT score. (Appendix 1).

Alopecia areata Physician Global Assessment (aaPGA): a static PGA score is widely used as a primary efficacy point of treatment success in many studies of dermatological diseases. The static aaPGA score represents an overall static evaluation of the alopecia, performed by the investigator at each visit. It utilizes a scale of 6-points, ranging from 0 (no regrowth) to 5 (100% regrowth), with 0:no regrowth; 1:<25% of regrowth; 2: 25%-49% of regrowth; 3:50%-74% of regrowth; 4:75%-99% of re- growth; 5:100% of regrowth). (Appendix 2).

Quality of life will be evaluated using the Alopecia Areata Symptom Impact scale (AASIS)[13] and the Alopecia Areata Quality of **L**ife Index (AA-QLI).[14] (Appendices 3 and 4).

Skin biopsies from the scalp will be performed on all subjects at Baseline and Week 24 or Early Termination visit (if prior to Week 24). At Baseline, biopsies will be obtained from involved and uninvolved areas of the scalp (except in the case of 100% involvement, when only 1 biopsy of involved scalp will be biopsied). At Week 24 or Early Termination visit, the biopsy will be performed in the vicinity of the involved area biopsied at baseline. An optional biopsy will be performed at Week 12, close to the area that was involved at baseline. All skin biopsies will be 4.5 mm in diameter.

At Baseline, serum will be obtained for DNA (1 PaxGene), RNA (2 PaxGene), and proteomic analysis (2 tubes serum). Studies of RNA, and proteomic analysis will further be performed at Weeks 12 and 24. Serum levels of various biomarkers such as interferon gamma, IL-2, IL-12, IL-13, IL-17A, IL-5, IL-10, IL-31 and IL-4 have been shown to be elevated in patients with alopecia areata. Thus, for mechanistic research purposes, we will assess Th1 (interferon gamma) and Th2 (IL-13) cytokines, as well as a panel of ten Th1 and Th2 chemokines (CXCL9, CXCL10, CCL17, CCL18, CCL22, CCL26, CCL2, CCL3, CCL4, CCL5) (using MSD) in serum before and after apremilast treatment.

INCLUSION CRITERIA:

Subjects must satisfy the following criteria to be enrolled in the study:

1. Males or females, 18 years or older at the time of signing the informed consent document.

2. Understand and voluntarily sign an informed consent document prior to any study-related assessments/procedures are conducted.

3. Able to adhere to the study visit schedule and other protocol requirements.

4. Subject with a diagnosis of patchy scalp alopecia areata present for at least 6 months, and up to a maximum of 10 years.

5. Patients with ≥50% and <95% total scalp hair loss at Baseline as measured using the SALT score to qualify as moderate to severe AA; and 95%-100% scalp hair loss to qualify as AA totalis/universalis.

6. 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).

7. 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:

   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

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

8. Male subjects (including those who have had a vasectomy) who engage in activity in which conception is possible must use barrier contraception (male latex condom or nonlatex condom NOT made out of natural [animal] membrane [for example, polyurethane]) while on IP and for at least 28 days after the last dose of IP.

9. No evidence of hair regrowth present at Baseline.

EXCLUSION CRITERIA:

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

1. Clinically significant (as determined by the investigator) cardiac, endocrine, pulmonary, neurologic, psychiatric, hepatic, renal, hematologic, or immunologic disease, or other major uncontrolled diseases that will affect the health of the subject during the study or interfere with the interpretation of study results.

2. Hepatitis B surface antigen positive at Screening (Visit 1).

3. Hepatitis C antibody positive at Screening (Visit 1).

4. History of positive human immunodeficiency virus (HIV), or congenital or acquired immunodeficiency (eg, Common Variable Immunodeficiency [CVID]). Subjects deemed at risk by the study investigator, may also undergo testing for

human immunodeficiency virus (HIV). Subjects deemed at risk include those with a history of injection drug use, homosexual subjects, and subjects with known sexual contact with an HIV positive partner.

