CLINICAL STUDY PROTOCOL

Protocol Number: ATI-50002-AA-201
Amendment 8

A Randomized, Double-Blind, Vehicle-Controlled Multicenter Study to Evaluate the Safety, Tolerability and Efficacy of ATI-50002 Topical Solution Administered Twice-Daily for 6 Months in Adolescents and Adult Subjects with Stable Patchy Alopecia Areata with Optional Long-Term Open-Label Extension

Sponsor
Aclaris Therapeutics, Inc.
640 Lee Rd
Suite 200
Wayne, PA 19087
Telephone: 484-324-7933
Facsimile: 484-320-2344

Study Contact
Sue Moran, RN, MSN
Sr. Director, Clinical Development
Aclaris Therapeutics, Inc.
640 Lee Rd
Suite 200
Wayne, PA 19087
Telephone: 484-329-2129
Cell: 484-999-7492
E-mail: smoran@aclaristx.com

Medical Monitor
Evyan Cord-Cruz, M.D.
Director, Medical Affairs
Symbio, LLC
21 Perry Street
Port Jefferson, NY 11777
Telephone (Main Line): 631-403-5126
Telephone (Office): 516-338-0647
Telephone (Cell): 516-982-0677
Facsimile: 631-474-8534
E-mail: ecruz@symbioresearch.com

Safety Monitor:
Deborah S. Kelly, M.D.
Senior Medical Director, Safety, PV & Risk Management
Aclaris Therapeutics, Inc.
640 Lee Rd
Suite 200
Wayne, PA 19087
Telephone: 484-540-2262
Cell: 484-280-2262
Email: dkelly@aclaristx.com
ProPharma
Email: clinicalsafety@propharmagroup.com

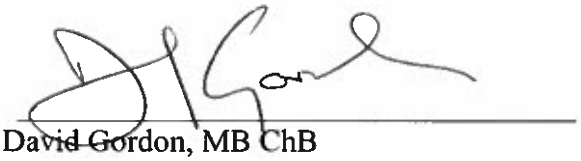
PROTOCOL APPROVAL SIGNATURE PAGE

Protocol Number: ATI-50002-AA-201 Amendment 8

Version 9.0: 09APR2019

Protocol Title: A Randomized, Double-Blind, Vehicle-Controlled Multicenter Study to Evaluate the Safety, Tolerability and Efficacy of ATI-50002 Topical Solution Administered Twice-Daily for 6 Months in Adolescents and Adult Subjects with Stable Patchy Alopecia Areata with Optional Long-Term Open-Label Extension

Sponsor Signatures:



David Gordon, MB ChB
Chief Medical Officer
Aclaris Therapeutics, Inc.

4-9-2019
Date

INVESTIGATOR SIGNATURE PAGE

Protocol Number: ATI-50002-AA-201 Amendment 8

Protocol Title: A Randomized, Double-Blind, Vehicle-Controlled Multicenter Study to Evaluate the Safety, Tolerability and Efficacy of ATI-50002 Topical Solution Administered Twice-Daily for 6 Months in Adolescents and Adult Subjects with Stable Patchy Alopecia Areata with Optional Long-Term Open-Label Extension

Version 9.0: 09APR2019

I have reviewed the above-titled protocol and agree that it contains all the information necessary to conduct the study as required. I will conduct the trial in accordance with the principles of ICH Good Clinical Practice and the Declaration of Helsinki.

I will maintain as confidential all written and verbal information provided to me by the Sponsor, including but not limited to, the protocol, case report forms, investigator's brochure, material supplied at investigator meetings, minutes of teleconferences, etc. Such materials will only be provided as necessary to site personnel involved in the conduct of the trial, involved IRBs or local regulatory authorities.

I will obtain written informed consent/ assent from each prospective trial subject or each prospective trial subject's legal representative prior to conducting any protocol-specified procedures. The Informed Consent/Assent Document used will have the approval of the IRB appropriate for my institution.

I will maintain adequate source documents and record all observations, treatments and procedures pertinent to trial subjects in their medical records. I will accurately complete the case report forms supplied by the Sponsor in a timely manner. I will ensure that my facilities and records will be available for inspection by representatives of the Sponsor, the IRB, and/or local regulatory authorities. I will ensure that I and my staff are available to meet with Sponsor representatives during regularly scheduled monitoring visits.

I will notify the Sponsor within 24 hours of any serious adverse events.

Investigator Signature:

_____ _____

Investigator signature Date

_____

Investigator printed name

1. AMENDMENT HISTORY

Protocol Version 2.0 dated 26JUL2017 was implemented prior to initiation of the study. Protocol Version 3.0 was amended to extend treatment from 3 to 6 months, add visits at Months 4, 5, and 6, allow subjects who normally shave their scalp to shave their scalp and maintain the same hair style, as long as they refrain from shaving at least 1 week prior to the study assessments or longer at the discretion of the investigator based on visible scalp hair growth and to add a requirement for sexually active males to use a barrier method of birth control (Inclusion criterion #13).

Protocol Version 4.0 dated 26APR2018 was amended to:

- clarify the definition of stable AA,
- update the ANC and platelet criteria to less than the lower limit of normal,
- add a 6-month washout period for JAK inhibitors (oral and topical),
- reduce the timeframe a WOCBP should be on highly effective birth control from 90 days to 30 days prior to study entry, given the proven onset of contraceptive effectiveness,
- update Sponsor address,
- add Sponsor Safety Monitor Contact Information,
- minor clarifications and typographical edits,
- addition of the Global Impression of Change (Physician and Subject) Assessment.

Protocol Version 5.0 dated 11JUN2018 was amended to allow enrollment of a broader range of subjects with AA for whom there is a hypothesis to support an efficacious response to ATI-50002 by:

- Decreasing the minimum SALT score from 30% hair loss to 15% hair loss,
- Increasing the maximum duration of the current episode of AA from 7 years to 12 years,
- Allowing the inclusion of subjects with ophiasis pattern of AA.
- Changing the primary efficacy analysis from the "change from baseline" to the "mean relative percent change from baseline" in the SALT score at Week 24 (Visit 10).
- Adding that the statistical analysis will include a stratification of subjects based on baseline SALT score, where the number and range of each stratum will be determined by a blinded review of the distribution of the baseline scores, conducted prior to database unblinding.
- Revising the secondary analysis:
  - Changed the "mean change from baseline" to the "mean relative percent change from baseline" in the ALODEX score
  - Added the mean change from baseline in SALT score and ALODEX score at Week 24 (Visit 10)
  - Clarified that the proportion of subjects achieving ≥50% hair regrowth compared with baseline will be analyzed using a separate model for SALT and for ALODEX scores
- Updating the number of sites from approximately 20 to 27.

Protocol Version 6.0 dated 08AUG2018 was amended to change the area of the study medication application from the areas of the scalp with patchy alopecia areata including a ½ inch margin around the patchy areas to application to the entire scalp. Subjects will apply up to 4-mLs, twice-daily; once in the morning and approximately 12 hours.

Protocol Version 7.0 (Amendment 6, dated 10AUG2018) was amended to allow inclusion of subjects ≥ 12 years old (previously ≥ 18 years old).

Administrative Letter #3 dated 28AUG2018 provided the previously approved study medication application instructions for use when applying drug to the patchy areas only. Administrative Letter #4 dated 06SEP2018 clarified the study medication allocation instructions for subjects < 18 years of age. Administrative Letter #5 dated 14SEP2018 updated the title to include adolescents.

Protocol Version 8.0 (Amendment 7, dated 26SEP2018) allowed subjects who complete the double-blind portion of the study to have the option of enrolling into a long-term open-label extension (OLE) period to apply ATI-50002 Topical Solution 0.46%. The purpose is to allow subjects who may have received placebo an opportunity to apply active treatment and subjects on active treatment to extend the treatment period. Amendments 1-8 have been incorporated into the final protocol dated 09APR2019

2. AMENDMENT RATIONALE

The primary purpose of the update to Protocol Version 9.0 (Amendment 8, dated 09APR2019) is to clarify that the primary analysis would be conducted on the ITT population. Additionally, other changes to the statistical methods are detailed in the following table.

3. PROTOCOL CHANGES

Protocol Version	Date	Section	Revisions
9.0	09APR2019	Title Page	Updated Safety Monitor
		Endpoints -Synopsis -Statistical Sections	Clarified that the primary endpoint and the secondary endpoints are “Percent change from baseline” and not “Mean relative percent change from baseline”.
		Sample Size and Power Calculations -Synopsis, -Section 11.1	Added a power statement describing the treatment difference (24-points) that we can detect with 80% power.
		Statistical Methods, -Synopsis -Section 11.4.1	Specified that the primary analysis would be on the ITT population, using LOCF for missing data and based upon a mixed effect model repeated measures (MMRM).
		Secondary Efficacy Analyses -Synopsis -Section 11.4.2	Deleted: Dichotomous variables will be analyzed using Chi-Square tests. Ordinal and continuous variables will be analyzed using appropriate Analysis of Variance models.
		Safety Data -Synopsis -Section 11.5	Deleted: Overall incidence of adverse events will be compared between groups using a chi-square test.
		Section 11.4	Deleted the statement that the PP population would be used for all efficacy analyses.
		Section 11.4.2	Included a secondary sensitivity analysis on the primary endpoint using the PP population. Added secondary responder efficacy endpoints to be specified in the SAP Specified that all secondary efficacy endpoints would be analyzed using the ITT population

1. SYNOPSIS

Protocol Number: ATI-50002-AA-201	**Protocol Title:** A Randomized, Double-Blind, Vehicle-Controlled Multicenter Study to Evaluate the Safety, Tolerability and Efficacy of ATI-50002 Topical Solution Administered Twice-Daily for 6 Months in Adolescents and Adult Subjects with Stable Patchy Alopecia Areata with Optional Long-Term Open-Label Extension
Sponsor: Aclaris Therapeutics Inc.	**Phase of Development:** Phase 2

Study Medication Description:
Double-Blind: ATI-50002 Topical Solution, 0.46%, ATI-50002 Topical Solution, 0.12% or Vehicle Topical Solution
Open-Label: ATI-50002 Topical Solution, 0.46%

Study Objectives:
Primary:

- To assess safety, tolerability and efficacy of ATI-50002 Topical Solution, 0.46% and 0.12% compared to vehicle in subjects with stable patchy alopecia areata (AA)

Secondary:

- To evaluate the psychometric performance of the key clinical outcome assessments and questionnaires with respect to reliability, construct-related validity, and sensitivity to change
- To generate score interpretation estimates for the clinical outcome assessments to guide the definition of responders in pivotal trials

Exploratory: (Select Sites Only)

- At select clinical sites, to explore the effects of ATI-50002 induced Janus kinase (JAK) 1/3 inhibition on the Alopecia Areata Disease Activity Index (ALADIN) scores, peribulbar infiltrate, and other inflammatory biomarkers

Long-term Objective:
To assess the safety, tolerability and efficacy of ATI-50002 Topical Solution, 0.46% in subjects with stable patchy alopecia areata.

Study Design:
Double-blind Period: This Phase 2, multicenter, randomized study is designed to evaluate the safety, tolerability and efficacy of ATI-50002 Topical Solution in subjects with stable patchy alopecia areata (AA). Subjects will be required to have a clinical diagnosis of alopecia areata (AA) with at least 15% up to 95% total scalp hair loss for a duration of at least 6 months up to and including 12 years. A total of 120 subjects will be randomized. Subjects will be randomized to a 1:1:1 ratio:

- ATI-50002 Topical Solution, 0.46%, BID for 24 weeks (6 months)
- ATI-50002 Topical Solution, 0.12%, BID for 24 weeks (6 months)
- Vehicle Topical Solution BID for 24 weeks (6 months)

During the screening period, subjects will be assessed for eligibility into the study. Subjects will apply up to 4 mLs of study medication to the entire scalp twice a day for 24 weeks. Assessment of response to treatment will be performed at Week 4 (Visit 5), Week 8 (Visit 6), Week 12 (Visit 7), Week 16 (Visit 8), Week 20 (Visit 9), Week 24 (Visit 10), and post-treatment Week 28 (Visit 11). Safety and tolerability will be evaluated at each study visit by assessment of adverse events and vital signs, and at select visits, ECGs and clinical laboratory tests will be completed. The primary analysis will be conducted after all subjects complete the double-blind visits.

Open-Label Extension (OLE): Subjects who complete Visit 10 or Visit 11 (Week 24 or 28) will be eligible to enter a 28 week OLE. In the OLE, safety, tolerability and efficacy will be assessed. All subjects enrolled in the OLE will apply ATI-50002 Topical Solution, 0.46% to the entire scalp or patchy areas with a ½" margin, twice daily for 24 weeks (6 months). In addition, subjects with eyebrow loss may apply study medication to the entire eyebrow(s) twice daily for 24 weeks. Subjects will be followed for safety, tolerability and efficacy as detailed in Section 6.3. Subjects who decline or are not eligible for the OLE should be seen for the Visit 11 (Post-treatment) assessments. Subjects who entered the OLE and for administrative reasons had a gap between completion of Visit 10 or Visit 11 and entry into the open-label should return for the OLE Visit 1 and have the procedures detailed in the Schedule of Assessments.

Number of Subjects (planned):
120 subjects will be enrolled into the study.

Number of Study Sites:
This study will be conducted at approximately 27 U.S. clinical sites.

Double-blind Period
Inclusion Criteria:
Subjects must meet the following criteria to be eligible for participation in the study.

1. Able to comprehend and willing to sign an Informed Consent/Assent Form (ICF).
2. Male or non-pregnant, non-nursing female ≥12 years old at the time of informed consent/ assent.
3. Have a clinical diagnosis of stable patchy alopecia areata (AA) defined as no current areas of spontaneous regrowth.
4. Have a Severity of Alopecia Tool (SALT) score of at least 15% up to 95% total scalp hair loss determined by the study investigator at baseline.
5. Have an assessment of "no hair" or "a little hair" in the target scalp patch based on both the Clinician and Subject Alopecia Scalp Appearance Assessment (AAA).
6. Have a duration of the current episode of scalp hair loss of a minimum of 6 months and a maximum of 12 years.
7. If a woman of childbearing potential (WOCBP), must have a negative serum pregnancy test at Screening (Visit 1) and a negative urine pregnancy test at Baseline (Visit 2) and agree to: use a highly effective method of birth control for the duration of the study; not be planning a pregnancy during the study duration and use contraception for 30 days after last application of study medication (Refer to Section 10).
8. Be in good general health and free of any known disease state or physical condition which, in the investigator's opinion, might impair evaluation of the subject or which might expose the subject to an unacceptable risk by study participation.
9. Be willing to maintain the same hair style throughout the study period. Subjects who shave their scalp must be willing to refrain from shaving their scalp for at least one week or longer prior to each study visit, as determined by the investigator based on visible scalp hair growth. Hair trimming outside the treatment areas to maintain the current hair style is permitted.
10. Be willing and able to follow all study instructions and to attend all study visits.
11. Subjects taking hormonal replacement therapies must be on stable doses for 6 months prior to enrollment and remain on the same maintenance dose throughout the study.
12. Subjects taking thyroid replacement medication must be on stable doses for 6 months prior to enrollment and remain on the same maintenance dose throughout the study.
13. Sexually active male subjects must agree to use a barrier method of contraception from the first application of study medication to at least 30 days after the last application of study medication.

Exclusion Criteria:

Subjects are excluded from this study if any 1 or more of the following criteria is met:

1. Females who are nursing, pregnant, or planning to become pregnant for the duration of the study and up to 30 days after the last application of study medication.
2. Diffuse alopecia areata.
3. Concomitant hair loss disorder (by history or physical exam) such as androgenetic alopecia or scarring alopecia (*e.g.*, cicatricial alopecia, frontal fibrosing alopecia, etc.). Subjects without a prior known history of androgenic alopecia (AGA) or other patterned hair loss disorder who exhibit AGA or other patterned hair loss with regrowth during the study will be allowed to continue in the study.
4. Active skin disease on the scalp (such as psoriasis or seborrheic dermatitis) or a history of skin disease on the scalp that in the opinion of the investigator would interfere with the study assessments of efficacy or safety.
5. Active scalp trauma or other condition affecting the scalp that, in the investigator's opinion, may affect the course of AA or interfere with the study conduct or evaluations.
6. The presence of a permanent or difficult to remove hairpiece or wig that will, in the opinion of the investigator, interfere with study assessments if not removed at each visit.
7. History of, or current, severe, progressive or uncontrolled renal, hepatic, gastrointestinal, pulmonary, cardiovascular, genitourinary (renal disease) or hematological disease, neurologic or cerebral disorders, infectious disease or coagulation disorders that, as determined by the Investigator, would preclude participation in and completion of study assessments.
8. History of, current or suspected systemic or cutaneous malignancy and /or lymphoproliferative disease, other than subjects with: a history of adequately treated and well healed and completely cleared non-melanoma skin cancers (basal or squamous cell carcinoma) treated successfully at least 1 year prior to study entry with no evidence of disease.
9. Evidence of active or latent bacterial (including tuberculosis) or viral infections at the time of enrollment, or history of incompletely treated or untreated tuberculosis. Subjects who have completed therapy for latent tuberculosis may participate.
10. History of serious local infection (*e.g.*, cellulitis, abscess) or systemic infection including but not limited to a history of treated infection (*e.g.*, pneumonia, septicemia) within 3 months prior to baseline. Subjects on an antibiotic for a nonserious, acute local infection must complete the course prior to enrollment into the study.
11. Positive for HIV, Hepatitis B or C. Subjects with serologic evidence of Hepatitis B vaccination (Hep B surface Ab without the presence of Hep B surface Ag) will be allowed to participate.
12. Herpes zoster or cytomegalovirus (CMV) that resolved less than 2 months before study enrollment. Subjects with a history of frequent outbreaks of Herpes Simplex Virus (defined as 4 or more outbreaks a year).
13. Clinically significant laboratory abnormalities at screening that, in the opinion of the Investigator, would make the subject a poor candidate for the study.
14. Subjects with absolute neutrophil count <1,800/mL, or platelet count <130,000/mL.
15. Subject unable to comply with the following required washout periods:

Therapy/Medication	Washout Period
Systemic Therapies	
Disease Modifying Anti-Rheumatic Drugs (DMARDS), Biologics or immunosuppressants, such as: anakinra, adalimumab, azathioprine, corticosteroids, cyclosporine, etanercept, infliximab, methotrexate, TNF inhibitors, ustekinumab	1 month or 5 half-lives whichever is greater
Plaquenil	2 months

JAK inhibitors (oral or topical)	6 months
Intralesional Steroids (Scalp)	1 month
Anthralin, bimatoprost, corticosteroids, diphencyprone, diphenylcycloprophenone (DPCP), Squaric acid dibutylester (SADBE), minoxidil, pimecrolimus, tacrolimus	1 month
Phototherapy, Laser Therapy, Excimer Laser	3 months

16. Participation in an investigational drug or device trial in which administration of an investigational drug or device occurred within 30 days or 5 half-lives (whichever is longer) of Screening (Visit 1). Subjects who have participated in a study of an investigational drug, device or biologic agent for alopecia areata within 1 year of screening will be eligible to participate only with individual permission from the Medical Monitor.
17. Sensitivity to any of the ingredients in the study medications.
18. History of or current alcohol or drug abuse within 2 years of assessment for study enrollment.
19. Unwillingness to refrain from weaves, hair extensions, or shaving of the scalp for at least one week or longer prior to each study visit, as determined by the investigator based on visible scalp hair growth for the duration of the study.
20. Screening ECG findings of:
    - QTcF >450msec for males or >470msec for females (use of the ECG algorithm is acceptable for this purpose)
    - Clinically Significant Heart rate < 45 or Heart Rate > 100 beats/minute
    - Rhythm disturbance other than sinus arrhythmia or ectopic supraventricular rhythm (ectopic atrial rhythm)
    - Conduction disturbance including PR >240msec, pre-excitation (delta wave and PR <120msec), second degree or higher AV block
    - Acute or chronic signs of ischemia
    - Left Bundle Branch Block
    - Prior myocardial infarction

Open-Label Extension
Inclusion Criteria:

1. Subject must be able to comprehend and willing to sign the OLE Informed Consent/Assent Form (ICF).
2. Subject must continue to meet the entry criteria from the double-blind period. The SALT score and duration of disease are based on the double-blind period baseline visit.
3. Subject has not experienced any clinically significant AEs, SAEs or tolerability issues that met study discontinuation criteria in the double-blind period.
4. Subject is capable of regrowing scalp hair in the opinion of the investigator.
5. WOCBP must have a negative UPT at entry into OLE.

