CLINICAL STUDY PROTOCOL
Protocol Number: ATI-50002-AA-202

A Randomized, Double-blind, Vehicle-controlled Multicenter Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ATI-50002 Topical Solution Administered Twice-Daily for 28 Days in Adult Subjects with Alopecia Universalis and Alopecia Totalis with a 12-Month Long-term Open-label Extension

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PROTOCOL APPROVAL SIGNATURE PAGE

Protocol Number: ATI-50002-AA-202

Version 3.0: 14AUG2018

Protocol Title: A Randomized, Double-blind, Vehicle-controlled Multicenter Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ATI-50002 Topical Solution Administered Twice-Daily for 28 Days in Adult Subjects with Alopecia Universalis and Alopecia Totalis with a 12-Month Long-term Open-label Extension.

Sponsor Signatures:

_____ _____

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

Date

_____ _____

Christopher Powala
Chief Regulatory and Development Officer
Aclaris Therapeutics, Inc.

Date

INVESTIGATOR SIGNATURE PAGE

Protocol Number: ATI-50002-AA-202

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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 from each prospective trial subject or each prospective trial subject's legal representative prior to conducting any protocol-specified procedures. The Informed Consent 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. Following this notification, a written report describing the serious adverse event will be provided to the Sponsor as soon as possible, but no later than five days following the initial notification.

Investigator Signature:

_____ _____

Investigator signature Date

_____

Investigator printed name

AMENDMENT HISTORY:

Protocol Amendment 1, Version 2.0, dated 06FEB2018 added a 6-month open-label extension to allow subjects to receive ATI-50002 Topical Solution, 0.46%. In addition, to assist with enrollment and to facilitate enrollment of subjects less than 60 years of age, the amendment specified that after the first 6 subjects were enrolled in the study, the blood PK samples were no longer collected. Administrative Letters 1 through 5 were incorporated into Protocol Amendment 1. Administrative Letter #1 (dated October 2, 2017) outlined the change from a single center to a multicenter study, clarified that M689 would not be measured and that the method for the detection of ATI-50002 in the scalp was a qualified method. Administrative Letter #2 (dated December 19, 2017) added superficial tape stripping of the scalp biopsy site for pharmacokinetic analysis to remove any residual study medication in the upper layers of the stratum corneum prior to biopsy. Administrative Letter #3 (dated January 22, 2018) allowed for a decrease in the volume of study medication applied from 4 mL to 1.5 mL to 4 mL, and Administrative Letter #4 (dated January 29, 2018) updated the address for Aclaris Therapeutics, Inc. Administrative Letter #5 (dated 03AUG2018) clarified the treatment duration of the Open Label Extension (OLE) portion of the study for subjects with a gap between Visit 7 and entry into the OLE due to an administrative delay.

AMENDMENT RATIONALE:

The primary purpose of Amendment 2, Version 3.0 dated 14AUG2018 is to increase the duration of the open-label extension (OLE) to 12 months.

Protocol Version	Date	Section	Revision
Version 1.0	26JUL2017	NA	NA
Version 2.0	06FEB2018	Title page Protocol Approval Signature Page Investigator Signature Page Synopsis	Replaced single center with multicenter in the title and added 6-Month Long-term open label Extension
		Title page	Removed Julian McKay-Wiggan
		Title page Section 10.2.2, Procedure for Reporting Adverse Events	Updated the Medical Monitor to: Terrence Chew, MD, RAC
		Synopsis, Number of Study Sites Section 6.1, Number of Subjects	Updated site number to two.
		Synopsis, Study Design, Endpoints, Statistical Methods Section 7.1 Overall Study Design Section 9.3.2 Blood Samples Section 12.4 Pharmacokinetic Analysis	Concentrations of M689 in the blood will not be measured.
		Synopsis: Endpoints section	Clarified that the bioanalytical methods for the skin biopsy are qualified and not fully validated methods.
		Section 9.3.1, Scalp Biopsy Appendix 5, Scalp Biopsy	Added tape stripping of the area of the scalp that will be biopsied for pharmacokinetic analysis with 5 D-Squame circular discs.
		Synopsis: Study Design, Study Medication Administration Section 7.1 Overall Study Design Section 8.3 Application of Study Medication Appendix 1, Subject Instructions	Changed the volume of study medication applied to the scalp from 4 mL to a minimum of 1.5 mL up to a maximum of 4 mL of study medication.