5. Active TB or a history of inadequately treated TB.

6. Active substance abuse or a history of substance abuse within six months prior to Screening.

7. Pregnant or breast feeding.

8. History of allergy to any component of the IP.

9. Major surgery within eight weeks prior to Screening (Visit 1) and/or planned surgery during the length of the study.

10. Malignancy or history of malignancy, except for:

    a. treated (ie, cured) basal cell or squamous cell in situ skin carcinomas;

    b. treated (ie, cured) cervical intraepithelial neoplasia (CIN) or carcinoma in situ of the cervix with no evidence of recurrence within 5 years prior to Screening (Visit 1).

11. Unstable asthma (eg, acute episodes of exacerbation [nocturnal episodes, sudden episodes triggered by unidentifiable factors] despite a stable regimen of anti-asthmatic medications); prior episode(s) of life-threatening asthma; or asthma that requires inhaled budesonide or equivalent at >1200 µg/day or fluticasone propionate at > 880 µg/day along with another anti-asthmatic drug such as a long-acting beta-agonist.

12. A history of and/or concurrent condition of serious hypersensitivity (eg, anaphylaxis) to drugs, foods, or other allergens without access to emergency rescue medication such as epinephrine.

13. Persistent or recurring bacterial infection requiring systemic antibiotics, or clinically significant viral or fungal infections, within two weeks of Screening (Visit 1). Any treatment for such infections must have been completed at least two weeks prior to the Screening Visit and no new/recurrent infections should have occurred prior to the Baseline Visit.

14. Active skin infection requiring systemic antimicrobials at Baseline/Randomization (Visit 2).

15. Skin lesion(s) due to conditions other than AA that would interfere with the study specified assessments.

16. Prior treatment with apremilast, or participation in a clinical study involving apremilast.

17. Use of phototherapy (ie, UVB, UVA) or systemic immunosuppressive drugs (including, but not limited to, cyclosprine, corticosteroids, mycophenolate mofetil, azathioprine, Methotrexate, or tacrolimus), or oral preparations of herbal immunomodulatory medications within four weeks prior to Baseline/Randomization (Visit 2).

18. Use of interferon-γ within 12 weeks prior to Baseline/Randomization (Visit 2).

19. Use of abatacept, adalimumab, certolizumab pegol, etanercept, golimumab, infliximab, or tocilizumab within 12 weeks prior to Baseline/Randomization (Visit 2).

20. Use of oral janus kinase (JAK) inhibitors (e.g. tofacitinib, ruxolitinib) within 12 weeks prior to Baseline/Randomization (Visit 2).

21. Use of omalizumab, rituximab, ustekinumab, alefacept, briakinumab, or other therapeutic antibody products within 24 weeks prior to Baseline/Randomization (Visit 2).

22. Use of any investigational drug within four weeks or five PK or PD half lives (whichever is longer) prior to Baseline/Randomization (Visit 2).

23. Use of topical corticosteroid preparations, topical calcineurin inhibitors, or other topical preparations with immunomodulatory properties within 2 weeks prior to Baseline/Randomization (Visit 2).

24. Prior history of suicide attempt at any time in the subject's lifetime prior to Baseline (Visit 2) or major psychiatric illness requiring hospitalization within 3 years prior to Baseline (Visit 2).

25. History of male or female pattern hair loss Ludwig stage III or Hamilton > stage V.

26. Patients in whom the diagnosis of alopecia areata is in question, or subjects with scarring alopecia.

RISKS OF APREMILAST

As of 10 May 2014, apremilast has been given to about 5200 subjects (people). The following are the most commonly seen risks, discomforts and side effects in subjects who have taken apremilast: headache including tension headache, stuffiness or infections of the nose and throat (upper respiratory tract infections including nasopharyngitis), stomach upset (nausea), vomiting and diarrhea. Most of these side effects were mild to moderate in intensity and resolved with continued treatment.

Similar side effects have been observed in studies that are running now. About 8 out of every 100 subjects treated have discontinued the study drug because of side effects.