Duration of Treatment
Double-blind Period

The duration of the screening period could be up to 30 days. Once randomized, the maximum duration of study treatment is from Baseline (Visit 2) to the Post-treatment follow-up visit at Week 28 (Visit 11) is anticipated to be a maximum of 200 days (197 days + 3-day window).

Open-Label Extension

The maximum duration of the of study treatment in the OLE is 176 days (169 days + 7-day window). The maximum duration of the study participation from entry into the OLE to completion of the post-treatment follow up visit is 204 days.

Endpoints

Primary Efficacy:

- Percent change from baseline in the Severity of Alopecia Tool (SALT) score at Week 24 (Visit 10), also described as mean percent regrowth

Secondary Efficacy*:*

- Percent change from baseline in the Alopecia Density and Extent Score (ALODEX) score at Week 24 (Visit 10), also described as mean percent regrowth
- Mean change from baseline in SALT score at Week 24 (Visit 10)
- Mean change from baseline in ALODEX score at Week 24 (Visit 10)
- Proportion of subjects in each treatment arm achieving ≥ 50% hair regrowth compared with baseline using a separate model for SALT and for ALODEX scores
- Change from baseline in the Alopecia Scalp Appearance Assessment (AAA) for Patchy AA (Patient-reported outcome [PRO]) at Week 24 (Visit 10)
- Change from baseline in the Alopecia Scalp Appearance Assessment (AAA) for Patchy AA (Clinician-reported outcome [ClinRO]) at Week 24 (Visit 10)
- Change from baseline in the Physicians Global Impression of Severity (PhGIS) of Patchy AA at Week 24 (Visit 10)
- Change from baseline in the Subject Global Impression of Severity (SGIS) of Patchy AA at Week 24 (Visit 10)
- Change from baseline in the subject reported Alopecia Impact Assessment (AIA) at Week 24 (Visit 10)
- Subject Global Impression of Treatment Satisfaction at Week 24 (Visit 10)
- Change in Dermatology Life Quality Index (DLQI) total score between Baseline and Week 24 (Visit 10)
- Global Impression of Change (Clinician and Subject) at Week 24 (Visit 10)

Exploratory:

- At select clinical sites, the change from baseline in the effects of ATI-50002 induced Janus kinase (JAK) 1/3 inhibition on the ALADIN scores, peribulbar infiltrate and other inflammatory biomarkers

Safety*:*

- Safety variables to be assessed include: adverse events, clinical laboratory tests (hematology, clinical chemistry, and urinalysis), vital sign measurements (systolic and diastolic blood pressures, respiration rate, heart rate, and oral body temperature), and electrocardiograms.

Long-term:

- Safety variables, efficacy variables [(change from baseline in SALT, ALODEX at long-term visits), (change from baseline in AAA-SR, AAA-CR, SGITS)].

Study Medication Administration

Subjects will apply up to a maximum of 4 mL of topical solution to the entire scalp twice-daily as directed.

Prior to amendment 5, subjects were instructed to treat patches of active alopecia areata and not the entire scalp. Subjects who signed the ICF prior to amendment 5 and do not consent to twice-daily applications to the entire scalp may continue in the study. These subjects should be given the option to

apply the study medication to the entire scalp at night only, and the patchy areas during the day or to the patchy alopecia areas and a ½" margin around the areas twice-daily. The areas being treated must be identified in the source documentation and the eCRF as; patchy areas only twice-daily, entire scalp twice-daily or patchy areas only once-daily, and entire scalp once-daily.
In the OLE, in addition to applying study medication to the entire scalp twice-daily (or patchy areas with a ½" margin if the subject did not agree to apply to the entire scalp) subjects with eyebrow loss will be allowed to apply a thin film of study medication twice-daily to the affected eyebrow(s) as directed.

Statistical Methods
The primary analysis will be conducted when all subjects have completed the double-blind period.

Sample Size/ Power Calculations
The planned sample size is 120 subjects. Sincethis is the first multicenter study evaluating the effect of ATI-50002 Topical Solution in subjects with stable patchy alopecia areata, the sample size was based upon feasibility issue rather than on a formal power calculation. However, data from 120 subjects (40 per group), utilizing LOCF for missing data, provides 80% power to detect a 24-point difference in percent change from baseline in SALT score between any two treatment groups. This power computation was based upon the assumed standard deviation of 38%. Data from a previous study NCT02197455 showed that the standard deviation in percent change from baseline in the SALT score is 30.3%. The 38% standard deviation assumption used for this power computation was chosen to account for the increase in variance due to the lower baseline SALT entry criteria for this study as compared to NCT02197455.

Statistical Methods
The primary efficacy variable will be the percent change from baseline in the SALT score at Week 24 (Visit 10). This represents the percentage of hair regrowth. It will be calculated as the mean of the changes from baseline (Visit 2) SALT score to Week 24 (Visit 10) SALT score, divided by the baseline SALT score and expressed as a percentage. Missing data for the primary endpoint will be imputed using Last Observation Carried Forward (LOCF) methodology. The analysis will be conducted on the relative percent change from baseline over time including all visits using a mixed effect modelrepeated measures (MMRM). The adjusted mean (LS Mean) for percent change from baseline in the SALT score at each visit, estimated from the MMRM, with the estimated standard error and 95% confidence interval (CI), will be presented in tabular and graphic format. The difference in the adjusted means between treatment groups and the associated 95% CI of the difference will be provided. No adjustment for multiplicity will be made. The model will include fixed effect terms for treatment, study visit, baseline SALT score group (<50, >=50), and treatment by SALT group interaction. The primary analysis will be based on the Intent-to-Treat (ITT) population. Statistical significance for primary efficacy endpoint will be declared if at least one treatment group comparison at the Week 24 visit has a two-sided p-value less than or equal to 0.05.

Secondary Efficacy Analyses
The secondary efficacy endpoints include: the percent change from baseline in the ALODEX score (percent regrowth); the mean change from baseline in SALT and the mean change from baseline in ALODEX, the proportion of subjects achieving a ≥ 50% hair regrowth compared with baseline using a separate model for SALT and for ALODEX scores; change from baseline in the AAA (Clinician and Subject), PhGIS, SGIS, AIA, treatment satisfaction questionnaires; and DLQI. Global Impression of Change (Clinician and Subject) at Week 24 (Visit 10) will be used in the psychometric evaluation of the scores from the AAA (CR and SR), respectively. The mean percent change in ALODEX score analysis

will use the same methodology as specified for the primary efficacy analysis. Other parameters will be analyzed as detailed in the statistical analysis plan.

Data collected in this trial will be used to evaluate the psychometric properties of the newly developed patient-reported and clinician-reported outcome (PRO and ClinRO) questionnaires. A measurement-focused statistical analysis plan will detail the data analytic strategy used to achieve the measurement property and score interpretation objectives. The anticipated psychometric evaluation analyses for the newly developed PRO and ClinRO questionnaires may include the following: score description, score reliability, score construct-related validity and score interpretation.

Exploratory Biomarker Analyses (Select Sites Only)
ALADIN Scores (Select Sites Only)
The cytotoxic T lymphocyte infiltration (CTL), Interferon (IFN-gamma), and hair keratin (KRT) ALADIN scores will be calculated for each scalp biopsy sample as described in Xing et al, 2014, Jabbari 2016. Change in ALADIN scores within individual subjects will be assessed between Baseline (Visit 2) and Week 4 (Visit 5), and Week 24 (Visit 10).

Histology (Select Sites Only)
Scalp punch-biopsies will be formalin-fixed and paraffin-embedded and will be sectioned both vertically and horizontally by a qualified dermatopathologist. Specimens will be stained with hematoxylin and eosin (H&E) for routine histopathologic evaluation that will include (but is not limited to) diagnosis and evaluation of the presence, quantity, location, and quality of any hair, hair follicles, and inflammatory infiltrate present. Additionally, immunohistochemical (IHC) stains will be employed to identify immune cell populations (*e.g.*, Lymphocytes, cytotoxic T-lymphocytes [CTLs]), and hair-specific keratins. IHC stains to assess inflammatory responses will include (but not be limited to) CD8, HLA-DR and ICAM-1. Data will be presented as percent change from baseline in the number of cells/ high power field in regions of interest (peribulbar areas) and in percent change from baseline in the number of cells/mm^2.

Blood for Immunology (Select Sites Only)
Blood samples will be stained with cell surface antibodies for fluorescence acquisition cell sorting (FACS) analysis. FACS experiments will allow for assessment of the subset and activation status of immune cells involved in AA.

Clinical Correlative Studies (Select Sites Only)
Previous studies in human AA have reported increased NKG2DL expression and augmented expression of IFN response genes in the target hair follicle end organ, and elevated NKG2D expression in circulating CD8 T-cells and NK cells (Ito et al, 2008; Petukhova et al, 2010).

The study will include assessment of AA biomarkers at baseline and during treatment to correlate treatment and disease status with:

1. Histological improvement with reduced T-cell peribulbar infiltrates.
2. Declines in circulating and peribulbar CD8+NKG2D+ infiltration and in hair follicle NKG2DL expression.
3. Reduced IFN inflammatory biomarkers in the skin and blood.

Safety Data
Safety analyses will include descriptive statistics calculated on the safety parameters using the safety population. The proportion of subjects with treatment-emergent adverse events will be tabulated and presented by treatment and Medical Dictionary for Regulatory Activities (MedDRA) System Organ

Class. Vital signs and clinically significant abnormal laboratory results will also be tabulated and presented by treatment group.

Data from all randomized and treated subjects will be presented and summarized. Safety summaries by study treatment group will include listings by study medication of adverse events incidences within each MedDRA System Organ Class, and changes from pre-application values in vital signs. Adverse event summaries will be presented by study medication showing the proportion of subjects experiencing adverse events, both overall and by MedDRA System Organ Class.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
AA	Alopecia Areata
AAA	Alopecia Scalp Appearance Assessment
Ab	Antibody
AE	Adverse Event
Ag	Antigen
AGA	Androgenic Alopecia
AIA	Alopecia Impact Assessment
ALADIN	Alopecia Areata Disease Activity Index
ALODEX	Alopecia Density and Extent Score
ALT	Alanine aminotransferase
ANC	Absolute Neutrophil Count
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
AT	Alopecia Totalis
AU	Alopecia Universalis
BCC	Basal Cell Carcinoma
BID, b.i.d.	Twice-daily
BUN	Blood Urea Nitrogen
°C	Degrees Centigrade
CD	Cluster of Differentiation
CI	Confidence Interval
ClinRO	Clinician Reported Outcome
CMV	Cytomegalovirus
CR	Clinician Rating
CRA	Clinical Research Associate
CRO	Contract Research Organization
CS	Clinically Significant
CTL	Cytotoxic T-lymphocytes
dL	deciliter
DLQI	Dermatology Life Quality Index
DMARDs	Disease Modifying Anti-Rheumatic Drugs
DMSO	Dimethyl Sulfoxide
DPCP	Diphenylcyclopropenone
e.g.	for example (Latin; *exempla gratia*)
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
°F	Degrees Fahrenheit

Abbreviation	Term
FACS	Fluorescence Acquisition Cell Sorting
FDA	Food and Drug Administration
G/g	Gram
GCP	Good Clinical Practice
HCl	Hydrochloride
HDL	High Density Lipoprotein
HepB	Hepatitis B
HCV	Hepatitis C Virus
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human Immunodeficiency Virus
H&E	Hematoxylin and Eosin
HLA-DR	Human Leukocyte Antigen- antigen D Related
ICAM-1	Intercellular Adhesion Molecule 1
ICF	Informed Consent Form
ICH	International Conference on Harmonization
i.e.	that is (Latin; *id est*)
IFN	Interferon
IHC	Immunohistochemical
IL	Interleukin
IUD	Intrauterine Device
IUS	Intrauterine Hormone Releasing System
ITT	Intent-to-Treat
IRB	Institutional Review Board
JAK	Janus Kinase
KER	Hair keratin panel
KRT	Hair Keratin
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
MedDRA	Medical Dictionary for Regulatory Activities
MHC	Major Histocompatibility Complex
mL	Milliliter
Mm	Millimeter
MMRM	Mixed Effect Model Repeated Measures
NCS	Not Clinically Significant
NK/ NKG	Natural Killer/ Natural Killer Group
NKG2D	A Transmembrane Protein
NMSC	Nonmelanoma Skin Cancer
OTC	Over-The-Counter
PDT	Photodynamic Therapy

Abbreviation	Term
PEG	Polyethylene glycol
PhGIC	Physician Global Impression of Change
PhGIS	Physicians Global Impression of Severity
PP	Per-protocol
PRO	Patient-Reported Outcome
PUVA	Psoralen and Ultraviolet A
SADBE	Squaric Acid Dibutyl Ester
SAE	Serious Adverse Event
SALT	Severity of Alopecia Tool
SCC	Squamous Cell Carcinoma
SGIC	Subject Global Impression of Change
SGIS	Subject Global Impression of Severity
SGITS	Subject Global Impression of Treatment Satisfaction
SI	Subject identifier
SN	Subject Number
SOP	Standard Operating Procedure
SR	Subject Rating
STAT	Signal Transducer and Activator of Transcription
T3, T4, TSH	Triiodothyronine, Thyroxine, Thyroid Stimulating Hormone
TIBC	Total Iron Binding Capacity
TEAE	Treatment Emergent Adverse Event
TNF	Tumor Necrosis Factor
Tyk2	Tyrosine Kinase 2
UPT	Urine Pregnancy Test
US	United States
UVA	Ultraviolet A
UVB	Ultraviolet B
WBC	White Blood Cell
WOCBP	Women of childbearing potential

2. INTRODUCTION

Aclaris Therapeutics, Inc. is developing ATI-50002 Topical Solution for the treatment of stable patchy alopecia areata. ATI-50002 is a potent highly selective inhibitor of Janus kinase 1 (JAK1) and Janus kinase 3 (JAK3).

2.1. Summary

Overview of Alopecia Areata

Alopecia areata (AA) is an autoimmune dermatologic condition, which, in its mildest form, is typically characterized by patchy non-scarring hair loss on the scalp and/or body. More severe forms of AA include total scalp hair loss, known as alopecia totalis (AT), and total loss of all the hair on the scalp and body- importantly, including loss of eyebrows, eyelashes, and intranasal hair- known as alopecia universalis (AU). While spontaneous regrowth of hair is common in the milder form of AA (patchy), where the hair loss may wax and wane, in patients with the extensive hair loss of AT or AU, spontaneous hair regrowth is rare. AA affects both males and females across all ethnic groups, with a lifetime risk of 1.7% (Safavi 1995). About two-thirds of affected individuals are 30 years old or younger at the time of disease onset.

The course of AA is unpredictable and while up to 50% of patients may recover within 1 year even without treatment, most patients will have more than one episode of hair loss (Price 2008). Factors portending a poorer prognosis for regrowth are more extensive hair loss presentations (extensive AA, AT, AU), an ophiasis pattern of hair loss, a long duration of hair loss, a positive family history, the presence of other autoimmune diseases, nail involvement, and young age of first onset (Tosti 2006, Weise 1996). In children, the disease may have a tendency towards worsening with time even if the initial presentation was mild, and the progressively disfiguring nature of the disease can be psychologically devastating. AA is highly associated with numerous psychiatric comorbidities including adjustment disorders, anxiety disorders and depression in both children and adults, and an effective treatment for AT and AU, the more severe forms of the disease, represents a significant unmet medical need (Bilgic 2014, Ruiz-Doblado 2003, Alkhalifah 2010).

The clinical development of innovative therapies in AA has lagged far behind other autoimmune conditions and there are currently no evidence-based treatments for AA. A Cochrane database review highlighted that few therapies for AA have been comprehensively evaluated in randomized clinical trials and that no treatment has demonstrated significant benefit compared to placebo according to evidence-based assessments (Delamere, 2008). This lack of good evidence-based data remains a challenge for physicians attempting to select efficacious treatments for their patients and, as a result, numerous approaches to treatment exist and are typically based on considerations such as the age of the patient, the extent and/or duration of the disease, patient expectations, cost considerations (both time and financial resources) and physician preferences and experience.

Common treatments for the less severe (patchy) form of AA include corticosteroids, either topically applied or injected intralesionally into the alopecic areas, or the induction of an allergic reaction at the site of hair loss using a topical contact sensitizing agent- an approach known as topical immunotherapy- typically with the topical contact sensitizers diphenylcycloprophenone (DPCP), squaric acid dibutyl ester (SADBE), or treatment with topical anthralin. While these

same treatment options may be utilized for the more severe forms of AA, their use in the more severe forms of AA is limited not only due to limited efficacy, but also because of the impracticality of using them over extensive body surface areas. Additional treatments used for extensive forms of AA (AT, AU) have included systemic steroids (pulsed or chronically administered), immunosuppressive agents such as cyclosporine or methotrexate, phototherapy with psoralen +UVA (PUVA), narrow-band UVB, photodynamic therapy (PDT), laser therapy (*e.g.*, excimer laser, fractional photothermolysis lasers), prostaglandin analogs, etanercept, bexarotene and others, all with varying degrees of success and each with its inherent risk of adverse effects and unproven efficacy (Alkhalifah 2010, Price 1999, Hordinsky 2015, Strober 2005). Most recently, however, a breakthrough in the understanding of the pathophysiology of AA and several case reports in the literature have suggested that a group of inhibitors of the JAK-STAT pathway, the Janus Kinase (JAK) inhibitors, or "jakinibs" may be efficacious in the treatment of AA even in its most severe phenotypes, AT and AU (Jabbari, 2015, Pieri, 2015, Xing 2014).

The JAKs are members of a family of tyrosine kinases that are involved in cytokine receptor signaling. The JAK family of enzymes (JAK1, JAK2, JAK3, Tyk2) plays an essential role in regulating the signaling process of most cytokines in cells by linking the cytokine-induced signaling from the cell surface membrane receptors to signal transducers and activators of transcription, or STATs, within the cells. Once these JAK receptors are activated by the binding of a cytokine to the appropriate receptor, they initiate a JAK-STAT signaling pathway which can modify gene expression and modulate important regulatory functions in the cell, including regulating immune and inflammatory responses. JAK1 and JAK3 are constitutively associated with the alpha chain and the common gamma chain (γc), respectively, of the receptors for interleukin-2 (IL2), interleukin-4 (IL-4), interleukin-7 (IL-7) interleukin-9 (IL-9), interleukin-15 (IL-15), and interleukin-21 (IL-21). When these cytokines bind to their respective receptors, JAK1 and JAK3 are activated and initiate a signaling cascade that drives key inflammatory events, including lymphocyte activation and proliferation. The JAK inhibitors can block the cytokine receptor signaling pathways, (in this instance JAK1 and JAK3) blocking JAK-STAT transcription activation, and can therefore modulate inflammatory or immune responses, which can be beneficial in a variety of disease states, particularly, as recently reported, AA (Xing 2014). In that report, pharmacologic inhibition of JAK kinase signaling (JAK-STAT signaling) was reported to promote hair growth in both genetic mouse models of alopecia and in human patients.

Immunopathology & Pathophysiology of AA

Alopecia areata (AA) results from an autoimmune attack on the hair follicles that results in growing anagen-phase terminal hairs being induced to prematurely enter the telogen-phase and then shed. In its most acute state, AA demonstrates a histopathologically characteristic white cell infiltrate- the so called "swarm of bees"- encircling the human hair follicle, though more chronic forms typically demonstrate a sparser infiltrate (Jabbari 2016, Whiting 2003). Though the exact autoantigens expressed in the perifollicular epithelium that allow these specific T-cells to infiltrate the normally immunologically privileged hair follicle have been previously unknown, the T-cells that home to the hair follicle have been demonstrated to consist of both CD4 and CD8 cells. Most recent studies have further characterized a specific subpopulation of activated NKG2D-bearing CD8+ T-cells as being prominent in the peribulbar infiltrate, and it is now currently felt that these CD8+NKG2D+ effector T-cells preferentially localize to dermal sheath cells aberrantly expressing high levels of major histocompatibility complex (MHC) molecules and NKG2D ligands.