Protocol Version	**Date**	**Section**	**Revision**
		Synopsis, multiple sections Applicable sections throughout the protocol	Clarified Double-blind period and Added Open-Label Extension information, including additional visits, study medication and statistical analyses.
		Synopsis, Inclusion Criteria Section 6.3, Inclusion Criteria	Added Inclusion Criteria 11: 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.
		Synopsis, Exclusion Criteria Section 6.5.2, Prohibited and/ or Restricted Medications	Clarified Exclusion Criterion #15; JAK inhibitor exclusion is for both topical and oral. Clarified Exclusion Criterion #16; alopecia areata includes (AA, AU, AT)
		Section 3.1, Summary	Clarified the purpose of the study is to determine if topical application of ATI-50002 will result in regrowth of the scalp hair loss and if applicable, eyebrow hair loss due to AU and AT.
		Section 4.1, Study Rationale	Added a description of how subjects will enter the open label extension and nonclinical justification for 6-month treatment duration.
		Synopsis, Study Design, Statistical Methods Section 7.1, Overall Study Design Section 7.3, Schedule of Assessments Section 12.4, PK analyses	Added that only the first 6 subjects enrolled will have the pharmacokinetic blood samples will not be collected.
		Section 7.3, Schedule of Assessments	Update Table to reflect additional study visits for open-label extension.
		Section 8.8, Other Study Supplies	Added ECG equipment and supplies for PD samples.
		Section 8.10, Study Medication Packaging	Added subjects must complete double-blind treatment to be eligible for entry into the open-label extension, Clarified controlled room temperature conditions for study medication storage, Described open-label study medication supplies.
		Section 9.4, Eyebrow Assessments (Open-Label Extension)	Added the Clinician's Eyebrow Assessment and Subject's Eyebrow Assessments at Day 29 and monthly throughout the open-label extension period.
		Section 10.2.4.1, Study Medication Interruption	Replaced hold with interrupt and clarified that Study medication can be resumed after the abnormal laboratory value returns to normal or baseline, if in the opinion of the Investigator, it is in the best interest of the subject to continue.
		Section 11.2 Highly Effective Methods of Birth Control	Added Obstruction of fallopian tubes via medical device (Essure™).

Protocol Version	**Date**	**Section**	**Revision**
		Section 12.5.4 Secondary Analyses	Added efficacy and satisfaction analyses consistent with potential hair regrowth from longer treatment duration.
		Appendix 1, Subject Instructions for Study Medication Application to the Scalp	Added study subjects should avoid strenuous exercise that would cause profuse sweating for at least 6 hours after study medication application. Added instructions for avoidance of exposure of scalp to excessive natural or artificial ultraviolet radiation and sunscreen information.
		Appendix 2, Subject Instructions for Study Medication Application to the Eyebrow(s)	Added study medication instructions for subjects with eyebrow loss who are participating in the open-label extension.
		Appendix 3, Alopecia Areata History	Simplified the AA history
		Protocol	Minor editorial changes and corrections of typographical errors were made.
Version 3.0	14AUG2018	Synopsis Study Design Duration of Treatment Endpoints Study Medication Administration Section 4.1, Study Rationale Section 6.5.1 Permitted Concomitant Therapies and Over the Counter Products (OTC) Section 6.8, Subject Withdrawal criteria Section 7.1, Study Design Section 7.3, Schedule of Assessments Section 7.5, Duration of the Study Section 8.1, Investigational Study Medication Section 8.2, Subject Randomization Section 8.4, Study Medication Weights Section 8.5, Treatment Compliance Section 9.1.1, SALT Score Section 9.1.2, ALODEX Score Section 9.4, Eyebrow Assessments Section 9.4.1, Clinician's Eyebrow Assessment Section 9.4.2, Subject's Eyebrow Assessment Section 9.5.2, Physical Examination Section 9.5.3, Clinical Laboratory Assessments Section 9.5.4, Pregnancy Tests Section 9.6.2, Photographic Assessment	Revised the duration of open-label treatment from 6 months to 12 months and added two additional visits (Visit 15 and 16). Post-treatment follow-up Visit 14 is now Visit 16. Maximum duration of study participation is 400 days (393 + 7 days).

Protocol Version	**Date**	**Section**	**Revision**
		Synopsis, Efficacy Assessments Section 12.5.4, Secondary Analyses	Added Week 40 and 52 to the SALT, ALODEX, Subject Satisfaction, CEA and SEA analyses
		Section 3.1, Summary, Overview of Alopecia Areata	Revised statement from AA is "the most prevalent" to "a prevalent" autoimmune disease in the United States
		Section 4.2 Study Rationale	Removed "6 month update" from the 9-month minipig study with dermal administration since full 9-month report is completed. Added update from currently enrolled subjects.
		Section 8.7, Study Medication Accountability	Added language to allow sites with a written drug destruction/disposal policy to dispose of unused and used investigational product on-site rather than returning to Aclaris.
		Section 8.10 Study Medication Packaging, Labeling, Storage and Security	Revised number of bottles to be dispensed.