The following side effects are the ones that may be associated with the use of apremilast:

- **Very common:** diarrhea, nausea (stomach upset), vomiting.
- **Common:** Upper abdominal (stomach) pain, indigestion, frequent bowel movement, heartburn, fatigue, bronchitis (infection of the tubes to the lungs), redness/swelling/pain in the sinuses, inflammation or infections of the nose and throat, weight loss, decreased appetite, back pain, headache (including tension and migraine), difficulty sleeping, depression, cough, rash, dizziness, weakness, flu, muscle pain, numbness, itchiness.
- **Uncommon:** allergic reaction.

Reports of various types of cancers, heart problems, and serious infections have been found from apremilast studies. However, these events in patients being treated with apremilast happened as often as those being treated with placebo (sugar pill).

Drugs in the same family as apremilast have been shown to produce inflammation around the vessels of the skin (vasculitis) in rats and mice. Skin vasculitis has been rarely reported equally in patients taking apremilast or placebo.

Depression and weight loss have been reported with the use of apremilast, thus, subjects will be questioned about any mood changes and weight loss at each visit.

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.

The Principal Investigator will provide safety data to an independent Data and Safety Monitoring Board every 12 months. This DSMB will be comprised of board certified dermatologists who are not directly related to this study. The DSMB will review the data and provide a report of their findings to the Principal Investigator.

EARLY TERMINATION

Any individual whose health or well being may be threatened by continuation in this study will be discontinued by the investigator.

If a female subject becomes pregnant at anytime during the study, she will be discontinued immediately. She will be asked to provide the investigator with medical updates throughout her pregnancy and on the final outcome of the pregnancy.

Any subject who chooses to discontinue the study, or is discontinued from the study by the investigator will be asked to return for a final visit to have some or all of the following procedures: limited physical examination; blood test; pregnancy test; clinical photography; assessment of regrowth pattern; hair evaluation; SALT assessment; aaPGA; questionnaires (AASIS and AA-QoL); assessment of concomitant medication and adverse events; biopsy of scalp.

ADVERSE EVENTS

An adverse event (AE) is any noxious, unintended, or untoward medical occurrence occurring at any dose that may appear or worsen in a subject during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values (as specified by the criteria below), regardless of etiology. Any medical condition that was present prior to study treatment and that remains unchanged or improved should not be recorded as an AE. If there is a worsening of that medical condition this should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the Case Report From rather than the individual signs or symptoms of the diagnosis or syndrome.

All AEs will be recorded by the Investigator(s) from the time of signing the informed consent through the end of the designated follow-up period.

Abnormal laboratory values defined as adverse events

An abnormal laboratory value is considered to be an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study.
- Requires treatment, modification/interruption of study drug dose, or any other therapeutic intervention.
- Is judged by the Investigator(s) to be of significant clinical importance.

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE.

Serious adverse event

A serious adverse event (SAE) is any AE which:

- Results in death
- Is life-threatening (i.e., in the opinion of the Investigator(s) the subject is at immediate risk of death from the AE)
- Requires inpatient hospitalization or prolongation of existing hospitalization

- Results in persistent or significant disability/incapacity (a substantial disruption of the subject's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect
- Constitutes an important medical event

Important medical events are defined as those occurrences that may not be immediately life threatening or result in death, hospitalization, or disability, but may jeopardize the subject or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

Events not considered to be SAEs are hospitalizations which: were planned before entry into the clinical study; are for elective treatment of a condition unrelated to the studied indication or its treatment; occur on an emergency outpatient basis and do not result in admission (unless fulfilling other criteria above); are part of the normal treatment or monitoring of the studied indication and are not associated with any deterioration in condition.

If an AE is considered serious, both the AE pages of the CRF and the SAE Report Form must be completed.

For each SAE, the Investigator(s) will provide information on severity, start and stop dates, relationship to study drug, action taken regarding study drug, and outcome.