Interferons, as key activators of the MHC locus and of the cellular immune response, appear to play a key role in eliminating the immunologic privilege of the hair follicle and in inducing and maintaining the pathologic inflammatory response in AA. This is also seen in the C3H-HeJ mouse model of AA, in which IFN-γ is required for pathogenesis, and in which administration of IFN-γ accelerates disease. (Gilhar 2005, Hirota 2003).

AA has been viewed as a Th1-driven disease and, consistent with a pathogenic cellular immune response, elevated Th1 cytokines/ chemokines (IFN-induced chemokines [IP-10/CXCL10]) are seen in the peripheral blood of AA patients and IFN-inducible gene signatures have been described in the skin of AA patients and may correlate with disease activity (Arca 2004, Barahmani 2009, Kuwano 2007). Additionally, transcriptional profiles in human AA patients have shown a Type I IFN response in lesional biopsies and Th1 skewing and elevated IFN response cytokines/chemokines in both the peripheral blood and in reviewed scalp biopsies (Jabbari 2015, Xing 2014, Jabbari 2016). The cellular source of IFN-γ is hypothesized to be the T-cells, as in the AA mouse model IFN-gamma producing CD8+NKG2D+ cells dominate the dermal HF infiltrate, and in human AA, IFN-γ producing cells were identified in 4 of 5 dermal crawl-out assays (Christiano 2016). Additionally, data implicate IL- 15 in driving activation of IFN-producing CD8 T-cells (Xing 2014).

Thus, preclinical and preliminary clinical information, as discussed above, strongly suggests that the primary pathophysiologic mechanism in AA (including AT and AU) is a cytokine mediated (primarily through T-lymphocyte induced upregulation of IL-15 and IFN gamma) induction of and prolonged maintenance of the telogen stage of the hair cycle. Inhibitors of the JAK/STAT pathway, particularly JAK1 and JAK3, are known to downregulate the effects of both IFN-gamma (through the inhibition at JAK1), and IL-15 (through inhibition at both JAK1 and JAK 3), and several published case reports have demonstrated the potential for compounds that are JAK1/3 inhibitors to induce hair growth in patients with AA (Kim 2017, Craiglow 2014 and 2017, Gupta 2016, Scheinberg 2016). As ATI-50002 is a potent inhibitor at JAK 1 and JAK 3, it is strongly suggested that ATI-50002 may be effective in the treatment of AA.

Among patients with AA, patients with higher disease burdens are unlikely to have satisfactory outcomes with current therapies. Aclaris Therapeutics, Inc. is developing ATI-50002 as a topical treatment for stable patchy AA. Aclaris is also developing ATI-50001 for the treatment of alopecia universalis (AU) and alopecia totalis (AT). ATI-50001 is an oral prodrug that is rapidly converted presystemically to ATI-50002, a potent highly selective inhibitor of Janus kinase 1 (JAK1) and Janus kinase 3 (JAK3). This study will evaluate whether or not the topically applied JAK1/JAK3 inhibitor, ATI-50002, will attenuate both IL-15 and IFN-gamma signaling, thereby blocking re-activation of CD8+NKG2D+ memory T-cells, and aborting the cytotoxic T-cell inflammatory response underlying AA.

In a previous Phase 1 study in healthy volunteers, single ascending oral doses of ATI-50001 of 50 mg to 500 mg and multiple ascending doses of up to 400 mg BID were well-tolerated. There were no SAEs. All treatment emergent adverse events (TEAEs) were transient and mild in intensity with the exception of 4 TEAEs: facial bone fracture, headache (2), and catheter site pain, which were classified as moderate. The most frequently reported drug related TEAEs occurring in > 1% of subjects were abdominal pain (10%), flatulence (7%), diarrhea (6%) and headache (6%).

Three subjects showed mildly elevated ALT or AST concentrations, which in two subjects were considered not clinically significant. For the other subject, the mild ALT elevation was reported as a treatment emergent adverse event possibly related to the study drug. No clinically significant laboratory abnormalities were observed. There were no clinically significant findings from 12-lead ECGs or vital signs assessments.

ATI-50001 was not detected in plasma and there was a dose-related increase in plasma of the active metabolite ATI-50002. The half-life of ATI-50002, after 14 days of dosing, was approximately 9.0 hours. Systemic levels of ATI-50002, following oral doses of ATI-50001 in healthy volunteers transiently reduced pSTAT5 activity in *ex vivo* IL-2 stimulated lymphocytes, indicating inhibition of the JAK signaling pathway. Upon multiple dosing, pSTAT5 activity showed a more sustained inhibition during the dosing period.

Non-clinical studies conducted with oral administration of ATI-50001 and topical administration of ATI-50002 support the topical administration of ATI-50002.

3. STUDY RATIONALE

3.1. Study Rationale

Preclinical and preliminary clinical information, as discussed above, strongly suggests that the primary pathophysiologic mechanism in AA (including AT and AU) is a cytokine-mediated (primarily through T-lymphocyte induced upregulation of IL-15 and IFN gamma) induction of and prolonged maintenance of the telogen stage of the hair cycle. Inhibitors of the JAK/STAT pathway, particularly JAK1 and JAK3, are known to downregulate the effects of both IFN-gamma (through the inhibition at JAK1), and IL-15 (through inhibition at both JAK1 and JAK 3), and several published case reports have demonstrated the potential for compounds that are JAK1/3 inhibitors to induce hair growth in patients with AA. As ATI-50002 is a potent inhibitor at JAK 1 and JAK 3, it is strongly suggested that ATI-50002 may be effective in the treatment of AA.

Topical treatment with ATI-50002 Topical Solution on the areas of a patient's scalp with alopecic patches only may result in undertreatment of disease and potentially lead to sub-optimal efficacy. Discussion with experts in the treatment of alopecia has led Aclaris to the conclusion that by only treating sites of visible disease, areas of sub-clinical, but active disease may be untreated. These sub-clinical patches may either (a) develop into new patches of disease, or (b) harbor a focus of inflammation that impacts other sites on the scalp.

Patchy Alopecia Areata can be a frustrating disease, both to the patient and prescriber. Physicians tell of disease that they describe as "whack a mole", meaning that treatment can be successful in one patch, only for new patches to emerge elsewhere. Reviews describe how regrowth in one region of the scalp may be associated with expanding areas of alopecia elsewhere (Pratt, 2017). This dynamic picture of disease suggests that areas of seemingly non-active disease likely have sub-clinical ongoing auto-immune inflammation.

If areas of untreated inflammation are present, the question is whether they can impact the health of other areas of scalp. The C3H/HeJ mouse model of alopecia provides strong evidence that disease in one area of skin can indeed impact another (McElwee, 1998). In this model, an alopecia phenotype is transmitted when skin from alopecia affected mice is grafted onto normal C3H/HeJ mice. This implies that the inflammatory process at one site can spread and lead to disease at another. Consequently, subjects in this study will be instructed to apply study medication to the entire scalp twice-daily to increase the probability that patients with patchy alopecia in ATI-50002-AA-201 will achieve good efficacy.

This study will evaluate the safety, tolerability and efficacy of ATI-50002 Topical Solution, 0.12% and 0.46% compared to vehicle solution applied twice-daily to the entire scalp for 6 months. In addition, at select clinical sites, the study will explore the effects of ATI-50002 induced Janus kinase (JAK)1/3 inhibition on the ALADIN scores, peribulbar infiltrate and other inflammatory biomarkers from scalp biopsies and blood samples. The Alopecia Areata Disease Activity Index (ALADIN) is a three-dimensional quantitative composite gene expression score that will be used in this study to track disease severity and explore signs of response to treatment (Xing et al 2014).

Subjects with patchy scalp hair loss due to AA ranging from 15% to 95% based on the SALT score will be included in this study. In prior published studies, assessment of efficacy of treatment of AA was based on hair regrowth as determined by changes in the SALT score (Olsen 2016). Both the SALT and ALODEX scores will be measured electronically using an iPad. The SALT score is a measurement based on the SALT I diagram, that quantifies the amount of scalp without any hair. The ALODEX score is a measurement of the amount of scalp with hair loss and is based on the SALT II diagram (Olsen, submitted). The SALT II diagram breaks the scalp surface area from the SALT I diagram into 1% scalp surface areas and allows the investigator to assign a density rating to each area from 0 = no hair loss to 100 = complete baldness. The ALODEX score is a summation of all density ratings in each 1% scalp area. By assessing hair density in each of the 1% scalp areas, the use of the SALT II visual aid allows the following to be tracked: amount of absolute hair loss (ALODEX score), specific areas of baldness (all 1% areas are numbered), small changes in density that may otherwise go undetected with original SALT I score, percentage of scalp surface involved with any hair loss and thus needing to be treated topically, new areas of hair loss present since baseline of any given treatment, and lesional scoring by identifying target areas.

Hair loss will be quantified using both the SALT and ALODEX assessments at baseline and monthly during treatment. The change from baseline in the SALT and ALODEX scores will be used to determine hair regrowth. In addition to the SALT and ALODEX assessment tools, Aclaris is developing Clinician and Patient Reported outcome tools (ClinRO and PRO) to assess hair regrowth that is clinically meaningful to subjects.

These ClinRO and PRO tools will measure the signs, symptoms, and impacts of AA for use as secondary efficacy endpoints. The following PRO and ClinRO questionnaires designed to measure the scalp appearance, the impact and the global satisfaction with treatment from the perspective of the patient and the clinician, where appropriate, are included in the protocol:

- Scalp based appearance PRO and ClinRO
    - Alopecia Scalp Appearance Assessment (AAA) for Patchy AA: Subject Rating [SR]

  - Alopecia Scalp Appearance Assessment (AAA) for Patchy AA: Clinician Rating [CR]
- Impact of alopecia PRO
  - Alopecia Impact Assessment (AIA)
- Satisfaction with treatment PRO
  - Subject Global Impression of Treatment Satisfaction (SGITS)

Subjects will be enrolled with a range of 15% to 95% hair loss over the entire scalp surface area based on the SALT score at baseline. A baseline ALODEX score will also be obtained but will not be used as an inclusion criterion. The scalp appearance assessments in development (ClinRO and PRO) will assess hair loss on a scale from "no hair" to a "full head of hair". A target patch (identified by the subject as their most bothersome area of hair loss) will be identified at baseline. By identifying a target patch, subjects with less extensive hair loss overall can be enrolled using the same rating on the Alopecia Scalp Appearance Assessment of "no hair" or "a little hair" at baseline. In addition to the ClinRO and PRO assessments being completed for a target patch, the same scalp appearance assessments will be completed for hair loss over a subject's entire scalp surface area.

Because this trial will not be stratified in terms of ethnic background or gender of subjects, there is no consistent measure of hair color, caliber, growth rate or hair density that can be used across such a diverse population of subjects. Each of these parameters is intrinsically determined within a given subject, and in subjects with more severe hair loss, there will be little to no normal hair at the start of the study. Therefore, it will be impossible to establish each subject's individual baseline measure of healthy hair in order to monitor restoration of these parameters over the study. Further, it is not possible to compare these parameters across individuals in the study.

The ALODEX and SALT assessments are based on terminal hair growth. Another parameter that will be considered will be the normalization of exclamation point hairs. These two features should be trackable irrespective of the subjects' intrinsic hair color, caliber or density, and should give a meaningful indicator of response to treatment.

The 6-month topical treatment period is supported by the results of 26-week mouse and 39-week dog oral toxicity studies with systemic administration and a 6-month update from the 9-month minipig study with dermal administration.

4. STUDY OBJECTIVES

4.1. Primary Objective

The primary objective is to assess the safety, tolerability and efficacy of ATI-50002 Topical Solution, 0.46% and 0.12% compared to vehicle in subjects with stable patchy alopecia areata (AA).

4.2. Secondary Objectives

Secondary objectives are:

- To evaluate the psychometric performance of the key clinical outcome assessments questionnaires with respect to reliability, construct-related validity, and sensitivity to change
- To generate score interpretation estimates for the clinical outcome assessments to guide the definition of responders in pivotal trials

4.3. Exploratory (Select Sites Only):

At select clinical sites, to explore the effects of ATI-50002 induced Janus kinase (JAK) 1/3 inhibition on the ALADIN scores, peribulbar infiltrate and other inflammatory biomarkers.

4.4. Long-term Objective

To assess the safety, tolerability and efficacy of ATI-50002 Topical Solution, 0.46% in subjects with stable patchy alopecia areata.

5. SELECTION AND DISPOSITION OF STUDY POPULATION

5.1. Number of Subjects and Study Sites

A total of 120 subjects will be enrolled at approximately 27 US sites.

5.2. Study Population Characteristics

Male and female subjects, 12 years of age or older, with a clinical diagnosis of stable patchy alopecia areata of the scalp, who meet all the inclusion criteria and none of the exclusion criteria, will be eligible to enroll in this study.

5.3. Double-Blind Inclusion Criteria

Subjects must meet the following criteria to be eligible for participation in the study:

1. Able to comprehend and willing to sign an Informed Consent/Assent Form (ICF).
2. Male or non-pregnant, non-nursing female ≥ 12 years old at the time of informed consent/ assent.
3. Have a clinical diagnosis of stable patchy alopecia areata (AA) defined as no current areas of spontaneous regrowth.
4. Have a Severity of Alopecia Tool (SALT) score of at least 15% up to 95% total scalp hair loss determined by the study investigator at baseline.
5. Have an assessment of “no hair” or “a little hair” in the target scalp patch based on both the Clinician and Subject Alopecia Scalp Appearance Assessment (AAA).
6. Have a duration of the current episode of scalp hair loss of a minimum of 6 months and a maximum of 12 years.
7. If a woman of childbearing potential (WOCBP), must have a negative serum pregnancy test at Screening (Visit 1) and a negative urine pregnancy test at Baseline (Visit 2) and agree to: use a highly effective method of birth control for the duration of the study; not be planning a pregnancy during the study duration and use contraception for 30 days after last application of study medication. (Refer to Section 10).

8. Be in good general health and free of any known disease state or physical condition which, in the investigator's opinion, might impair evaluation of the subject or which might expose the subject to an unacceptable risk by study participation.
9. Be willing to maintain the same hair style throughout the study period. Subjects who shave their scalp must be willing to refrain from shaving their scalp for at least one week or longer prior to each study visit, as determined by the investigator based on visible scalp hair growth. Hair trimming outside the treatment areas to maintain the current hair style is permitted.
10. Be willing and able to follow all study instructions and to attend all study visits.
11. Subjects taking hormonal replacement therapies must be on stable doses for 6 months prior to enrollment and remain on the same maintenance dose throughout the study.
12. Subjects taking thyroid replacement medication must be on stable doses for 6 months prior to enrollment and remain on the same maintenance dose throughout the study.
13. Sexually active male subjects must agree to use a barrier method of contraception from the first application of study medication to at least 30 days after the last application of study medication.

5.4. Double-Blind Exclusion Criteria

Subjects are excluded from this study if any 1 or more of the following criteria is met:

1. Females who are nursing, pregnant, or planning to become pregnant for the duration of the study and up to 30 days after the last application of study medication.
2. Diffuse alopecia areata.
3. Concomitant hair loss disorder (by history or physical exam) such as androgenetic alopecia, or scarring alopecia (*e.g.*, cicatricial alopecia, frontal fibrosing alopecia, etc.). Subjects without prior known history of androgenic alopecia (AGA) or other patterned hair loss disorder, who exhibit AGA or other patterned hair loss with regrowth during the study, will be allowed to continue in the study.
4. Active skin disease on the scalp (such as psoriasis or seborrheic dermatitis) or a history of skin disease on the scalp that in the opinion of the investigator would interfere with the study assessments of efficacy or safety.
5. Active scalp trauma or other condition affecting the scalp that, in the investigator's opinion, may affect the course of AA or interfere with the study conduct or evaluations.
6. The presence of a permanent or difficult to remove hairpiece or wig that will, in the opinion of the investigator, interfere with study assessments if not removed at each visit.
7. History of, or current, severe, progressive or uncontrolled renal, hepatic, gastrointestinal, pulmonary, cardiovascular, genitourinary (renal disease) or hematological disease, neurologic or cerebral disorders, infectious disease or coagulation disorders that, as determined by the Investigator, would preclude participation in and completion of study assessments.
8. History of, current or suspected systemic or cutaneous malignancy and /or lymphoproliferative disease, other than subjects with: a history of adequately treated and well healed and completely cleared non-melanoma skin cancers (basal or squamous cell carcinoma) treated successfully at least 1 year prior to study entry with no evidence of disease.
9. Evidence of active or latent bacterial (including tuberculosis) or viral infections at the time of enrollment or history of incompletely treated or untreated tuberculosis. Subjects who have completed therapy for latent tuberculosis may participate.

10. History of a serious local infection (*e.g.*, cellulitis, abscess) or systemic infection including but not limited to a history of treated infection (*e.g.*, pneumonia, septicemia) within 3 months prior to Baseline (Visit 2). Subjects on an antibiotic for a nonserious, acute local infection must complete the course prior to enrollment into the study.
11. Positive for HIV, Hepatitis B or C. Subjects with serologic evidence of Hepatitis B vaccination (Hep B surface Ab without the presence of Hep B sAg) will be allowed to participate.
12. Herpes zoster or cytomegalovirus (CMV) that resolved less than 2 months before study enrollment. Subjects with a history of frequent outbreaks of Herpes Simplex Virus (defined as 4 or more outbreaks a year).
13. Clinically significant laboratory abnormalities at screening that, in the opinion of the Investigator, would make the subject a poor candidate for the study.
14. Subjects with absolute neutrophil count <1,800/mL or platelet count <130,000/mL.
15. Subject unable to comply with the following required washout periods:

Therapy/Medication	Washout Period
Systemic Therapies	
Disease Modifying Anti-Rheumatic Drugs (DMARDS), Biologics or immunosuppressants, such as: anakinra, adalimumab, azathioprine, corticosteroids, cyclosporine, etanercept, infliximab, methotrexate, TNF inhibitors, ustekinumab	1 month or 5 half-lives whichever is greater
Plaquenil	2 months
JAK inhibitors (oral or topical)	6 months
Intralesional Steroids (Scalp)	1 month
Topical Treatments on the Scalp	
Anthralin, bimatoprost, corticosteroids, diphencyprone, diphenylcycloprophenone (DPCP), Squaric acid dibutylester (SADBE), minoxidil, pimecrolimus, tacrolimus	1 month
Phototherapy, Laser Therapy, Excimer Laser	3 months

16. Participation in an investigational drug or device trial in which administration of an investigational study drug or device occurred within 30 days or 5 half-lives (whichever is longer) of Screening (Visit 1). Subjects who have participated in a study of an investigational drug, device or biologic agent for alopecia areata within 1 year of screening will be eligible to participate only with individual permission from the Medical Monitor.
17. Sensitivity to any of the ingredients in the study medications.
18. History of or current alcohol or drug abuse within 2 years of assessment for study enrollment.
19. Unwillingness to refrain from weaves, hair extensions, or shaving of the scalp for at least one week or longer prior to each study visit, as determined by the investigator based on visible scalp hair growth the duration of the study.

20. Screening ECG findings of:
    - QTcF >450msec for males or >470msec for females (use of the ECG algorithm is acceptable for this purpose)
    - Clinically significant Heart rate < 45 or Heart Rate > 100 beats/minute
    - Rhythm disturbance other than sinus arrhythmia or ectopic supraventricular rhythm (ectopic atrial rhythm)
    - Conduction disturbance including PR >240msec, pre-excitation (delta wave and PR <120msec), second degree or higher AV block
    - Acute or chronic signs of ischemia
    - Left Bundle Branch Block
    - Prior myocardial infarction.

5.5. Open-Label Extension

At Week 24 (Visit 10) or Week 28 (Visit 11), the subject may be given the option to move into the OLE treatment if the subject meets the OLE entry criteria in Section 5.5.1.

- If the subject declines the OLE, Visit 11 (Post-treatment) will be the last double-blind study visit.
- If the subject chooses to continue into the OLE, she/he must sign an OLE ICF/Assent, complete Visit 10 or Visit 11, as appropriate, and OLE Visit 1, prior to being dispensed open-label drug and receiving continued study medication application instructions. The subject will be scheduled for OLE Visit 2.
- If the subject enrolls in open-label treatment within 30 days of completion of Week 24 (Visit 10) or Week 28 (Visit 11), OLE Visit 1 will be conducted to obtain consent/assent and perform the procedures in the Schedule of Assessments. This will be considered the official date that the subject initiated OLE treatment.
- If the subject enrolls in open-label treatment > 30 days after completion of Week 28, OLE Visit 1 will be conducted in order to obtain consent/ assent, and complete the procedures detailed in the Scheduled of Events. This will be considered the official date that the subject initiated OLE treatment and the scheduling of subsequent visits will be based on this date.

5.5.1. OLE Entry Criteria

1. Subject must be able to comprehend and willing to sign the OLE Informed Consent/Assent Form (ICF).
2. Subject must continue to meet the entry criteria from the double-blind period. The SALT score and duration of disease are based on the double-blind period baseline visit.
3. Subject has not experienced any clinically significant AEs, SAEs or tolerability issues that met the study discontinuation criteria in the double-blind period.
4. Subject is capable of regrowing scalp hair in the opinion of the investigator.
5. WOCBP must have a negative UPT at entry into OLE.

5.6. Previous and Concomitant Medications and Therapies

At Screening (Visit 1), the Investigator or designee will question the subject to ensure they have not used any excluded therapies (Section 5.6.2).

5.6.1. Permitted Concomitant Medications and Over the Counter Products (OTC)

Concomitant therapies are any new or existing therapies received from Screening (Visit 1) until Week 28 (Visit 11). Concomitant therapies include drug (e.g. prescription and over the counter [OTC]), and non-drug (e.g., chiropractic, physical therapy, energy-based treatments).

Subjects will be allowed to take medications not restricted by the protocol as long as they have been on a stable dose prior to study entry. Vitamins, minerals, and dietary supplements are permitted while on study if the subject has been on a stable dose prior to study entry and, in the opinion of the Investigator, will not affect the safety or efficacy of the subject during the study. Topical hair and scalp products (shampoos, conditioners, styling products) should be reviewed by the Investigator and are permitted if, in the Investigator's opinion, they will not affect the safety or efficacy of the subject during the study. Use of hair dyes is permitted during the study if the subject uses the same hair dye throughout the study. If in the opinion of the Investigator, the hair dye interferes with study assessments the subject may be directed to wait until after the study visit to dye his or her hair. Efforts should be made to keep the hair care regimen the same throughout the duration of the study.

Topical therapies such as topical corticosteroids are permitted if they are not applied on or near the scalp. Inhaled or intranasal corticosteroids are allowed in the study.

Prior permitted concomitant medications taken within 30 days of beginning treatment with ATI-50002 Topical Solution will be documented in the subject's source document and eCRF. In addition, any new permitted medications administered during protocol treatment and through end of study (Week 28 (Visit 11) or OLE Visit 6) will be documented in the subject's source document and eCRF.

5.6.2. Prohibited and/ or Restricted Medications

Any medication, shampoo or hair product known to affect hair growth in AA is prohibited throughout the study period. Subjects who are on a chronic stable dose of finasteride for benign prostatic hypertrophy for greater than 1 year are eligible for enrollment in the study as long as they maintain the same stable dose throughout the study. Since subjects with known AGA are excluded from the study, treatment with finasteride for alopecia during the study or within one year prior to study enrollment is prohibited. A list of medications and therapies that require a specific washout period prior to study entry and are not permitted during the study is in **Table 1**.

Table 1: Prohibited Medications

Therapy/Medication	Washout Period
Systemic Therapies	
Disease Modifying Anti-Rheumatic Drugs (DMARDS), Biologics or immunosuppressants, such as: anakinra, adalimumab, azathioprine, corticosteroids, cyclosporine, etanercept, infliximab, methotrexate, TNF inhibitors, ustekinumab	1 month or 5 half-lives whichever is greater
Plaquenil	2 months

JAK inhibitors (oral or topical)	6 months
Intralesional Steroids on the Scalp	1 month
Topical Treatments on the Scalp	
Anthralin, bimatoprost, corticosteroids, diphencyprone, diphenylcycloprophenone (DPCP), Squaric acid dibutylester (SADBE), minoxidil, pimecrolimus, tacrolimus	1 month
Phototherapy, Laser Therapy, Excimer Laser	3 months

5.7. Subject Identifier (SI)

The Investigator will assign a unique five-digit subject identifier (SI) to each subject at Screening (Visit 1).
The SI format will be NN-NNN, using leading zeroes as appropriate, where:

- The first 2 digits are the investigational center site number assigned by Aclaris
- The final 3 digits are the subject number (SN), assigned in ascending numerical order by the Investigator or designee, without omitting or repeating any number, starting with 001.

For example, the SI for the twenty-third subject that signs an informed consent/ assent form at site number 01 would be 01-023. The subject will be identified using the SI in all study documentation for the duration of the study.

5.8. Replacement Subjects

Subject enrollment will continue until approximately 120 subjects have been randomized. Subjects who are randomized and do not complete the study will not be replaced.

5.9. Subject Withdrawal Criteria

Subjects will be informed that they are free to withdraw from the study at any time and for any reason. The investigator may remove a subject from the study if, in the investigator's opinion, it is not in the best interest of the subject to continue the study. Examples of other reasons subjects may be discontinued from the study are: a change in compliance with an inclusion or exclusion criterion, occurrence of AEs, occurrence of pregnancy, use of a prohibited therapy or subject is unwilling or refuses to continue with the protocol defined study visits and/or subject withdraws consent/ assent (Refer to Section 9.2.4 for study medication discontinuation or termination criteria).

In case of premature discontinuation from double-blind study participation, all efforts will be made to perform all Visit 10 (Week 24) assessments. For premature discontinuations occurring in the OLE, all efforts will be made to perform all OLE Visit 6 assessments. The date the subject is withdrawn from the study and the reason for discontinuation must be recorded in the subject's electronic case report forms (eCRFs). All withdrawn subjects with ongoing AEs will be followed until the event has resolved or stabilized, until the subject is referred to the care of a local health care professional, or until a determination of a cause unrelated to the study medication or study procedures is made.

5.10. Study Termination

This study may be terminated prematurely in whole or in part due to a change in the benefit/risk profile for ATI-50002 Topical Solution such that continuation of the study would not be justified on medical or ethical grounds. This determination may be made by the Study Investigators in conjunction with the Sponsor, or by IRB or the U.S. Food and Drug Administration (FDA). The Sponsor may also elect to terminate the study if enrollment is sufficiently slow to prevent the completion of the study in an acceptable timeframe, or if ATI-50002 development is discontinued.

If the study is terminated prematurely, the Sponsor will notify the Study Investigators and the FDA. The Investigator must promptly notify all enrolled subjects and the IRB of study termination.

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

This Phase 2, multicenter, randomized study is designed to evaluate the safety, tolerability and efficacy of ATI-50002 Topical Solution in subjects with stable patchy alopecia areata (AA). Subjects will be required to have a clinical diagnosis of alopecia areata (AA) with at least 15% up to 95% total scalp hair loss for a duration of the current episode of scalp hair loss of a minimum of 6 months and a maximum of 12 years.

During the screening period, subjects will be assessed for eligibility into the study. Subjects will apply up to a maximum of 4 mLs of study medication to the entire scalp twice a day for up to 24 weeks in the double-blind period. Subjects who continue onto the OLE will apply ATI-50002-Topical Solution, 0.46% twice-daily for 24 weeks to the scalp and if applicable to eyebrow(s). Assessment of response to treatment will be performed at Week 4 (Visit 5), Week 8 (Visit 6), Week 12 (Visit 7), Week 16 (Visit 8), Week 20 (Visit 9), Week 24 (Visit 10), post-treatment Week 28 (Visit 11, if applicable), and OLE Visits 3-6. Safety and tolerability will be evaluated at each study visit by assessment of adverse events and vital signs, and at select visits, ECGs and clinical laboratory tests.

6.2. STUDY PROCEDURES

The investigator, a designated and appropriately trained staff member, or the subject will perform the study assessments according to the schedule of assessments (Section 6.3). The same staff member should perform the assessments for a given subject throughout the study. If this becomes impossible, an appropriate designee with overlapping experience with the subject and study should perform the assessments. The same lighting conditions and subject positioning should be used for all evaluations for a given subject.

6.3. Schedule of Assessments

Double-Blind Period	Screening	Baseline	Treatment								Post-Treatment
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10[14]	Visit 11[14]
Week		0	1	2	4	8	12	16	20	24	28
Treatment Day	-30 to 0	1	8	15	29	57	85	113	141	169	197
Treatment Window(days)	N/A	N/A	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3
Informed consent/ assent[1]	X									X[14]	X[14]
Inclusion/exclusion criteria	X	X									
Physical exam[2]	X									X	
Demographics & medical history	X										
Alopecia Areata History	X										
Vital signs[3]	X	X	X	X	X	X	X	X	X	X	X
Clinical CBC, Chemistry, Virology, Serum Pregnancy, Urinalysis[4]	X[4]	X		X	X	X	X	X	X	X	X
Urine pregnancy test[5]		X			X	X	X	X	X	X	X
ECG	X	X		X	X	X	X	X	X	X	X
SALT Score (prior to ALODEX)[6]		X			X	X	X	X	X	X	X
ALODEX Score (after SALT)[7]		X			X	X	X	X	X	X	X
Target Scalp Patch Identification[8]		X									
Assessment of hair quality[9]		X			X	X	X	X	X	X	X
Alopecia Appearance Assessment (AAA): Clinician Rating (CR) **All Patches, Target Patch**[10]		X			X		X			X	
Alopecia Appearance Assessment (AAA): Subject Rating (SR) **All Patches, Target Patch**[10]		X			X		X			X	
Physician Global Impression of Severity (PhGIS)[10]		X			X		X			X	
Physician Global Impression of Change (PhGIC)										X	
Subject Global Impression of Severity (SGIS) [10]		X			X		X			X	
Subject Global Impression of Change (SGIC)										X	
Subject Global Impression Treatment Satisfaction (SGITS)							X			X	
DLQI[10]		X			X		X			X	
Alopecia Impact Assessment (AIA): Subject Rating[10]		X			X		X			X	
Photography (complete scalp and target patch) [10]		X			X	X	X	X	X	X	X
Subject randomization		X									
Subject instructions[11]		X	X	X	X	X	X	X	X	X	
Dispense, collect, weigh study medication, assess compliance [12]		X	X	X	X	X	X	X	X	X	
In office study medication application[13]		X									
Concomitant therapies		X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X

Double-Blind Period	Screening	Baseline	Treatment								Post-Treatment
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10[14]	Visit 11[14]
Week		0	1	2	4	8	12	16	20	24	28
Treatment Day	-30 to 0	1	8	15	29	57	85	113	141	169	197
Treatment Window(days)	N/A	N/A	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3
Select Sites Only[16]											
4 mm Punch biopsy[17]		X			X					X	
Suture removal, if applicable[18] (Approximately 2-weeks after punch biopsy)				X	X[16]					X[16]	
Blood for immunologic studies[19]		X			X					X	
Schedule Telephone Call to occur the Day Prior to Visit 5, and Visit 10[20]				X					X		

Open-Label Extension	Open-Label Treatment						Post-Treatment
	OLE Visit 1		OLE Visit 2	OLE Visit 3	OLE Visit 4	OLE Visit 5	OLE Visit 6
Week	OLE Visit 1 occurs at Visit 10 or 11	Visit 11 (Week28) was:	2	4	12	24	28
Treatment Day		≤ 30 Days / > 30 Days	15	29	85	169	197
Treatment Window(days)	NA	NA / NA	± 3	± 3	± 7	± 7	± 7
Informed Consent/ Assent	X	X / X					
Inclusion/exclusion criteria	X	X / X					
Physical exam[2]		/ X					X
Medical History Update		/ X					
Vital signs[3]		X / X	X	X	X	X	X
Clinical CBC, Chemistry, Virology, Serum Pregnancy, Urinalysis[4]		/ X	X	X	X	X	X
Urine pregnancy test[5]		X / X	X	X	X	X	X
ECG							X
SALT Score (prior to ALODEX)[6]		X[15] / X		X	X	X	X
ALODEX Score (after SALT)[7]		X[15] / X		X	X	X	X
Alopecia Appearance Assessment (AAA): Clinician Rating (CR) **All Patches, Target Patch**					X	X	X
Alopecia Appearance Assessment (AAA): Subject Rating (SR) **All Patches, Target Patch**					X	X	X
Subject Global Impression Treatment Satisfaction (SGITS)						X	
Photography (complete scalp and target patch)		X[15]		X	X	X	X
Subject instructions[11]	X	X	X	X	X	X	
Dispense and weigh study medication[12]	X	X	X	X	X	X (Weigh only)	
Collect study medication, assess compliance			X	X	X	X	
Concomitant therapies		X	X	X	X	X	X
Adverse events		X	X	X	X	X	X

[1]A written, signed ICF/ assent must be obtained from each subject prior to performing any study related procedure (*i.e.*, prior to performing vital signs, standardized photography, biopsies, clinical laboratory sampling or urine pregnancy tests.) An OLE ICF must be obtained prior to performing any OLE assessments.

[2] A physical exam includes: general appearance, extremities, examination of head, eyes, ears, nose and throat, respiratory, cardiovascular, abdominal, neurological, musculoskeletal, lymphatic and skin assessment.

[3]Vitals signs include: oral or ear temperature, blood pressure, heart rate, respiration rate (height and weight at baseline only).

[4] Clinical laboratory sampling include: CBC, Chemistry with lipids, Urinalysis. Quantiferon Gold, HIV, Hepatitis B and C, and Serum Pregnancy should be done at screening visit only.

[5] UPT must be performed in WOCBP prior to randomization at Baseline (Visit 2) and must be negative for the subject to continue in the study. UPTs in WOCBP must also be obtained at Visits 5, 6, 7, 8, 9, 10, 11 and if applicable, OLE Visit 1, and OLE Visit 2-6. WOCBP must have a negative serum pregnancy test at Screening (Visit 1) and a negative UPT at baseline (Visit 2) prior to randomization. WOCBP must have a negative UPT prior to continuation into the OLE.
[6] SALT score must be determined prior to ALODEX using device provided. At Baseline (Visit 2), SALT score should be determined prior to application of study medication.
[7] ALODEX score determined after SALT score using device provided. At Baseline (Visit 2), ALODEX score should be determined prior to application of study medication.
[8] The subject will be instructed to identify the most bothersome patchy area- this will be the Target Patch. At Baseline (Visit 2), the Target Patch should be identified prior to application of study medication.
[9] The investigator will assess active hair loss through the hair pull test and the presence of exclamation point hairs. At Baseline (Visit 2), the assessment should be determined prior to application of study medication.
[10] At Baseline (Visit 2), study assessments must be performed prior to application of study medication.
[11] The study staff must instruct the subject to apply study medication according to the instructions in APPENDIX 1, 2 and 3.
[12] The study staff must weigh the study medication bottle(s) with cap prior to dispensing to the subject and after the study medication bottles are returned to the investigational site. Staff should review the usage based on the weight of the bottle and counsel the subject as necessary.
[13] At Baseline (Visit 2), the first application of study medication should be performed by the subject under the supervision of the study staff and the subject should be observed for 20 minutes after the initial application.
[14]At Week 24 (Visit 10) or Week 28 (Visit 11), the subject may be given the option to move into the OLE treatment. • If the subject declines the OLE, Visit 11 (Post-treatment) will be the last double-blind study visit. • If the subject chooses to continue into the OLE, she/he must sign an OLE ICF, complete Visit 10 or Visit 11 as appropriate and OLE Visit 1, prior to being dispensed open-label drug and receiving study medication application instructions. The subject will be scheduled for OLE Visit 2. • If the subject enrolls in open-label treatment within 30 days of completion of Week 24 (Visit 10) or Week 28 (Visit 11), OLE Visit 1 will be conducted to obtain consent/assent and perform the procedures in the Schedule of Assessments. This will be considered the official date that the subject initiated OLE treatment. • If the subject enrolls in open-label treatment > 30 days after completion of Week 28, OLE Visit 1 will be conducted to obtain consent/ assent, and complete the procedures detailed in the Scheduled of Events. This will be considered the official date that the subject initiated OLE treatment and the scheduling of subsequent visits will be based on this date.
[15] SALT, ALODEX and Photography is performed at OLE Visit 1 (if Week 28 is < 30 days) only if there is a significant change from the last double-blind study visit.
Select Sites Only[16]
[16] At Baseline (Visit 2) the scalp biopsy and blood samples for immunologic evaluations must be performed prior to study medication application. At Week 4 (Visit 5), and Week 24 (Visit 10), the scalp biopsy and blood samples for immunologic evaluations must be performed prior to study medication application. The evening prior to the Week 4 (Visit 5), and Week 24 (Visit 10) visits, the subject should be instructed to apply study medication approximately 12 hours prior to Week 4 (Visit 5), and Week 24 (Visit 10) visit time and to note the time of application. Subjects should be instructed not to apply study medication before the Week 4 (Visit 5), and Week 24 (Visit 10) visits.
[17] The biopsy will be performed by the investigator or designee using a 4mm punch biopsy per instructions in APPENDIX 11. The Baseline/Day 1 (Visit 2) biopsy must be obtained prior to the first application of study medication. Subjects must be instructed not to apply study medication to the biopsy site until instructed to by the investigator or designee. Study assessments including photography should be completed prior to the biopsy. (Select sites only)
[18] Sutures will be removed approximately 2 weeks following biopsy unless absorbable sutures are used precluding the need for suture removal. (Select sites only)
[19] Blood samples for immunologic studies will be obtained prior to study medication application on Baseline (Day 1/ Visit 2), Week 4 (Visit 5), and Week 24 (Visit 10). (Select sites only)
[20] A member of the study staff should schedule a reminder call with the subject to occur on **the day before** Week 4 (Visit 5), Week 8 (Visit 7), and Week 24 (Visit 10) to remind the subject to apply the study medication approximately 12 hours prior to the visit and to hold the morning dose of study medication. The subject should be instructed to document the time of application and quantity of the dose applied.

6.4. Duration of the Study

Double-blind Period

The anticipated time for study enrollment is 6 months. The duration of the screening period could be up to 30 days. Once randomized, the maximum duration of double-blind study treatment from Baseline (Visit 2) to the post-treatment follow-up visit at Week 28 (Visit 11) is anticipated to be a maximum of 200 days (197 days + 3-day window).

Open-Label Extension

The maximum duration of the of study treatment in the OLE is 176 days (168 days + 7-day window). The maximum duration of the study participation from entry into the OLE to completion of the post-treatment follow up visit is 204 days.

7. STUDY TREATMENT

7.1. Investigational Study Medication

The study medications for this study are ATI-50002 Topical Solution in dosage strengths of 0.46% and 0.12% and Vehicle Topical Solution. The study medications are formulated as thin clear solutions that are indistinguishable as packaged and labelled. The inactive ingredients include: water, transcutol P, propylene glycol, PEG400, dimethyl sulfoxide (DMSO), kolliphor CS 20, benzyl alcohol, poloxamer 188, and povidone K30.

STUDY MEDICATION INFORMATION				
Study medication name	**Double-Blind Period**			**Open-Label Period**
	ATI-50002	**ATI-50002**	**Vehicle**	**ATI-50002**
Dosage Strength	0.46%	0.12%	-	0.46%
Manufacturer	PMRS, Inc., Horsham, PA			
Pharmaceutical Form	Topical Solution			
Container	Amber Glass Bottle, 120 mL with screw cap			
Storage Conditions	59°F to 77°F (15°C to 25°C)			
Dose regimen				
Route	Topical			
Frequency	Twice-daily			
Duration of administration	24 weeks			24 Weeks
Other supplies	Disposable, single-use droppers will be provided.			