Classification of severity

For both AEs and SAEs, the investigator(s) must assess the severity of the event. The AEs will be evaluated for severity according to the following scale:

Grade 1 = Mild

Grade 2 = Moderate

Grade 3 = Severe

Classification of Relationship/Causality of adverse events (SAE/AE) to study drug

The Investigator(s) must determine the relationship between the administration of study drug and the occurrence of an AE/SAE as Not Suspected or Suspected as defined below:

Not suspected:	The temporal relationship of the adverse event to study drug administration makes **a causal relationship unlikely or remote**, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event
Suspected:	The temporal relationship of the adverse event to study drug administration makes **a causal relationship possible,** and other medications, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.

Immediate reporting of serious adverse events

Any AE that meets the any criterion for a SAE requires the completion of an SAE Report Form in addition to being recorded on the AE pages of the CRF. The Investigator(s) is required to ensure that the data on these forms is accurate and consistent. This applies to all SAEs, regardless of relationship to study drug, that occur during the study, those made known to the Investigator(s) within 30 days after a subject's last dose of study drug, and those made known to the investigator(s) at anytime that are suspected of being related to study drug.

The SAE must be reported immediately (i.e., within 24 hours of the Investigators' knowledge of the event) to Celgene Safety by facsimile or email. A written report (prepared by the Investigator(s) using an SAE Report Form or a 3500A Medwatch form is to be faxed to Safety (see below for contact information).

Celgene Drug Safety Contact Information:

Celgene Corporation
Global Drug Safety and Risk Management
Connell Corporate Park
300 Connell Dr. Suite 6000
Berkeley Heights, NJ 07922
Fax:(908) 673-9115
E-mail: drugsafety@celgene.com

The SAE report should provide a detailed description of the SAE. If a subject has died and an autopsy has been performed, copies of the autopsy report and death certificate are to be sent to Celgene as soon as these become available. Any follow-up data will be detailed in a subsequent SAE Report Form or Medwatch form and sent to Celgene.

The Investigator(s) is responsible for informing the Institutional Review Board/Ethics Committee (IRB/IEC) of the SAE and providing them with all relevant initial and follow-up information about the event. The Investigator(s) must keep copies of all SAE information, including correspondence with Celgene and the IRB/IEC, on file. All SAEs that have not resolved upon discontinuation of the subject's participation in the study must be followed until either the event resolves completely, stabilizes, resolves with sequelae, or returns to baseline (if a baseline value is available).

Contraception Education

The risks to a fetus or to a nursing child from apremilast are not known at this time. Results of the animal and in vitro studies can be found in the IB.

All females of childbearing potential (FCBP) must use one of the approved contraceptive options as described under inclusion criterion # 7 while on investigational product and for at least 28 days after administration of the last dose of the investigational product. Male subjects (including those who have had a vasectomy) who engage in activity in which conception is possible must adhere to inclusion criterion # 8 while on investigational product and for at last 28 days after administration of the last dose of investigational product.

When a subject's contraceptive methods or ability to become pregnant changes at any time during the study, the Investigator will educate the subject regarding options and correct and consistent use of effective contraceptive methods in order to successfully prevent pregnancy.

Pregnancies

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject or the female partner of a male subject occurring while the subject is on study drug, or within 30 days of the subject's last dose of study drug, are considered immediately reportable events. Study drug is to be discontinued immediately and the subject instructed to return any unused portion of the study drug to the investigator(s). The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Celgene Safety immediately via facsimile or email using the Pregnancy Report form provided by Celgene.

The female should be referred to an obstetrician-gynecologist experienced in reproductive toxicity for further evaluation and counseling.

The Investigator(s) will follow the female subject until completion of the pregnancy, and must notify Celgene Safety of the outcome of the pregnancy as a follow-up on the follow up Pregnancy Reporting form.

If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., spontaneous or therapeutic abortion [any congenital anomaly detected in an aborted fetus is to be documented], stillbirth, neonatal death, or congenital anomaly [including that in an aborted fetus]), the Investigator(s) should follow the procedures for reporting SAEs (i.e., report the event to Celgene Safety by facsimile within 24 hours of the Investigator's knowledge of the event).