7.2. Subject Randomization

Prior to the start of the study, Aclaris Therapeutics, Inc. or a designated third party will generate a list of randomization numbers that shall be transmitted to the assigned clinical packaging organization for study medication labeling. The randomization list will be stored with access limited to designated personnel for study medication labeling. The randomization list will be made available, as appropriate, to unblind the database.

In the double-blind period, subjects will be assigned to 1 of the 3 treatment groups in a random manner and at a 1:1:1 ratio. At Baseline (Visit 2), an investigational center staff member will assign study medication to eligible subjects by selecting an appropriate Subject Kit. The staff member must select Subject Kits in chronological sequence and in an ascending numerical order starting with the lowest available Subject Kit number. No Subject Kit number may be omitted or reused. The Subject Kit number is the randomization number.

The investigational staff member randomizing the subject will enter the subject identifier, subject initials, and date randomized on both parts of the Subject Kit label, remove the tear-off part, attach it to the subject's study medication eCRF label page and record the Subject Kit number in the subject's eCRF.

In the open-label period, all subjects will receive the dosage strength of 0.46%.

7.3. Application of Study Medication

For participants < 18 years of age, the parent and study staff should assess the subject's ability to apply study medication to the scalp for the duration of the study. For subjects who are not able to apply study medication to their scalp, a parent will apply the study medication using gloves. Subjects or their parents will be instructed to apply a thin film of ATI-50002 Topical Solution or matching vehicle up to 4-mLs, twice-daily; once in the morning and approximately 12 hours later to the entire scalp following the instructions in APPENDIX 1. The subject or parent must wash her/his hands thoroughly before and after each study drug application. At each study visit, subjects should bring the study medication including unused bottles back to the site. The disposable droppers should be disposed of at the subject's home.

Following review of study medication instructions, subjects or their parents will apply the first dose of study medication in the office under the instruction and supervision of the study staff. During the Baseline visit (Visit 2), the study staff member will:

- Dispense the appropriate study medication bottle.
- Weigh the bottle with the cap prior to the first study medication application.
- Instruct the subject on the appropriate application technique following instructions in APPENDIX 1.
- For select sites only: Instruct the subject not to apply study medication to the biopsy site.
- Observe the subject's first study medication application to ensure proper coverage and monitor the subject for at least 20 minutes after application.
- Record the quantity in mLs of study medication the subject applied to cover the entire scalp in the source document, eCRF and on the subject instruction sheet.
- After the first application, the study staff should weigh the study bottle with the cap.
- Provide feedback on the application procedure, if needed.

In the OLE, in addition to applying study medication to the entire scalp twice-daily (or patchy areas with a ½" margin if the subject did not agree to apply to the entire scalp) subjects with eyebrow loss will be allowed to apply a thin film of study medication twice-daily to the affected eyebrow(s) as detailed in APPENDIX 3. Subjects should be instructed to apply the study medication to the entire natural eyebrow(s) and not just the patches.

7.3.1. Subjects Who Signed Consent Prior to Amendment 5

Subjects who signed the ICF prior to amendment 5 and do not consent to twice-daily applications to the entire scalp may continue in the study. These subjects should be given the option to apply the study medication to the entire scalp at night only, and the patchy areas during the day or to the patchy alopecia areas and a ½" margin around the areas twice-daily. The areas being treated must be identified in the source documentation and the eCRF as:

- patchy areas only twice-daily,
- entire scalp twice-daily or
- patchy areas only once-daily, and entire scalp once-daily.

If the subject develops a new patch of hair loss during the study (e.g. areas of the scalp with active shedding) the investigator will instruct the subject to treat the new area of hair loss. The investigator must incorporate the new area of hair loss, once it is clinically evident, into the SALT and ALODEX score assessments. The investigator must determine if additional study medication is required to cover the new patchy area(s) and if applicable, document the new amount of study medication to be applied in the source documents and on the subject instruction sheet. If a subject believes symptoms indicate a new area of impending hair loss, but no hair loss is clinically apparent, the area will be noted in the source document, but will not be treated or included in SALT or ALODEX assessment until clinically apparent as either thinned or alopecic patch. New patches of hair loss that develop during the study are not considered an adverse event.

7.4. Study Medication Weights

A study staff member will weigh the study medication bottles with the cap prior to dispensing to the subject and after collection of the study medication bottles during study treatment visits. Bottles that are not dispensed to the subject do not need to be weighed.

7.5. Treatment Compliance

The investigator or designee will be responsible for monitoring subject compliance through questioning the subject, documenting missed doses, if any, weighing the bottle before dispensing and after return and visual inspection of the quantity in the study medication bottles (used and unused). Study staff will counsel the subjects, as required to make sure subjects are compliant with study medication applications.

7.6. Dose Modification

If the subject experiences application site AE(s), study medication may be stopped for up to a total of 3 days or the frequency of study medication application may be reduced from twice-daily to once-daily for up to a total of 6 days. Dose modifications greater than these must be reviewed with the Medical Monitor. Refer to Section 9.2.4 for Study Medication Interruption or Discontinuation due to related SAEs or specific abnormal laboratory values.

7.7. Study Medication Accountability

The Principal Investigator or designee is responsible for ensuring accountability for the investigational agent, including reconciliation of medications and maintenance of medication records. Upon receipt of study medication, the clinical site will check for accurate delivery and acknowledge receipt by signing (or initialing) and dating the documentation provided. One copy of this document will be returned to Aclaris Therapeutics, Inc. (or designee) and one copy will be maintained in the study file. In addition, an accurate study medication disposition record will be kept, specifying the amount dispensed for each subject and the date of dispensing. This inventory record will be available for inspection at any time. At the completion of the study, the original inventory record will be available for review by Aclaris Therapeutics, Inc. upon request. Final medication accountability will be performed by the study monitor at the completion of the study and all used and unused study medication bottles will be returned to Aclaris Therapeutics, Inc. or designee for disposal.

7.8. Other Study Supplies

Aclaris Therapeutics, Inc. or a third party vendor will provide:

- Supplies and instructions for collecting, labeling, shipping and result reporting for the clinical laboratory tests and urine pregnancy tests.
- A scale to weigh the study medication bottles.
- Equipment, supplies and training for taking standardized photographs.
- An iPad for recording the SALT and ALODEX score.
- Hair clips and Insulated lunch bags.
- Equipment, supplies and training for performing standardized ECGs.
- Select sites only: Supplies and instructions for collecting the scalp biopsies and blood tests.
- Gloves

7.9. Blinding

Blinding of study medication is important for validity of this study. This double-blind period of the study uses a double-blind design. The study medications are indistinguishable in appearance, as packaged and labeled.

7.9.1. Unblinding the Study Medication

During the double-blind period, the blinding may be broken ONLY in the event of a medical emergency, in which knowledge of the study medication identity is critical to the management of the subject's course of treatment. Before breaking the blind, the investigator shall determine that the information is necessary (*i.e.*, that is, will alter the subject's immediate course of treatment). In many cases, particularly when the emergency is clearly not study medication-related, the problem may be effectively managed by assuming that the subject is receiving an active study medication without the need for unblinding.

If the investigator deems it necessary to break the blind for a study subject, he/she will attempt to contact the Medical Monitor (protocol page 1) to obtain permission. If it is not possible to contact the Medical Monitor beforehand, contact her/him as soon as possible after breaking the blind for a subject.

To identify a subject's study medication, locate the second panel of the tear-off label from the Subject Kit attached to the subject's study medication label page and follow the instructions on the label. Record the date of unblinding, the reason for unblinding, and the initials of the investigational staff member who performed the unblinding on the subject's study medication label page. Any subject whose blind has been broken must be discontinued from the study (Section 5.9).

At the end of the study, the original study medication label pages will be returned to Aclaris Therapeutics, Inc. with a photocopy placed in the investigator's study file. The original study medication label pages will be available, upon request, to the site if needed to respond to a regulatory audit.

7.10. Study Medication Packaging, Labeling, Storage and Security

The study medication must be used by the study subjects only. Investigational site staff will explain the application of the study medication to subjects.

Study medication will be provided by Aclaris Therapeutics, Inc. and labeled according to regulations. Study medications must be stored in a secure area with limited access under appropriately controlled and monitored storage conditions. Study medication should be stored at controlled room temperature 59°F - 77°F (15°C – 25°C). Subjects will be instructed to store the study medication in the original glass bottle (in the carton provided) at room temperature, away from heat, moisture, direct light, and to keep it from freezing and out of the reach of children.

The study medication will be supplied in amber glass 120 mL bottles. Disposable droppers with 1 mL calibration mark will be provided in the study kits.

For the double-blind period, two Subject Kit boxes will be provided. Each Subject Kit box will contain 15 bottles in individual boxes and disposable droppers. Each kit will be labelled with a two-part, three panel, double blind label. One part (one-panel) of the label remains attached to the Subject Kit, the other part (two-panel tear-off) is separated and attached to the subject's study medication eCRF label page when the subject is randomized. Each box and bottle will be labeled with a single panel label.

For the open-label period, subjects will receive approximately 1-3 bottles of ATI-50002-Topical Solution, 0.46% per month. Each bottle is numbered and packaged in a carton and both the bottle and carton will be labelled with a single-panel label.

8. STUDY ASSESSMENTS

Note that all subject evaluations (Alopecia Appearance Assessment, Global Impression of Severity and questionnaires) should be performed prior to any investigator assessments. However, the investigator may assist in determining the location of the target patch prior to subject assessments.

8.1. Assessments of Efficacy

8.1.1. Severity Alopecia Tool (SALT) Score

The SALT score is a measurement of the amount of scalp without any terminal hair. The investigator will assess the SALT score using an iPad provided by Aclaris at baseline and monthly during the study. The SALT score must be determined prior to the ALODEX score. At the baseline visit (Visit 2), the SALT score must be determined prior to randomization and the score must be at least 15% to 95% total scalp hair loss. Equipment, supplies, training and the detailed Reference Guide will be provided to the investigational site prior to the initiation of subject enrollment.

8.1.2. Alopecia Density and Extent Score (ALODEX)

The ALODEX score is a measurement of the amount of scalp with terminal hair loss. The investigator will calculate the ALODEX score using the iPad provided by Aclaris at baseline and monthly during the study. The ALODEX score must be determined after the SALT score. At the baseline visit (Visit 2), the ALODEX score must be determined prior to randomization. Equipment, supplies, training and the detailed Reference Guide will be provided to the investigational site prior to the initiation of subject enrollment.

8.1.3. Alopecia Target Scalp Patch Identification

At the baseline visit (Visit 2), the investigator will ask the subject to identify the most bothersome scalp patchy area, which will be identified as the target patch. The target patch must be assessed by the subject as "no hair" or "a little hair" at Baseline (Visit 2). In subjects with extensive hair loss, the target patch may be a large scalp surface area. The target scalp patch must be identified prior to study medication application. The target patch will be documented photographically at baseline and monthly throughout the study. Equipment, supplies, training and the detailed Reference Guide will be provided to the investigational site prior to the initiation of subject enrollment.

8.1.4. Alopecia Appearance Assessment (AAA) for Patchy AA: Clinician and Subject

The appearance of the subject's patchy AA for both the target scalp patch and all affected patchy areas on the scalp will be assessed using the AAA by both the investigator and the subject at baseline (prior to study medication application) and at Day 29 (Visit 5), Day 85 (Visit 7), Day 169 (Visit 10), and OLE Visits 4-6 during study treatment. At the baseline visit, both the Clinician's and the Subject's AAA should be assessed as "no hair" or "a little hair". The Clinician's AAA is below and the Subject's AAA is in APPENDIX 6.

Alopecia Scalp Appearance Assessment for AA Patchy: Clinician Rating (CR)

Instructions for item 1: Please mark an "X" in the box (☒) that best describes the appearance of the **subject's target patch** right now. Please select the one response that best represents your answer.

☐ Full hair, scalp of the target patch completely covered with hair
☐ Most hair, scalp of the target patch mostly covered with hair

☐Some hair, scalp of the target patch somewhat covered with hair
☐A little hair, scalp of the target patch mostly exposed
☐No hair, scalp of the target patch completely exposed

Instructions for item 2: Please mark an "X" in the box (☒) that best describes the appearance of the subject's **whole scalp** right now. Please select the one response that best represents your answer.

☐Full hair, whole scalp completely covered with hair
☐Most hair, whole scalp mostly covered with hair
☐Some hair, whole scalp somewhat covered with hair
☐A little hair, whole scalp mostly exposed
☐No hair, whole scalp completely exposed

8.1.5. Global Impression of Severity

The severity of the subject's patchy AA will be assessed at baseline (Visit 2) (prior to study medication application) and at Week 4 (Visit 5), Week 12 (Visit 7), and Week 24 (Visit 10) during study treatment by both the investigator and the subject using the Global Impression of Severity Questionnaires. The Physician's scale is below and the subject's scale is in APPENDIX 7.

PHYSICIAN GLOBAL IMPRESSION OF SEVERITY (PhGIS)
Please mark an "X" in the box (☒) that best describes the severity of the patient's patchy alopecia areata right now.
Overall, how severe is the patient's patchy alopecia areata right now?
☐ Mild
☐ Moderate
☐ Severe
☐ Very Severe
☐ Extremely Severe

8.1.6. Global Impression of Change

The investigator will assess their overall impression of change for the subject's alopecia at Week 24 (Visit 10) using the 7-point Physician Global Impression of Change (PhGIC) detailed below.

Compared to the subject's hair loss at Baseline [prior to study medication initiation], the subject's AA, AT or AU is (Please mark an "X" in the box (☐):

☐1=Very much improved since the initiation of treatment;

☐2=Much improved;

☐3=Minimally improved;

☐4=No change from baseline (the initiation of treatment);

☐5=Minimally worse;

☐6= Much worse;

☐7=Very much worse since the initiation of treatment.

The investigator or study staff will instruct subjects to answer the Subject Global Impression of Change (SGIC) questionnaire at their final study visit to report their overall impression of change for their condition. Subjects will assess change in the severity of their condition on a 7-point scale from Very much improved to Very much worse; the SGIC can be found in APPENDIX 12.

8.1.7. Hair Quality Assessments

Hair quality will be assessed by the investigator using the hair pull test including the presence of exclamation point hairs at baseline (prior to study medication application) and then monthly during the double-blind period study.

8.1.7.1 Hair Pull Test

The hair pull test is performed at the edge of the alopecic patch as follows:

- Pinch 25 to 50 hairs between non-gloved thumb and forefinger and exert slow, gentle traction while sliding fingers up.
- Resulting extracted hairs should be examined with magnification and counted.
  - Normal: 1 to 2 hairs dislodged
  - Abnormal: > 2 hairs dislodged
  - Broken hairs (structural disorder)
  - Broken-off hair at the borders of an alopecic patch that are easily removable (in alopecia areata)

8.2. Biomarker Assessment (Selected Sites only)

Scalp biopsies and blood samples for immunologic evaluation will be obtained prior to study medication application at Baseline (Visit 2), Week 4 (Visit 5), and Week 24 (Visit 10). The timing of the Week 4 (Visit 5), and Week 24 (Visit 10) samples should be approximately 12 hours after the previous evening study medication application. The study staff should instruct the subject not to apply the study medication the morning of the Week 4 (Visit 5), and Week 24 (Visit 10) visits and to apply the study medication approximately 12 hours prior to the study visit. The subject should also be instructed to record the dose applied and the time. All study assessments (i.e. SALT and ALODEX score, AAA, PhGIS, SGIS, DLQI, AIA and photography) should be performed prior to the scalp biopsies.

8.2.1. Scalp Biopsy (Selected Sites Only)

Scalp biopsies will be obtained at Baseline (Visit 2) before study medication application and at Week 4 (Visit 5), and Week 24 (Visit 10) 12 hours after the evening dose of study medication for histology and RNA sequencing (APPENDIX 11). An additional unscheduled biopsy may be obtained with the subject's consent/ assent based on the investigator's assessment of worsening of alopecia or other clinically significant finding. The study staff should call the subject on the day prior to the Week 4 (Visit 5), and Week 24 (Visit 10) visits to instruct the subject to apply the study medication in the evening approximately 12 hours prior to the Week 4 (Visit 5), and Week 24 (Visit 10) visits. The subject should also be instructed to record the dose applied and the time.

8.2.2. Blood for Immunologic Evaluation (T-cells) (Selected Sites Only)

The blood samples for immunologic evaluation will be obtained at Baseline (Visit 2) and at Week 4 (Visit 5), and Week 24 (Visit 10) for measurement of the following tests including:

- T, B, NK lymphocytes, $CD4^+$, $CD8^+$ T-cells
- T-regulatory and T-cell subtypes
- RNA seq
- TCR seq

8.3. Assessment of Subject Satisfaction and Patchy AA Impact

8.3.1. Subject Global Impression of Treatment Satisfaction

The investigator or study staff will instruct the subject to answer this questionnaire in relation to their satisfaction with the outcome of the study medication response. Subjects will assess their satisfaction with the outcome of the study treatment in all treated patchy areas on a 7-point satisfaction scale from extremely satisfied to extremely dissatisfied at Week 12 (Visit 7) Week 24 (Visit 10), and if continuing in the open label extension, OLE Visit 5.
(APPENDIX 8).

8.3.2. Alopecia Impact Assessment (AIA): Subject Rating

The Investigator or study staff will instruct the subject to answer the AIA questionnaire during the study visit at Baseline (Visit 2), Week 4 (Visit 5), Week 12 (Visit 7), and Week 24 (Visit 10) (APPENDIX 9).

8.3.3. Dermatology Life Quality Index (DLQI)

The impact of patchy AA on the quality of life of subject will be assessed using the DLQI (APPENDIX 10) at Baseline (Visit 2), Week 4 (Visit 5), Week 12 (Visit 7), and Week 24 (Visit 10). The investigator or study staff will instruct the subject to answer the questions based on the scalp hair loss instead of skin.

8.4. Assessment of Safety

Safety will be assessed throughout the study by the investigator or a designated and appropriately trained staff member.

8.4.1. Vital signs

Vital signs will be measured at each visit during the study. The following items will be measured:

- Body temperature, (oral or ear)
- Pulse rate
- Respiration rate
- Blood pressure (systolic and diastolic) after the subject sits quietly for at least 5 minutes
- Height (at Visit 2 only)
- Weight (at Visit 2 only)

Any measure that is, in the opinion of the investigator, abnormal AND clinically significant (CS) must be recorded as history if found prior to the first study medication application, or as an AE if found after the first study medication application begins.

A systolic blood pressure >140mm Hg or a diastolic blood pressure >90 mmHg is considered abnormal and therefore must be defined as CS or not clinically significant (NCS) in the eCRF. A weight >300 lbs. is considered abnormal and therefore must be defined as CS or NCS in the eCRF.

8.4.2. Physical Examination

The investigator or designee will perform a physical examination for all body systems (general appearance, extremities, examination of head, eyes, ears, nose and throat, respiratory, cardiovascular, abdominal, neurological, musculoskeletal, lymphatic and skin assessment) at Screening (Visit 1), end of study Week 24 (Visit 10) and if applicable, OLE Visit 1 and OLE Visit 6.

8.4.3. Clinical Laboratory Assessments

A qualified staff member will collect non-fasting samples for clinical laboratory analysis at screening and baseline and then monthly throughout the study. Samples will be sent to ACM Global Laboratories for analysis. Refer to the study specific laboratory manual for handling and shipping instructions. The following tests will be conducted:

Chemistry Panel	**Complete Blood Count**
Albumin	Hematocrit
Alkaline phosphatase	Hemoglobin
Alanine aminotransferase (ALT)	Platelet count
Aspartate aminotransferase (AST)	Red blood cell morphology
Blood urea nitrogen (BUN)	Red blood cell count
Bicarbonate	White blood cell count
Calcium	White blood cell differential
Chloride	% & absolute
Creatinine	Basophils
Glucose	Eosinophils
Lactate dehydrogenase (LDH)	Lymphocytes
Phosphorus	Monocytes
Potassium	Neutrophils
Sodium	

Total bilirubin	**Urinalysis**
Total protein	
Uric acid	
Total cholesterol, LDL, HDL, Triglycerides	
Creatine Phosphokinase (CPK)	
Screening Only	
Virology (HepB, HCV, HIV)	
Quantiferon Gold	
Total Iron Binding Capacity (TIBC)	
Serum iron	
Serum Ferritin	
T3/T4, TSH	
Serum pregnancy	

The following clinical laboratory tests: TIBC, serum iron, serum ferritin, T3/T4, TSH are drawn at screening to rule out any underlying conditions that could be causing alopecia. The results of the clinical laboratory tests will be reported on ACM Global Laboratory's standard reports. The investigator must note NCS or CS to define the clinical relevance of any result that is outside the normal range for the laboratory. The investigator must date and initial every laboratory report.
The investigator or subinvestigator must review all the Screening (Visit 1) laboratory test results against the study entry criteria for each subject prior to Baseline (Visit 2). The investigator must report all laboratory results that are BOTH outside the normal range for the laboratory AND, in the opinion of the investigator, CS as medical history if found prior to the first study medication treatment or as an AE if found after the first study medication treatment begins. Throughout the study, the investigator must review all laboratory reports in a timely manner.

8.4.4. Pregnancy tests

Subjects who are WOCBP must have a negative serum pregnancy test result at Screening (Visit 1) to continue in the study, and a negative UPT at Baseline (Visit 2) prior to randomization. In addition, the investigator or designee will perform a urine pregnancy test for subjects who are WOCBP at monthly visits during the double-blind portion of the study and during the OLE at OLE Visit 1 (if Visit 28 > 30 days from entry to OLE), and OLE 2-6. The UPT kits provided by ACM Global Laboratories have a minimum sensitivity of 25-mIU ß-HCG/milliliter (mL) of urine. If the result of any post-treatment urine pregnancy test is positive, the subject will be withdrawn from the study and the subject's pregnancy documented and followed.

8.4.5. ECGs

Standard 12-lead ECGs will be performed by a qualified staff member at Screening (Visit 1), Baseline (Visit 2), Day 15 (Visit 4) and monthly visits during the double-blind period study and if subject is continuing in the OLE, at OLE Visit 6. The ECGs must be obtained using a standard 12-lead ECG with a 10mm/mV amplitude, at 25mm/sec and a 5 to 10-second duration. To ensure a steady heart rate the subject must rest quietly in the supine position for at least 5 minutes prior to performing the ECG.

A central lab, eResearch Technology, Inc. (ERT), will provide ECG equipment, supplies and site training. In addition, ERT will process ECGs received by the sites and report results via a secure study portal. The ECG results will be interpreted by a qualified health professional (evaluator) and the interpretation reported either directly on the tracing or in a separate report. The evaluator will interpret the results of every ECG and define the ECG as "normal" or "abnormal". Variations such as minor ST changes (*i.e.*, <0.5mm depression) and early re-polarization are considered normal.

The investigator must review the evaluator's interpretation of each subject's screening ECG prior to Baseline (Visit 2). The investigator will review the evaluator's interpretation of all ECG reports in a timely manner and comment on the clinical relevance of any result that is defined by the evaluator as abnormal.

Any abnormalities that are, in the opinion of the investigator, clinically significant, must be reported as history if found prior to the start of the first study medication application or as an AE if found after the start of the first study medication application (see Section 9.1.1).

8.5. Other Assessments

8.5.1. Demographics, Medical History and Alopecia Areata History

During the screening visit, the investigator or designee will interview each subject to obtain demographic information including date of birth, sex at birth, Fitzpatrick skin type, race and, if appropriate, ethnicity. The investigator or designee will interview each subject to obtain medical history information related to all medical conditions, surgeries and disease states that, at screening: are ongoing, require concomitant therapy or are, in the opinion of the investigator, relevant to the subject's study participation. In addition, the medical history of women who are not of childbearing potential should reflect the reason e.g. post-menopausal for 1 year or greater, bilateral tubal ligation, or hysterectomy. The investigator or designee will also obtain an AA history at screening (APPENDIX 2).

8.5.2. Photographic Assessment

A qualified investigational staff member will take standardized photographs of the scalp at baseline and then monthly during the double-blind period and if the subject is continuing in the OLE, OLE Visits 3-6 and OLE Visit 1 (according to the Schedule of Assessments), if there is a significant change from the last double-blind study visit. The photographs are to document the baseline hair loss and hair growth during treatment. In addition, a target patch will be identified and photographed. Equipment, supplies, training and detailed instructions for obtaining and managing photographs will be provided by Canfield Scientific to the investigational center prior to the initiation of subject enrollment.

9. ADVERSE EVENTS

Adverse events will be monitored throughout the study and reported on the appropriate Aclaris Therapeutics, Inc. AE eCRF.

9.1. Definitions

9.1.1. Adverse events (AE)

An AE is any untoward medical occurrence in a subject administered a study medication(s) and which does not necessarily have a causal relationship with the study medication. An AE can therefore be any unfavorable and unintended sign or symptom associated with the use of a study medication (including an abnormal laboratory finding), whether or not related to the study medication.

Thus, any new, clinically significant worsening of an existing sign, symptom or disease, should be considered an AE.

Every new episode or clinically significant worsening of a chronic condition (*e.g.*, headaches, seasonal allergies, depression, or hypertension) should be reported as a separate AE, even if the condition is reported in the subject's medical history.

The investigator should, when certain, report a diagnosis rather than the signs, symptoms or clinically significant abnormal laboratory values associated with the AE. Otherwise, signs, symptoms or abnormal laboratory values may be used to describe the AE.

Any CS abnormality discovered prior to the first study medication treatment should be reported as medical history, not as an AE.

9.1.2. Serious adverse event (SAE)

A Serious Adverse Event is any untoward medical occurrence that at any dose:

- Results in death
- Is life threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect
- Is an important medical event.

The term "life threatening" refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event that hypothetically might have caused death if it were more severe.

Inpatient hospitalization is considered to have occurred if the subject is admitted to the hospital on an in-patient basis, even if released the same day. Prolongation of hospitalization is defined as an additional night stay in the hospital. Hospitalization for a diagnostic test (even if related to an AE) or elective hospitalization that was planned before study enrollment (signing the ICF) are not themselves reasons for an event to be defined as a SAE.

Important medical events are those that may not be immediately life threatening, result in death or hospitalization, but are clearly of major clinical significance and may jeopardize the subject or require intervention to prevent one of the outcomes listed in the SAE definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an

emergency room or at home for allergic bronchospasm, blood dyscrasia or convulsions that do not result in hospitalization.

9.1.3. Unexpected adverse event

An AE is considered unexpected if it is not listed in the Investigator Brochure or is not listed at the specificity or severity that has been observed.

9.1.4. Adverse event reporting period

The investigator must start reporting non-serious AEs with the subject's first study medication application and continue reporting until the end of the subject's last study visit. Reporting for SAEs must start when the subject signs the ICF and continue until the end of the subject's last visit.

9.1.5. Severity

The investigator is to define the severity of each AE using the following definitions as a guideline. The investigator will consider the range of the possible severity of the event and identify the severity that is the most appropriate according to her/his medical judgment.

Mild – Awareness of signs or symptom, but easily tolerated

Moderate – Discomfort, enough to cause interference with usual activity

Severe – Incapacitating with inability to perform usual activity.

9.1.6. Relationship to study medication

The investigator will determine if there is a reasonable causal relationship between the study medication and an AE or not. The investigator will use her/his best medical judgment and consider all relevant factors (*e.g.*, temporal relationship, location of the event, the subject's relevant medical history, concomitant therapies and concurrent conditions) to determine the relationship of the AE to the study medication. The investigator will define the relationship of an AE to the study medication by selecting one of the following categories:

Related – There is a reasonable causal relationship between the study medication and the AE.

Not Related – There is not a reasonable causal relationship between the study medication and the AE.

The expression "reasonable causal relationship" is meant to convey in general that there are facts (evidence) or arguments to suggest a causal relationship (International Conference on Harmonization [ICH] E2A).

9.2. Reporting Procedures

9.2.1. Procedures for reporting adverse events

At each post-enrollment visit, the investigator will question the subject to elicit AEs using a non-directive question such as "Has there been any change in your health since the previous study visit?" If appropriate, based on the subject's response to non-directed questioning to elicit AEs, the investigator will follow-up with directed questions and appropriate evaluations.

Any AE noted during the reporting period must be reported in the source documents and on the appropriate AE eCRF. AEs that are defined as "Not Related" to the study medications will be followed until they are resolved or until the subject's last study visit. AEs that are defined as "Related" to the study medications will be followed until they are resolved or, if not resolved after the subject's last study visit, until in the opinion of the investigator, the AE reaches a clinically stable outcome with or without sequelae.

9.2.2. Procedure for reporting a serious adverse event

Upon becoming aware of a SAE occurring during the AE reporting period, whether or not related to the study medications, the investigator must:

1. Take the appropriate medical action to ensure the subject's safety.
2. Immediately inform the Safety Monitor of the SAE by email, ensuring that the subject information is deidentified (only subject initials and subject number) to:
    - **ProPharma, Email:** clinicalsafety@propharmagroup.com**.**
    - **Fax: (866)-681-1063**
3. Print a copy of the email confirmation from ProPharma and place in the study file.
4. Within 24-hours complete, as fully as possible, an AE eCRF and an SAE form; e-mail the forms and any other relevant information (*e.g.*, concomitant medication eCRF, medical history eCRF, laboratory test results) to ProPharma (Aclaris Therapeutics, Inc. Safety Monitor).
5. Monitor and document the progress of the SAE until it resolves or, if not resolved after the subject's last study visit, until in the opinion of the investigator the AE reaches a clinically stable outcome with or without sequelae AND the investigator and Aclaris Therapeutics, Inc. Safety and Medical Monitor agree that the SAE is satisfactorily resolved.
6. Inform the Aclaris Therapeutics, Inc. Safety Monitor of SAE updates, via telephone, followed by an SAE form update sent by e-mail.
7. Comply with the appropriate regulatory requirements and Aclaris Therapeutics, Inc. instructions regarding reporting of the SAE to the responsible Institutional Review Board (IRB) or Ethics Committee (EC).

9.2.3. Safety Monitoring Discontinuation Criteria

Any subject who develops any of the following ECG findings during the active treatment phase will be instructed to stop study medication and will be withdrawn from the study:

- A post-study medication ECG result where the evaluator's interpretation shows any of the following:
    - Clinically significant rhythm disturbance other than sinus rhythm or ectopic supraventricular rhythm (ectopic atrial rhythm)
    - Clinically significant conduction disturbance including PR >240msec, pre-excitation (delta wave and PR <120msec), second degree or higher AV block
    - New finding of QRS>120ms (if not present at screen. For example, subjects with Right Bundle Branch Block at screening would not need to be withdrawn from the study if their subsequent ECGs remained unchanged).
    - Evidence of QT-interval prolongation, defined as an increase in the QT_cF interval>60ms from Visit 1
    - Acute signs of ischemia or infarction
    - Any ECG abnormality which may, in the opinion of the investigator, represent a new medical issue of concern

Site staff must perform protocol-required procedures for trial discontinuation and follow-up.

9.2.4. Study Medication Interruption and Discontinuation Criteria

9.2.4.1 Study Medication Interruption

Treatment with ATI-50002 Topical Solution should be temporarily interrupted in the event of severe adverse events considered related to ATI-50002, or in the event of one or more of the abnormal laboratory values in Table 2.

Table 2: Study Medication Interruption Criteria

Laboratory Test	Hold Study Medication if:	Resume Study Medication if:
WBC count	$< 2 \times 10^9$/L	$\geq 2.5 \times 10^9$/L
ANC	$< 1 \times 10^9$/L	$\geq 1.5 \times 10^9$/L
Lymphocyte count	$< 0.5 \times 10^9$/L	$\geq 0.75 \times 10^9$/L
Platelet count	$< 75 \times 10^9$/L	$\geq 100 \times 10^9$/L
Hemoglobin	< 8 g/dL or a decrease > 2g/dL	≥ 10 g/dL
AST or ALT	> 3 x ULN	< 2 x ULN or within 20% of Baseline values
Serum creatinine	>2 x ULN	<1.5 x ULN or within 10% of Baseline value

If a subject has one or more of the abnormal laboratory values noted in Table 2, the investigator or designee upon receipt and review of the central laboratory report should instruct the subject to hold study medication applications. The investigator or designee should ask the subject about symptoms, concomitant illnesses and medications and repeat the test(s) as soon as possible. The Medical Monitor must be notified of dose interruptions due to SAEs considered related to study medication or laboratory abnormalities noted in Table 2.

If the retest confirms the abnormal laboratory value, then the study medication should continue to be held followed by repeat testing once a week or sooner at the discretion of the investigator. The subject should be followed until the laboratory abnormality(s) return to normal or to baseline values.

9.2.4.2 Study Medication Discontinuation

Study medication should be permanently discontinued in the event of any of the following:

- Severe infection requiring parental antimicrobial therapy or hospitalization
- Symptomatic herpes zoster
- Malignancy – except for non-melanoma skin cancer (SCC or BCC) not in or near the treatment area
- Anaphylactic or severe allergic reaction
- WBC Count: $< 1 \times 10^9$/L or second occurrence of $< 2 \times 10^9$/L
- ANC:$< 0.5 \times 10^9$/L or second occurrence of $< 1 \times 10^9$/L
- Lymphocyte count:$< 0.3 \times 10^9$/L or second occurrence of $< 0.5 \times 10^9$/L
- Platelet count: $< 50 \times 10^9$/L or second event of $< 75 \times 10^9$/L - in each case, value should be confirmed by retesting before treatment discontinuation

- Hemoglobin: < 6.5 g/dL or second occurrence of < 8 g/dL - in each case, value should be confirmed by retesting before treatment discontinuation
- AST or ALT:
  - > 5 x ULN persisting for 2-weeks of study medication interruption or second event of > 5 x ULN
  - > 3 x ULN with total bilirubin >2 x ULN or symptoms of hepatocellular injury (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/ or eosinophilia (>5%).
- Serum creatinine: > 2 x ULN persisting for >2 weeks of treatment discontinuation or second occurrence of > 2 x ULN

The continued treatment of subjects who experience other serious or severe adverse events considered related to study treatment should be discussed with the Sponsor's medical monitor.

10. PREGNANCY

10.1. Definition of Women of Child Bearing Potential (WOCBP)

WOCBP includes any female who has experienced menarche and who has not undergone successful surgical sterilization (*e.g.*, hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal. Postmenopausal is defined as ≥12 months with no menses without an alternative medical cause. WOCBP must have a negative serum pregnancy test at screening and a negative UPT at baseline prior to randomization.

10.2. Highly Effective Methods of Birth Control

The Investigator or subinvestigator will discuss the potential risk factors associated with pregnancy and the importance of maintaining a highly effective method of contraception throughout the study with all WOCBP (for example, those which result in a low failure rate - i.e., less than 1% per year-when used consistently and correctly). All WOCBP must use a highly effective method of birth control during the study and for 30 days after the final application of study medication in a manner such that risk of failure is minimized.

Highly effective methods include:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - oral
  - intravaginal
  - transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable
  - implantable
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- vasectomized partner[1]
- sexual abstinence[2]

[1]Vasectomized partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomized partner has received medical assessment of the surgical success.
[2] Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

WOCBP must be on a highly effective method of birth control for the following timeframes prior to study entry:

- Implants (on a stable dose for ≥30 days)
- Injectables (on a stable dose for ≥30 days)
- Patches (on a stable dose for ≥30 days)
- Combined oral contraceptives (on a stable dose for ≥30 days)
- Intrauterine devices (inserted for ≥30 days).

Prior to trial enrollment, WOCBP must be advised of the importance of avoiding pregnancy during trial participation and of the potential risk factors associated with pregnancy while in the study. The subject must sign an informed consent/ assent form documenting this discussion. During the trial, all WOCBP will be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period).

If a subject or investigator suspects that the subject may be pregnant prior to study medication administration, the study medication must be withheld until the results of a pregnancy test are available. If pregnancy is confirmed, the subject must not receive study medication and must be discharged from the study.

If, following study medication administration, it is determined that the subject may have been or was pregnant at the time of study medication exposure (including at least 2 days after study medication administration) the investigator must immediately notify the Aclaris Therapeutics, Inc. Medical Monitor and record the event on a pregnancy surveillance form. While not an AE or SAE, the investigator must report every pregnancy using a pregnancy surveillance form and follow the reporting procedures described for SAE reporting.
Protocol-required procedures for trial discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (*e.g.*, x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the investigator must report to Aclaris Therapeutics, Inc.'s Medical Monitor on the pregnancy surveillance form, follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants should be followed for a minimum of six weeks.

11. STATISTICAL ANALYSES

The primary analysis will be conducted when all subjects have completed the double-blind period.

11.1. Sample Size and Power Calculations

The planned sample size is 120 subjects. Since this is the first multicenter study evaluating the effect of ATI-50002 Topical Solution in subjects with stable patchy alopecia areata, the sample size was based upon feasibility issues rather than on a formal power calculation. However, data from 120 subjects (40 per group), utilizing LOCF for missing data, provides 80% power to detect a 24-point difference in percent change from baseline in SALT score between any two treatment groups. This power computation was based upon the assumed standard deviation of 38%. Data from a previous study NCT02197455 showed that the standard deviation in percent change from baseline in the SALT score is 30.3%. The 38% standard deviation assumption used for this power computation was chosen to account for the increase in variance due to the lower baseline SALT entry criteria for this study as compared to NCT02197455.

11.2. Analysis Populations

The Intent to Treat (ITT) population is defined as all subjects who are randomized. The Safety population is defined as all randomized subjects who applied at least 1 application of study medication. The Per-Protocol (PP) population is defined as all subjects who are randomized and do not have any major protocol violations. Major protocol violations are defined as:

1. Inclusion or Exclusion criteria not met
2. Concomitant medications taken during the study that interfere with efficacy
3. Did not apply at least 75% of study medication
4. Did not complete Week 24 (Visit 10).

11.3. Demographic and Baseline Characteristics

Subject demographic and baseline characteristics, including medical and alopecia history, prior medications and therapies and physical examination findings will be summarized using descriptive statistics.

For continuous variables, descriptive statistics (number, mean, standard deviation, standard error, median, minimum, and maximum) will be provided. For categorical variables, subject counts and percentages will be provided. Categories for missing data will be presented, if necessary.

11.4. Efficacy Analyses

11.4.1. Primary Efficacy Analyses

The primary efficacy variable will be the percent change from baseline in the SALT score at Week 24 (Visit 10). This represents the percentage of hair regrowth. It will be calculated as the mean of the changes from baseline (Visit 2) SALT score to Week 24 (Visit 10) SALT score, divided by the baseline SALT score and expressed as a percentage. Missing data for the primary endpoint will be imputed using Last Observation Carried Forward (LOCF) methodology. The analysis will be conducted on the relative percent change from baseline over time including all visits using a mixed effect model repeated measures (MMRM). The adjusted mean (LS Mean) for percent change from baseline in the SALT score at each visit, estimated from the MMRM, with the estimated standard error and 95% confidence interval (CI), will be presented in tabular and graphic

format. The difference in the adjusted means between treatment groups and the associated 95% CI of the difference will be provided. No adjustment for multiplicity will be made. The model will include fixed effect terms for treatment, study visit, baseline SALT score group (<50, >=50), and treatment by SALT group interaction. The primary analysis will be based on the Intent-to-Treat (ITT) population. Statistical significance for primary efficacy endpoint will be declared if at least one treatment group comparison at the Week 24 visit has a two-sided p-value less than or equal to 0.05.

11.4.2. Secondary Efficacy Analyses

A secondary sensitivity analysis of the primary endpoint will be conducted using the PP population. This analysis will use the same methodology as described for the primary analysis just applied to the PP population.