In the case of a live "normal" birth, Celgene Safety should be advised by facsimile within 24 hours of the Investigator's knowledge of the event.

All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the Investigator(s) suspects is related to the in utero exposure to the study drug should also be reported to Celgene Safety by facsimile within 24 hours of the Investigators' knowledge of the event.

If the female is found not to be pregnant, any determination regarding the subject's continued participation in the study will be determined by the Investigator(s).

Proposed Mechanistic studies:

In order to identify the molecular and cellular profiles of alopecia areata at Baseline, and identify treatment response biomarkers, we plan to perform lesional (LS) and nonlesional (NL) 4.5 mm punch biopsies at Baseline and after 24 weeks of treatment (LS only) as above (from the same area previously biopsied, but not from the scar).

Gene expression studies (RT-PCR and gene arrays), and immunohistochemistry will be performed.

The expression levels of markers of the different immune pathways, including: Th1 (IFN-gamma, CXCL9, CXCL10, MX1, IL-12RB1, IL-12RB2), Th17 (IL-17A, IL23p19, IL23p40, CCL20, CXCL1, elafin/PI3), Th2 (IL-13, IL-4, IL-5, IL-10, CCL17, CCL18, CCL22, CCL5), T22/IL-22 cytokine and IL-17/IL-22 regulated S100s genes (S100A12), Th9/IL-9, and general T-cell activation (IL-2, IL-15, IL-2RA, IL-15RA), JAK3, as well as IL-16, and IL-32, and keratin associated genes (Keratins 35, 75, 85, 86), will be evaluated using RT-PCR. We will also assess for modulation of inflammatory markers (i.e MMP12), and innate immune genes (IL-1b, IL-8) by RT-PCR.

Gene arrays will also be performed. The Affymetrix U133A Plus 2 gene array platform will be used.

Skin infiltration by T-cells, DCs, and Langerhans cells will be assessed by immunohistochemistry. The following markers will be used: CD3 and CD8 for T cells, Langerin for Langherans cells, CD11c for myeloid DCs. A similar score to ALADIN will be used, however, taking into account more hair and keratin biomarkers.

STATISTICAL ANALYSES:

Sample size considerations

This is an exploratory study therefore the sample size has been kept relatively low. Data collected from this study will be used to obtain estimates of differences and variability of the study outcomes. Therefore there will be no formal hypothesis testing and power calculation in this study.

Nevertheless, consider the primary outcome is the % of patients who achieve a 50% improvement in their SALT score after 24 weeks of treatment, with a sample size of 20:10 on drug and placebo groups respectively will allow to detect approximately 40% superiority. This calculation is based on the use of a one-tail Fisher's exact test at alpha=0.05 level, 80% power and was obtained with the software Gpower.

Impact of the sample size on the biomarkers outcomes: Gulati et.al. (2014))[3] reported a standard deviation equal to 3.45 for IFNg's $\log_2$FCH between baseline and after DPCP treatment at Week 14 in a cohort of 11 patients. Assuming this standard deviation, the sample size of 20 patients allows us to detect an effect size of 0.87 (which corresponds to a $\log_2$FCH larger than 3) with power of 95% in a 95% two-side t-Test for paired samples. Considering Bonferroni correction for 5 biomarkers, the same effect size could be detected with a power of 80%.

Statistical Analysis

Primary outcome: A one-tail Fisher exact test will be used to compare the percentage

of patients who achieve 50% improvement in the SALT score after 24 weeks of treatment between drug and placebo groups.

Secondary Outcomes:

A Mixed effect model repeated measures (MMRM) will be used to detect any overall differences in the treatment effect as compared to placebo. The differences in SALT score between Baseline values and those at Weeks 4, 8, 12, 16, 20, and 24 will be estimated considering the missing values for patients who prematurely discontinued the study. Changes after 24 weeks of treatment will be estimated using this model and presented as percentage.

The differences in semi-quantitative SALT scores between Baseline and after 24 weeks of treatment will be compared using a two-tailed Wilcoxon matched-pairs test.