The secondary efficacy endpoints include: the percent change from baseline in the ALODEX score (percent regrowth); the mean change from baseline in SALT and the mean change from baseline in ALODEX, the proportion of subjects achieving a ≥ 50% hair regrowth compared with baseline using a separate model for SALT and for ALODEX scores; change from baseline in the AAA (Clinician and Subject), PhGIS, SGIS, AIA, treatment satisfaction questionnaires; and DLQI. Various secondary responder efficacy endpoints will be defined based upon the SALT score, the ALODEX scores and the AAA (Clinician and Subject) questionnaires. Details of these responder definitions will be specified in the statistical analysis plan (SAP). Global Impression of Change (Clinician and Subject) at Week 24 (Visit 10) will be used in the psychometric evaluation of the scores from the AAA (CR and SR), respectively. All secondary efficacy endpoints will be analyzed using the ITT population. The analysis of the percent change in ALODEX score analysis will use the same methodology as specified for the primary efficacy analysis. Other parameters will be analyzed as detailed in the SAP.

11.5. Safety Analyses

Safety analyses will include descriptive statistics calculated on the safety parameters using the safety population. The proportion of subjects with treatment-emergent adverse events will be tabulated and presented by treatment and Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class. Vital signs and clinically significant abnormal laboratory results will also be tabulated and presented by treatment group.

Data from all randomized subjects will be presented and summarized. Safety summaries by study treatment group will include listings by study medication of adverse events incidences within each MedDRA System Organ Class, and changes from pre-application values in vital signs. Adverse event summaries will be presented by study medication showing the proportion of subjects experiencing adverse events, both overall and by MedDRA System Organ Class.

11.6. Open-Label Extension Analyses

OLE endpoints will include safety variables, efficacy variables [(change from baseline in SALT, ALODEX at long-term visits), (change from baseline in AAA-SR, AAA-CR, and SGITS)].

11.7. Exploratory Biomarker Analyses (Select Sites Only)

11.7.1. ALADIN Scores (Select Sites Only)

The cytotoxic T-lymphocyte infiltration (CTL), Interferon (IFN-gamma), and hair keratin (KRT) ALADIN scores will be calculated for each scalp biopsy sample as described in (Xing et al, 2014, Jabbari 2016). Change in ALADIN scores within individual subjects will be assessed between Baseline (Visit 2), Week 4 (Visit 5), and Week 24 (Visit 10).

11.7.2. Scalp Biopsy for Histology and Immunology (Select Sites Only)

Scalp punch-biopsies will be formalin-fixed and paraffin-embedded and will be sectioned both vertically and horizontally by a qualified dermatopathologist. Specimens will be stained with hematoxylin and eosin (H&E) for "routine" histopathologic evaluation that will include (but is not limited to) diagnosis and evaluation of the presence, quantity, location, and quality of any hair, hair follicles, and inflammatory infiltrate present. Additionally, immunohistochemical (IHC) stains will be employed to identify immune cell populations (*e.g.*, Lymphocytes, cytotoxic T-lymphocytes (CTLs)), and hair-specific keratins. IHC stains to assess inflammatory responses will include but not be limited to CD8, HLA-DR and ICAM-1. Data will be presented as percent change from baseline in the number of cells/ high power field in regions of interest (peribulbar areas) and in percent change from baseline in the number of cells/mm^2.

11.7.3. Blood for Immunology (Select Sites Only)

Blood samples will be stained with cell surface antibodies for fluorescence acquisition cell sorting (FAC) analysis. FACS experiments will allow for assessment of the subset and activation status of immune cells involved in AA.

11.7.4. Clinical Correlative Studies (Select Sites Only)

Previous studies in human AA have reported increased NKG2DL expression and augmented expression of IFN response genes in the target hair follicle end organ, and elevated NKG2D expression in circulating CD8 T-cells and NK cells (Ito et al, 2008; Petukhova et al, 2010).

The study will include assessment at select sites of AA biomarkers at baseline and during treatment to correlate treatment and disease status with:

1. Histological improvement with reduced T-cell peribulbar infiltrates.
2. Declines in circulating and peribulbar CD8+NKG2D+ infiltration and in follicular hair follicle NKG2DL expression.
3. Reduced IFN inflammatory biomarkers in the skin and blood.

12. TRAINING, MONITORING, DATA MANAGEMENT AND QUALITY ASSURANCE

12.1. Training

For each investigational center, there will be an initiation visit prior to enrolling any study subjects.

It is strongly recommended that all investigators, other evaluators, study nurses, study coordinators or other applicable personnel attend this visit. During this visit, participants will be trained to the protocol, study specific procedures, and the eCRFs. Those unable to attend the initiation visit must receive on-site training from an appropriately trained individual prior to participating in any of the procedures and evaluations in this study.

Clinical Research Associates (CRAs) and other applicable personnel will be trained prior to study initiation to familiarize CRAs with the disease, the Standard Operating Procedures (SOPs), the protocol and other study specific items. Team organization, communication and operational issues will also be discussed.

Aclaris Therapeutics, Inc. will provide an investigational center file to each center.

12.2. Monitoring

The conduct of the study will be closely monitored by representatives of Aclaris Therapeutics, Inc. to verify adherence to ICH Good Clinical Practice (GCP) guidelines, and applicable SOPs. Reports of these verifications will be archived with the study report. The investigator will allow the Aclaris Therapeutics, Inc. representative's designee and/or any regulatory agency to have direct access to all study records, eCRFs, corresponding subject medical records, study medication-dispensing records and study medication storage area, and any other documents considered source documentation. The investigator also agrees to assist the representative, if required.

12.3. Data Management

Data-management activities of this study will be subcontracted. Edit checks and review processes will be performed by the sub-contractor until all data clarifications are resolved. The data will be exported to be stored in SAS datasets (or equivalent) by the sub-contractor. After all data clarifications are resolved and subject's evaluability is determined, the database will be locked.

12.4. Quality Assurance

The study is conducted under the sponsorship of Aclaris Therapeutics, Inc. in compliance with the applicable regulatory requirements as well as applicable ICH guidelines, Declaration of Helsinki, and in respect of the Aclaris Therapeutics, Inc. and/or sub-contractor SOPs for study conduct and monitoring.

Audits may be carried out by Aclaris Therapeutics, Inc. or Aclaris Therapeutics, Inc.'s representatives, and inspections may be performed by regulatory authorities or IRB/ECs before, during or after the study. The investigator will provide the auditing/inspecting group direct access to all study records (*e.g.*, eCRFs, subject medical records, study medication dispensing records) and the investigational center study facilities. The investigator and study staff will be available and will assist the auditing/inspecting groups as appropriate.

13. ETHICS AND GENERAL STUDY CONDUCT CONSIDERATIONS

13.1. Institutional Review Board (IRB)/Ethics Committee (EC)

This protocol, informed consent/ assent form, any information provided to subjects, subject-recruiting advertisements, and any amendments to these items will receive IRB/EC approval prior to use.
The IRB/EC must receive a copy of the Investigator's Brochure, all protocol amendments, safety reports and other study related information as required by regulation or the IRB/EC procedures.

13.2. Ethical Conduct of the Study

The rights, safety and well-being of the subjects are the most important considerations in this study and take priority over the interests of society and science.

This study will be conducted in accordance with the ethical principles originating from the Declaration of Helsinki, the ICH E6 GCP guideline, local regulatory requirements and, at US investigational centers, in compliance with the HIPAA. The study will be conducted in compliance with the IRB/EC approved version of the protocol and any applicable amendments.

13.3. Subject Information and Consent/ Assent

All subjects who participate in this study must be fully informed about the study in accordance with the GCPs, federal regulations, local regulations and, at US investigational centers, with HIPAA. The ICF/ Assent will contain all the required elements in compliance with the current ICH E6 GCP guideline and local regulatory requirements. The investigator must have a defined process for obtaining voluntary informed consent from every subject.

The ICF/ Assent Form, approved by an IRB/EC, will be fully explained to the subject and the parent/ legal guardian, if applicable. Prior to any study related procedures, including washout from therapies, the subject will voluntarily sign and date the ICF/ Assent Form. The investigator must maintain each subject's ICF/ and if applicable the Assent Form in the investigational center's study file and must provide each subject and if applicable parent/ legal guardian with a copy of the signed and dated ICF/ Assent Form.

13.4. Study Conduct and Protocol Amendments

With the exception of eliminating an immediate hazard to a subject, the investigator should not deviate from the protocol or implement any changes without prior written approval from the Aclaris Therapeutics, Inc.'s representative or designee and prior review and documented approval from the IRB/EC.

Changes that involve only logistical or administrative changes are allowed. The investigator should document and explain any deviation from the protocol. A protocol deviation is a non-adherence to protocol-specific study procedures or schedules that does not increase the risk to a study subject and does not affect the scientific integrity of the study.

A protocol violation is defined as any divergence from the protocol-specific study procedures or schedules that may results in an increased risk to a study subject or that affect the scientific integrity of the study. All protocol violations must be reviewed by the Medical Monitor and reported to the IRB by the Investigator, as directed by the IRB-specific procedures.

13.5. Regulatory Documents

The investigator must maintain a study file containing current and complete regulatory documentation in compliance with the current ICH E6 GCP guideline. This file will be reviewed as part of the routine monitoring for this study.

13.6. Contractual Requirements

A contractual agreement will be signed between Aclaris Therapeutics, Inc. and each investigator. This document will contain supplemental information, including financial terms, confidentiality, study schedule, third party responsibility, and publication rights.

13.7. Data Collection and Archiving

13.7.1. Data collection

The Investigator must maintain required records for all study subjects. Data for this study will be recorded in the subject's source document and on the eCRFs. All data on these eCRFs should be recorded completely and promptly. A copy of the completed eCRFs for each subject will be retained by the investigational center.

Records of the subject’s participation in this study will be held confidential except as disclosure is required by law. The study doctor, the sponsor, persons working on behalf of the sponsor, and under certain circumstances, the United States Food and Drug Administration and the Institutional Review Board will be able to inspect and copy confidential study-related records that identify subjects by name. Therefore, absolute subject confidentiality cannot be guaranteed. If the results of this study are published or presented at meetings, the subject’s identity will not be revealed.

13.7.2. Source documentation

Investigators must keep accurate separate records (other than the eCRFs) of all subjects' visits that include all pertinent study related information. A statement should be made indicating that the subjects have been enrolled in this clinical study and have provided written informed consent/ assent. Any AEs must be completely documented. Source documentation includes results of any diagnostic tests conducted during the study.

13.7.3. Archiving

All pertinent data, samples, photographs, correspondence, original or amended protocol, reports and all other material relating to the study will be maintained securely in Aclaris Therapeutics, Inc. /contract research organization/investigator archives for the legally required duration for archiving.

The investigator should maintain the essential study documents as specified in ICH GCP, and in compliance with all regulatory requirements. The investigator should ensure these documents are protected from accidental destruction or disposal.

If the Investigator needs to re-assign responsibility for maintaining these documents (*e.g.*, due to retirement) it must be transferred to a person willing to accept this responsibility. The investigator must notify Aclaris Therapeutics, Inc., in writing, of the name and address of the new individual.

14. REFERENCES

ATI-50002 Topical Solution Investigator Brochure. Data on File at Aclaris Therapeutics, Inc.

Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update: Part II. Treatment. *J Am Acad Dermatol*. 2010;62(2):191-202.

Arca E, Muşabak U, Akar A, Erbil AH, Taştan HB. Interferon-gamma in alopecia areata. *Eur J Dermatol*. 2004 Jan-Feb;14(1):33-6.

Barahmani N, Schabath MB, Duvic M; National Alopecia Areata Registry. History of atopy or autoimmunity increases risk of alopecia areata. *J Am Acad Dermatol*. 2009 Oct;61(4):581-91.

Bilgiç Ö, Bilgiç A, Bahalı K, Bahali AG, Gürkan A, Yılmaz S. Psychiatric symptomatology and health-related quality of life in children and adolescents with alopecia areata. *J Eu Acad Dermatol Venereol.* 2014;28(11):1463-1468.

Christiano, A. 2016. Data on File.

Craiglow BG, King BA. Killing Two Birds with One Stone: Oral Tofacitinib Reverses Alopecia Universalis in a Patient with Plaque Psoriasis. *J Invest Dermatol.* 2014;134(12):2988-2990.

Craiglow BG, Liu LY, and King BA. Tofacitinib for the treatment of alopecia areata and variants in adolescents. *J Am Acad Dermatol.* 2017;76(1):29-32.

Delamere FM, Sladden MJ, Dobbins HM, Leonardi-Bee J. Interventions for alopecia areata. *Cochrane Database Syst Rev*. 2008;16(2).

Gilhar A, Kam Y, Assy B, Kalish RS. Alopecia areata induced in C3H/HeJ mice by interferon-gamma: evidence for loss of immune privilege. *J Invest Dermatol*. 2005;124(1):288-9.

Gupta AK. Efficacy of tofacitinib in treatment of alopecia universalis in two patients. *J Eur Acad Dermatol Venereol*. 2016;30(8):1373-1378.

Hirota R, Tajima S, Yoneda Y, Okada M, Tashiro J, Ueda K, Kubota T, Yoshida R. Induction of hair regrowth in the alopecia site of IFN-gamma knockout mice by allografting and IFN-gamma injection into the transplantation site. *J Interferon Cytokine Res*. 2003 Aug;23(8):433-9.

Hordinsky MK. Current Treatments for Alopecia Areata. *J Investig Dermatol Symp Proc*. 2015;17(2):44-46.

Ito, T., Ito, N., Saatoff, M., Hashizume, H., Fukamizu, H., et al. Maintenance of hair follicle immune privilege is linked to prevention of NK cell attack. *J. Investig Dermatol*. 2008:128; 1196–1206.

Jabbari A, Dai Z, Xing L, et al. Reversal of Alopecia Areata Following Treatment With the JAK1/2 Inhibitor Baricitinib. *EBioMedicine*. 2015;2(4):351-355.

Jabbari A, Cerise JE, Chen JC, Mackay-Wiggan J, Duvic M, Price V, Hordinsky M, Norris D, Clynes R, Christiano AM. Molecular signatures define alopecia areata subtypes and transcriptional biomarkers. *EBioMedicin*e. 2016 May;7:240-7.

Kim BY and Kim HS. Successful hair growth in a Korean patient with alopecia universalis following tofacitinib treatment. *Singapore Med. J.,* 2017 May; 58(5): 279-280.

Kuwano Y, Fujimoto M, Watanabe R, Ishiura N, Nakashima H, Ohno Y, Yano S, Yazawa N, Okochi H, Tamaki K. Serum chemokine profiles in patients with alopecia areata. *Br J Dermatol*. 2007:157(3):466-73.

McElwee KJ, Boggess D, King, B, Sundbrg JP. Experimental Induction of Alopecia Areata-Like Hair Loss in C3H/HeJ Mice Using Full-Thickness Skin Grafts. *J Invest Dermatol.* 1998. 111:797–803.

Olsen EA. Investigative guidelines for alopecia areata. *Dermatol Ther*. 2011:24;311-319.
Olsen EA and Canfield D. SALT II: A New Take on the Severity of Alopecia Tool (SALT) for Determining Scalp Hair Loss. *J Am Acad Dermatol*. 2016;75(6):1268-1270.
Olsen EA, Green C, Hordinsky M, Bergfeld W, McMichael A, Callender V, Washenik K, Cotsarelis G, Shapiro J, Roberts J, Wolfe S, Cherill D, Canfield D. Alopecia Density and Extent (ALODEX): A new method for assessing severity of hair loss in alopecia areata. Submitted for publication.
Petukhova, L., Duvic, M., Hordinsky, M., Norris, D., Price, V., et al. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity.
Nature. 2010:466, 113–117.
Pieri L, Guglielmelli P, Vannucchi AM. Ruxolitinib-induced reversal of alopecia universalis in a patient with essential thrombocythemia. *Am J Hematol*. 2015;90(1):82-83.
Pratt CH, King LE Jr, Messenger AG, Christiano AM, Sundberg JP. Alopecia areata. *Nat Rev Dis Primers*. 2017 16;3:17011.
Price VH. Treatment of Hair Loss. *N Eng J Med*. 1999;341(13):964-973.
Price VH, Hordinsky MK, Olsen EA, et al. Subcutaneous efalizumab is not effective in the treatment of alopecia areata. *J Am Acad Dermatol.* 2008;58(3):395-402.
Ruiz-Doblado S, Carrizosa A, García-Hernández MJ. Alopecia areata: psychiatric comorbidity and adjustment to illness. *Int J Dermatol.* 2003;42(6):434-437.
Safavi KH, Muller SA, Suman VJ, Moshell AN, Melton Iii LJ. Incidence of Alopecia Areata in Olmsted County, Minnesota, 1975 Through 1989. *Mayo Clinic Proc*. 1995;70(7):628-633.
Scheinberg M, and Ferreira SB. Reversal of Alopecia Universalis by Tofacinitinib: A Case Report; *Ann Intern Med*.; 2016 Nov 15; 165(10): 750-751. doi: 10.7326/L16-0125.
Strober BE, Siu K, Alexis AF, et al. Etanercept does not effectively treat moderate to severe alopecia areata: An open-label study. *J Am Acad of Dermatol*. 2005;52(6):1082-1084.
Tosti A, Bellavista S, Iorizzo M. Alopecia areata: a long term follow-up study of 191 patients. *J Am Acad Dermatol.* 2006;55(3):438-441.
Weise K, Kretzschmar L, John SM, Hamm H. Topical Immunotherapy in Alopecia areata: Anamnestic and Clinical Criteria of Prognostic Significance. *Dermatology.* 1996;192(2):129-133.
Whiting, D.A., 2003a. Histopathologic features of alopecia areata: a new look. Arch. Dermatol. 139, 1555–1559.
Whiting, D.A., 2003b. Histopathologic features of alopecia areata. Arch. Dermatol. 139, 1555–1559.
Xing L, Dai Z, Jabbari A, et al. Alopecia areata is driven by cytotoxic T lymphocytes and is reversed by JAK inhibition. *Nat Med.* 2014;20(9):1043-1049.

APPENDIX 1: Subject Instructions for Study Medication Application to the Patchy Areas of the Scalp

Preparation and General Instructions:

1. Gather a clean washcloth and towel, the study medication bottle, and disposable dropper.
1. Hair and scalp should be clean (free of any hair and scalp styling products), and dry or at least towel-dried before applying study medication. Ensure that your scalp is as dry as possible. A clean scalp will allow the study medication to penetrate down into the scalp to ensure you are getting the best application. The treatment areas of the scalp should be washed using your normal cleansing products at least an hour prior to study medication application, at least once a day to prevent accumulation of study medication on your skin.
2. Wash your hands with soap and water before and after using this study medication.
3. You will apply a thin layer of study medication to each area of scalp hair loss as instructed by the study doctor or the study staff. Keep applying study medication to the areas of hair loss throughout the study, even if scalp hair is regrowing in these areas. If you experience new areas of scalp hair loss (for example, areas of the scalp with active hair shedding and abnormal sensations that in your experience is a sign of scalp hair loss) discuss these new areas with the study Doctor so they can be appropriately documented before applying study medication.
4. **The amount of study medication you will apply during one treatment is a total of __ mL (__ -1mL droppers).**
5. Avoid study medication contact with the eye. If the study medication gets on any part of your body other than your scalp, rinse the area well with water.
6. You will apply study medication twice-a-day, approximately 8 to 12 hours apart.
7. Remember to bring your study medication bottles both used and unused to each study visit.

Instructions For Select Sites Only:

8. *Apply your study medication in the evening before your Week 4, and Week 24 visits and note the time. Do not apply your morning dose of study medication until after your office visit.*
9. *Do not apply study medication to the area where the biopsy was performed until the study doctor indicates you are allowed.*

Study Medication Application:

1. Draw up exactly 1 mL of study medication into the dropper. The medication level should be at the 1mL line.
2. During study medication application, keep your head tilted back to avoid any study medication running into your eyes.
3. Starting with your Target Patch, squeeze a few drops of study medication onto the center of the patch of your scalp and gently rub the study medication into your scalp. Keep applying a few drops and rubbing into your scalp until **the entire Target patch and about ½" margin around the patch is** covered with a thin film of study medication.
4. Draw up additional study medication in the dropper – 1mL at a time to cover the entire patchy areas on your scalp with hair loss.
5. Select the next area of hair loss, squeeze a few drops of study medication onto the center of the patchy area of hair loss, and gently rub the medication into your scalp. Keep applying a few drops until **the entire patch of scalp hair loss and about a ½ inch margin around the patch** is covered with a thin film of study medication. Repeat until all affected areas are covered with a thin film of medication using one dropper per treatment application.
6. Replace the screw top cap and make sure it is closed tightly. Dispose of the used dropper(s).
7. It is important to continue to apply study medication to the areas of hair loss throughout the study, even if there is hair growth in the area(s).
8. Wash your hands after using this product to prevent any residue being left on your hands.

9. Allow the study medication to dry for at least 15 minutes before you apply any styling products to the hair. All topical products applied to the hair and scalp must be reviewed and approved by your study doctor before use.
10. If you missed a dose or doses, record on your subject compliance record and tell the study staff at your next visit.
11. Do not wash your hair or scalp for at least 6 hours after applying study medication.

Wigs and Hairpieces

1. Wigs and hairpieces may be worn while participating in the study but must be appropriately managed.
2. Wigs or hairpieces should not be worn until the study medication is completely dry on the scalp. Thus, wigs or hairpieces should not be reapplied for at least 15 minutes after the study drug has been applied.
3. Wigs and hairpieces will need to be removed at each study visit to allow the study doctor to evaluate your scalp and hair loss. Any hairpieces that may be difficult to remove will prevent you from participating in the study. Do not have semi-permanent or difficult to remove hairpieces placed during the study. Hair "weaves" that only involve areas of the scalp that continue to have hair may be accepted on a case by case basis.
4. If scalp irritation develops during the study, it may be necessary to temporarily stop wearing of a hairpiece or wig. The study doctor will discuss this with you should scalp irritation develop.

Missed Doses: If you miss a dose of this study medicine, apply it as soon as possible. However, if it is almost time for your next dose, skip the missed dose, and go back to your regular dosing schedule.

Storage: Store the medicine in the original glass bottle, in the carton provided, at room temperature, away from heat, moisture, and direct light. Keep from freezing. Keep study medication and used droppers out of the reach of children.

APPENDIX 2: Parent/ Subject Instructions for Study Medication Application to the Entire Scalp

Preparation and General Instructions:

2. Gather a clean washcloth and towel, the study medication bottle, disposable dropper and gloves (parent application only). Parents must wear gloves when applying study medication to their child's scalp.
3. Hair and scalp should be clean (free of any hair and scalp styling products), and dry or at least towel-dried before applying study medication. Ensure that your scalp is as dry as possible. A clean scalp will allow the study medication to penetrate down into the scalp to ensure you are getting the best application. The scalp should be washed using your normal cleansing products at least an hour prior to study medication application, at least once a day to prevent accumulation of study medication on your skin.
4. Wash your hands with soap and water before and after using this study medication.
5. You will apply a thin layer of study medication to the entire scalp as instructed by the study doctor or the study staff. Once medication has been applied to all current patchy areas, part hair into approximately 4 sections (from front to back). Take each section one by one and apply up to 1mL to the scalp part and massage the study medication into the scalp from the part to the sides until the entire scalp is covered in a thin film of study medication. Keep applying study medication throughout the study, even if scalp hair is regrowing. If you experience new areas of scalp hair loss (for example, areas of the scalp with active hair shedding and abnormal sensations that in your experience is a sign of scalp hair loss) discuss these new areas with the study Doctor so they can be appropriately documented.
6. **The amount of study medication you will apply during one treatment is a total of __ mL (__ -1mL droppers).**
7. Avoid study medication contact with the eye. If the study medication gets on any part of your body other than your scalp, rinse the area well with water.
8. You will apply study medication twice-a-day, approximately 8 to 12 hours apart.
9. Remember to bring your study medication bottles both used and unused to each study visit.

Instructions For Select Sites Only:

10. *Apply your study medication in the evening before your Week 4, and Week 24 visits and note the time. Do not apply your morning dose of study medication until after your office visit.*
11. *Do not apply study medication to the area where the biopsy was performed until the study doctor indicates you are allowed.*

Study Medication Application:

1. Draw up exactly 1 mL of study medication into the dropper. The medication level should be at the 1mL line.
2. During study medication application, keep your head tilted back to avoid any study medication running into your eyes.
3. Starting with your Target Patch, squeeze a few drops of study medication onto the center of the patch of your scalp and gently rub the study medication into your scalp. Once medication has been applied to all current patchy areas, part hair into approximately 4 sections (from front to back). Apply up to 1 mL to the scalp of each parted section from front to back and massage the study medication into the scalp from the part to extending to the sides until the **entire scalp** is covered in a thin film of study medication.
4. Replace the screw top cap and make sure it is closed tightly. Dispose of the used dropper(s).
5. It is important to continue to apply study medication to the entire scalp throughout the study, even if there is hair growth.
6. Wash your hands after using this product to prevent any residue being left on your hands.

7. Allow the study medication to dry for at least 15 minutes before you apply any styling products to the hair. All topical products applied to the hair and scalp must be reviewed and approved by your study doctor before use.
8. If you missed a dose or doses, record on your subject compliance record and tell the study staff at your next visit.
9. Do not wash your hair or scalp for at least 6 hours after applying study medication.

Wigs and Hairpieces

1. Wigs and hairpieces may be worn while participating in the study but must be appropriately managed.
2. Wigs or hairpieces should not be worn until the study medication is completely dry on the scalp. Thus, wigs or hairpieces should not be reapplied for at least 15 minutes after the study drug has been applied.
3. Wigs and hairpieces will need to be removed at each study visit to allow the study doctor to evaluate your scalp and hair loss. Any hairpieces that may be difficult to remove will prevent you from participating in the study. Do not have semi-permanent or difficult to remove hairpieces placed during the study. Hair "weaves" that only involve areas of the scalp that continue to have hair may be accepted on a case by case basis.
4. If scalp irritation develops during the study, it may be necessary to temporarily stop wearing of a hairpiece or wig. The study doctor will discuss this with you should scalp irritation develop.

Missed Doses: If you miss a dose of this study medicine, apply it as soon as possible. However, if it is almost time for your next dose, skip the missed dose, and go back to your regular dosing schedule.

Storage: Store the medicine in the original glass bottle, in the carton provided, at room temperature, away from heat, moisture, and direct light. Keep from freezing. Keep study medication and used droppers out of the reach of children.

APPENDIX 3 : Parent/ Subject Instructions for Study Medication Application to the Eyebrow during the Open-Label Extension

General Instructions:

1. Before application of study medication, your eyebrow area should be clean (free of any makeup, moisturizers, sunscreen, etc.), and dry. This will allow the study medication to penetrate down into the skin to ensure you are getting the best application.
2. The study doctor will instruct you to apply study medication to one or both eyebrows. You will apply the study medication to the entire eyebrow area, both with and without eyebrow hair.
3. You will be asked to apply a thin layer of study medication to the affected eyebrow with an applicator as instructed by the study doctor or the study staff. Keep applying study medication to the affected eyebrow(s) throughout the study, even if hair is re-growing in these areas.
4. You will want the tip of your applicator to be saturated but not too much as to cause dripping. An applicator should only be dipped in the bottle once and then disposed of.
5. Keep the study medication out of your eyes. If the study medication gets in your eyes, rinse the area well with water for up to 15 minutes. Contact the study doctor for further advice on managing the eye exposure.
6. You will apply study medication twice-a-day, approximately 8 to 12 hours apart. Once you apply study medication, do not wash your face and eyebrow area or participate in strenuous exercise that would cause profuse sweating for at least 6 hours.
7. Remember to bring your study medication bottles, both used and unused, to each study visit.
8. Avoid exposing your face to excessive natural or artificial ultraviolet radiation (e.g., sunlight, tanning beds) and use sunscreen on the face including the eyebrows, if excessive sun exposure cannot be avoided.
9. Remove any products applied to the eyebrow area at least 1 hour before study visits. Do not apply study medication less than 6 hours before a study visit. If your visit is in the morning you should wait until after the visit to apply your study medication.
10. Each bottle of study medication should be used for 60 days only, even if there is remaining study medication.

Preparation for Study Medication Application

1. Gather a clean, dry washcloth or towel, the study medication bottle, disposable applicators and a mirror.
2. Wash your hands with soap and water before and after using this study medication.
3. Gently wash your eyebrow areas, ensuring your eyebrow areas are clean. Use your normal cleansing regime as approved by your study doctor. Do not use abrasive cleansers or materials on your face and eyebrow area.
4. Pat your face dry with a clean towel and then let it air dry until it is completely dry to the touch.

Study Medication Application:

1. Unscrew the cap from the bottle. Place the open bottle on a stable, level surface.
2. Dip a disposable applicator into the bottle of study medication for about 2 seconds. Tap the tip of the applicator twice inside the edge of the bottle to remove any excess study medication. The applicator should be saturated, but not dripping.
3. Tilt your head back and place your clean, dry washcloth over one eye. Swipe the applicator across your affected eyebrow ridge above the covered eye, applying a thin layer of study medication over the entire affected eyebrow area. Your eyebrow area should be wet, but not dripping wet. Dispose of the applicator. **Do not dip the same applicator in the study medication bottle more than once.**
4. If you need additional study medication to cover your entire affected eyebrow, use a new applicator and repeat the application process as described in #2 and #3.
5. If you are instructed by the study doctor to treat both eyebrows, apply study medication to your other eyebrow following instructions in #2, #3, and #4.

After Study Medication Application

1. Securely close the study medication bottle and dispose of any used applicators.
2. Wash your hands after using this product.
3. Allow the study medication to completely dry for at least 10 minutes.
4. Do not apply any products (moisturizers, sunscreens, cosmetics, etc.) to your eyebrow area until the study medication has completely dried, at least 30 minutes after applying study medication.
5. Do not wash your face and eyebrow areas or participate in strenuous exercise that would cause profuse sweating for at least 6 hours after applying the study medication.

Missed Doses

If you miss a dose of this study medication, apply it as soon as possible. However, if it is almost time for your next dose, skip the missed dose, and go back to your regular dosing schedule. Tell the study staff about any missed doses at your next study visit.

Storage

Store the study medication in the original glass bottle at room temperature, away from heat, moisture, and direct light. Do not refrigerate or freeze. Keep out of reach of children.

APPENDIX 4: Alopecia Areata History

The following AA history will be obtained:

1. Onset date of alopecia
2. Onset date of current episode of patchy alopecia areata
3. Did the subject use previous therapies for Patchy AA?
    a. If Yes, indicate which therapies
        1. Topical immunotherapy
        2. Corticosteroids
        3. Systemic Steroids
        4. DMARDS
        5. Biologics or immunosuppressants
        6. Plaquenil
        7. PDT
        8. Janus kinase inhibitors
        9. Phototherapy
        10. Laser therapy
        11. Narrow-band UVB
        12. Other

APPENDIX 5: Fitzpatrick's Skin Type Chart

Fitzpatrick Skin Type	
Description (Sunburn & Tanning History According to Skin Type)	**Skin Type**
Always Burns; never tans (pale white skin)	I
Burns easily; tans minimally (white skin)	II
Burns moderately; tans uniformly (light brown skin)	III
Burns minimally; always tans (moderate brown skin)	IV
Rarely burns; tans profusely (dark brown skin)	V
Never burns; deeply pigmented (dark black Skin)	VI

APPENDIX 6: Alopecia Scalp Appearance Assessment for AA Patchy (AAA) (Subject)

Prior to administering the questionnaires to the subject, review the target patch identified at baseline. Subject should be provided a handheld mirror to adequately view the target patch. Ensure that the subject clearly understands the full location of the target area (especially in cases where the patch may have regrown hair).

Alopecia Scalp Appearance Assessment for Patchy AA: Subject Rating (SR)

Instructions for item 1: Please mark an "X" in the box (☒) that best describes the appearance of the **target patch** right now. Please select the one response that best represents your answer.

☐ Full hair, scalp of the target patch completely covered with hair
☐ Most hair, scalp of the target patch mostly covered with hair
☐ Some hair, scalp of the target patch somewhat covered with hair
☐ A little hair, scalp of the target patch mostly exposed
☐ No hair, scalp of the target patch completely exposed

Instructions for item 2: Please mark an "X" in the box (☒) that best describes the appearance of your **whole scalp** right now. Please select the one response that best represents your answer.

☐ Full hair, whole scalp completely covered with hair
☐ Most hair, whole scalp mostly covered with hair
☐ Some hair, whole scalp somewhat covered with hair
☐ A little hair, whole scalp mostly exposed
☐ No hair, whole scalp completely exposed

APPENDIX 7: GLOBAL IMPRESSION OF SEVERITY - Subject

SUBJECT GLOBAL IMPRESSION OF SEVERITY (SGIS)

Please mark an "X" in the box (☒) that best describes the severity of your patchy alopecia areata right now.

Overall, how severe is your patchy alopecia areata right now?

☐ Mild
☐ Moderate
☐ Severe
☐ Very Severe
☐ Extremely Severe

APPENDIX 8: SUBJECT SATISFACTION QUESTIONNAIRE

The investigator or study staff will review with the subject that this questionnaire is related to satisfaction with the outcome of the treatment (study medication).

GLOBAL IMPRESSION OF TREATMENT SATISFACTION

Please mark an "X" in the box (☒) that best describes how satisfied you are with the treatment you received in this study for your patchy alopecia areata.

How satisfied or dissatisfied are you with the treatment you received in this study for your patchy alopecia areata?

☐ Extremely satisfied
☐ Moderately satisfied
☐ A little satisfied
☐ Neither satisfied or dissatisfied
☐ A little dissatisfied
☐ Moderately dissatisfied
☐ Extremely dissatisfied

APPENDIX 9: ALOPECIA IMPACT ASSESSMENT

Alopecia Impact Assessment (AIA)

Instructions: The following questions are about your alopecia. For each question, please select the box (☒) below the number that best describes your experience with alopecia during the past seven days. There are no right or wrong answers.

1. During the past seven days, how bothersome was it to cover your hair loss (e.g., wearing a wig, using makeup to fill in eyebrows, wearing hats)?	Not at all bothersome										Extremely bothersome		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
2. During the past seven days, how worried were you about your appearance due to your hair loss?	Not at all worried										Extremely worried		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
3. During the past seven days, how sad did you feel due to your hair loss?	Not at all sad										Extremely sad		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
4. During the past seven days, how much did your hair loss impact your confidence?	Not at all impacted										Extremely impacted		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
5. During the past seven days, how self-conscious did you feel due to your hair loss (e.g., feeling uncomfortable with hair/hair loss in public)?	Not at all self-conscious										Extremely self-conscious		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
6. During the past seven days, how embarrassed did you feel due to your hair loss (e.g., feeling awkward about, or ashamed of, hair loss)?	Not at all embarrassed										Extremely embarrassed		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

7. During the past seven days, how unattractive did you feel due to your hair loss?	Did not feel unattractive at all										Felt extremely unattractive		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
8. During the past seven days, how much did your hair loss limit your social activities (e.g., spending time with friends, going to a social event)?	Not at all limited										Extremely limited		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
9. During the past seven days, how much did your hair loss limit your physical activities (e.g., going to the gym, swimming, playing sports)?	Not at all limited										Extremely limited		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
10. During the past seven days, how bothersome was unwanted or negative attention from others due to your hair loss (e.g., staring, questions)?	Not at all bothersome										Extremely bothersome		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
11. During the past seven days, how bothersome was your experience of getting sweat in your eyes due to your hair loss?	Not at all bothersome										Extremely bothersome		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
12. During the past seven days, how bothersome was your experience of getting debris in your eyes due to your hair loss?	Not at all bothersome										Extremely bothersome		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	
13. During the past seven days, how bothersome was your experience getting debris in your nose due to your hair loss?	Not at all bothersome										Extremely bothersome		
		0	1	2	3	4	5	6	7	8	9	10	
		☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

APPENDIX 10 : DERMATOLOGY LIFE QUALITY INDEX

The Investigator or study staff will remind the subject to think of their scalp hair loss instead of "skin problem" and "skin" in the questions below.

DERMATOLOGY LIFE QUALITY INDEX

When completing the questionnaire, think of your hair loss in place of "skin problem" and "skin" in the questions below.

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

1. Over the last week, how **itchy**, **sore**, **painful** or **stinging** has your skin been?
   Very much
   A lot
   A little
   Not at all

2. Over the last week, how **embarrassed** or **self conscious** have you been because of your skin? Very much
   A lot
   A little
   Not at all

3. Over the last week, how much has your skin interfered with you going **shopping** or looking after your **home** or **garden**?
   Very much
   A lot
   A little
   Not at all
   Not relevant

4. Over the last week, how much has your skin influenced the **clothes**
   you wear?
   Very much
   A lot
   A little
   Not at all
   Not relevant

5. Over the last week, how much has your skin affected any **social** or **leisure** activities?
   Very much
   A lot
   A little
   Not at all
   Not relevant

6. Over the last week, how much has your skin made it difficult for you to do any **sport**?
   Very much
   A lot
   A little

Not at all
Not relevant

7. Over the last week, has your skin prevented you from **working** or **studying**?
   Yes, No, or Not relevant
   If "No", over the last week how much has your skin been a problem at **work** or **studying**?
   A lot
   A little
   Not at all

8. Over the last week, how much has your skin created problems with your **partner** or any of your **close friends** or **relatives**?
   Very much
   A lot
   A little
   Not at all
   Not relevant

9. Over the last week, how much has your skin caused any **sexual difficulties**?
   Very much
   A lot
   A little
   Not at all
   Not relevant

10. Over the last week, how much of a problem has the **treatment** for your skin been, for example by making your home messy, or by taking up time?
    Very much
    A lot
    A little
    Not at all
    Not relevant

Please check you have answered EVERY question. Thank you.

APPENDIX 11: Select Sites Only- Scalp Biopsy

All scalp punch biopsies will be performed by the investigator in a patchy area (not the Target area) at Baseline (Visit 2), Week 4 (Visit 5), and Week 24 (Visit 10) prior to application of study medication.

The biopsy site will be anesthetized with an injection of 1% lidocaine with epinephrine. The lidocaine with epinephrine may be neutralized with bicarbonate to prevent stinging. After approximately 1 minute, the physician will apply pressure to the biopsy site using a 4mm diameter skin punch (a sterile cylindrical tube with a sharp edge). The punch is twisted until the blade of the skin punch has pierced the epidermis and dermis of the skin and enters the subcutaneous fat. Depending on the thickness of the skin in the area being biopsied, the cylindrical blade may be buried to the hub (approximately 6mm in depth). After the blade has sufficiently cored or carved out a cylinder of skin, the skin punch is removed. Nontraumatic forceps are used to gently grasp the cored skin, pulling upward to remove the core and reveal the subcutaneous fat. Scissors are used to cut the cored tissue free from the underlying subcutaneous fat. Care should be taken to ensure that the specimen is cut well below the level of the hair follicle. The specimen is placed immediately into the appropriate media. Once the specimen has been removed, pressure is applied to the biopsy site with a sterile 2 x 2 gauze. The biopsy site is then closed with several simple interrupted sutures. Either an absorbable or nonabsorbable suture may be used at the investigator's discretion. Antibiotic ointment is applied and the area is covered with a standard Band-Aid or sterile gauze and paper tape. If the presence of adjacent hair makes adhesion of a bandage difficult, antibiotic ointment will be used without a covering. When necessary, a small pressure dressing may be applied. Subjects will be instructed in wound care, to avoid applying study medication to the biopsy site until the sutures are removed and will be advised to call the research unit if they have any concerning signs or symptoms during healing. A follow-up visit will be scheduled approximately 2 weeks later, to remove sutures and examine the healing process of the biopsy site to ensure it is healing appropriately.

APPENDIX 12: Subject Global Impression of Change (SGIC)

(Please mark an "X" in the box (☐):

1. Compared to your hair loss at the beginning of the study [before starting study medication], your alopecia is?

☐ 1=Very much improved since starting study medication

☐ 2=Much improved

☐ 3=A little improved

☐ 4=No change since starting study medication

☐ 5=A little worse

☐ 6= Much worse

☐ 7=Very much worse since starting study medication