The change from Baseline in cellular and molecular markers in skin biopsies after treatment will be analyzed using two-tailed t-Student test for paired samples.

Similar analysis will be carried out for the secondary endpoints at Week 48.

MECHANISTIC STUDIES STATISTICAL ANALYSES:

For comparison of expression levels of lesional, and non-lesional AA skin, we will use RT-PCR, and gene-arrays. RT-PCR values will be normalized to the housekeeping gene hARP (validated in all our AD studies) and log2-transformed prior to analysis. Microarray data will be preprocessed using standard pipeline (exhaustively used by our group in a large number of studies) and log-2 transformed expressions. Adjustments by batch effect and clinical variables will be carried out if needed using ComBat. Mixed effect model on R's *limma* framework will 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. Similar approaches will be carried out for blood analyses.

Gene expression changes in Th2/IL-13, "T22"/IL-22, S100A7 and S100A8, Th1/IFN-gamma, and Th17/IL-17A will be jointly correlated with clinical responses by multivariate analysis using multivariate u-statistics and R package muStat, as we have previously

reported.

The change from Baseline in the Alopecia Areata Symptom Impact Scale (AASIS) at Week 24 and the change from Baseline in the Alopecia Areata Quality of Life questionnaire (AA-QoL) at Week 24 will be evaluated using a two-sided Wilcoxon signed rank test for each item. To analyze the score created by summarizing a group of items a two-tailed t-Student test for paired samples will be used.

Safety and Tolerability Analysis

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. For local subcutaneous adverse events, the rate of AEs will be compared between groups. The proportion of lesions in each treatment group reporting adverse events that occur in ~ 5% in either treatment group will be compared using the Fisher's exact test.

LABORATORY SPECIMENS

All blood and urine samples will be processed through the Mount Sinai Center for Clinical Laboratories, One Gustave Levy Place, New York, NY 10029.

INSTITUTIONAL REVIEW BOARD

Prior to beginning this study, approval for all study related documents (protocol, consent form, advertising) would be obtained from the Icahn School of Medicine at Mount Sinai Program for the Protection of Human Subjects (Institutional Review Board), One Gustave Levy Place, Box 1081, New York, NY 10029.

Flow Chart

Procedures	**Screening (Day -28 to Day -1)**	**Baseline Day 0**	**Week 4**	**Week 8**	**Week 12**	**Week 16**	**Week 20**	**Week 24**	**Week 28**	**Week 32**	**Week 36**	**Week 40**	**Week 44**	**Week 48 / Early Term**	**Week 52 F-UP**
Informed consent	X														
Inclusion/exclusion criteria	X	X													
Past Medical History (including previous therapies for AA) and Demographic Data collection	X														
Limited Physical Examination	X							X						X	
HBsAg and Hepatitis C antibody	X														
Chemistry and hematology	X														
Blood for mechanistic studies		X			X			X						X[3]	
DNA analysis		X													
RNA and proteomic analysis		X			X			X						X[3]	
SALT assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
aaPGA			X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of regrowth pattern	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical photographs		X		X	X			X	X	X	X	X	X	X	
AASIS and AA-QoL		X			X			X			X			X	
Pregnancy Test[1]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Study Product Dispensing		X	X	X	X	X	X	X	X	X	X	X	X		
Scalp Biopsies[2]		X			X[4]			X						X[3]	
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events (including assessment of mood changes and weight loss)		X	X	X	X	X	X	X	X	X	X	X	X	X	X

1 Serum pregnancy test for females of childbearing potential at screening and urine test at other visits.

2 Two biopsies will be collected at Baseline (affected and nonaffected areas). In case of 100% hair loss, only one biopsy of the affected area will be biopsied. One biopsy will be collected at Week 24 from the vicinity where the affected skin biopsy was taken at Baseline.

3 To be collected only if early termination visit is being performed prior to Week 24.

4 Optional: One biopsy will be taken at Week 12 (from the vicinity of the affected area biopsied at baseline) if consented to by subject.

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