1. SYNOPSIS

Protocol Number: ATI-50002-AA-202	**Protocol Title:** Double-blind, Vehicle-controlled Multicenter Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ATI-50002 Topical Solution Administered Twice-Daily for 28 Days in Adult Subjects with Alopecia Universalis and Alopecia Totalis with a 12-Month, Long-Term, Open-Label Extension
Sponsor: Aclaris Therapeutics Inc.	**Phase of Development:** Phase 2

Study Medication Description:
ATI-50002 Topical Solution, 0.46%
Vehicle Topical Solution

Study Objectives:

Primary:

To assess safety, tolerability, pharmacokinetics and pharmacodynamics of ATI-50002 Topical Solution, 0.46% compared to vehicle in subjects with alopecia universalis (AU) and alopecia totalis (AT)

Secondary Objectives:

To assess the efficacy of ATI-50002 Topical Solution, 0.46% for the treatment of scalp hair loss and eyebrow in subjects with AU or AT.

To assess subject satisfaction following treatment with ATI-50002 Topical Solution, 0.46% for the treatment of scalp hair loss in subjects with AU or AT.

Study Design:

During the double-blind period, this Phase 2, multicenter, randomized study is designed to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of ATI-50002 Topical Solution in subjects with AU and AT. Subjects will be required to have a clinical diagnosis of stable, clinically typical, AU or AT for a duration of at least 6 months up to and including seven years. In the double-blind phase, a total of approximately 12 subjects will be randomized to a 2:1 ratio:

- ATI-50002 Topical Solution, 0.46%, BID for 28 days (8 subjects)
- Vehicle Solution BID for 28 days (4 subjects)

During the screening period, subjects will be assessed for eligibility into the study. Subjects who meet all the entry criteria will be randomized to ATI-50002 Topical Solution, 0.46% or Vehicle Topical Solution and will apply a minimum of 1.5 mLs up to a maximum of 4 mLs of study medication to the entire scalp twice-a-day (BID) for 28 days (except for Day 1 and Day 28 when study medication will only be applied once a day). For the first 6 subjects enrolled in the study, blood samples for concentrations of ATI-50002 will be collected on Day 1 (Visit 2) and Day 28 (Visit 6) at pre-dose and 1, 2, 4, 8 hours post dose and on Day 2 (Visit 3) and Day 29 (Visit 7) at 24 hours post-dose. Blood samples for immunology will be collected on Day 1 (Visit 2) before study medication application and on Day 29 (Visit 7), 24 hours after study medication application on Day 28 (Visit 6). A 4-mm punch biopsy of the scalp will be obtained to determine the concentration of ATI-50002 in the scalp skin on Day 2 (Visit 3) and on Day 29 (Visit 7) 24 hours after study medication application on Day 1 (Visit 2) and on Day 28 (Visit 6). A 4-mm punch biopsy will be obtained to determine histology and immunology, prior to study medication application on Day 1 (Visit 2) and on Day 29 (Visit 7), 24 hours after study medication application on Day 28 (Visit 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 will be completed.

Open-Label Extension:
On Day 29 (Visit 7) subjects who complete Visit 7, continue to meet the entry criteria, have no clinically significant AEs or tolerability issues and in the opinion of the investigator have experienced or are capable of re-growing scalp hair will be eligible to enter a 12-month open-label extension. In the open-label extension, safety, tolerability and efficacy will be assessed. All subjects enrolled in the open-label extension will apply ATI-50002 Topical Solution, 0.46% to the entire scalp, twice daily for 52 weeks (12 months). In addition, subjects with eyebrow loss may apply a thin film of study medication to the affected eyebrow(s) twice-daily for 52 weeks (12 months). Subjects will be followed for safety, tolerability and efficacy as detailed in Section 7.3. Subjects who decline or are not eligible for the open-label extension should be seen for the Visit 16 (Post-treatment) assessments 30 days following the Day 28 (Visit 6). The duration of the open-label extension (OLE) should be based on the date of the visit that the subject initiated OLE treatment rather than the days from Baseline (Visit 2). For subjects who entered the open-label extension and had a gap between Visit 7 and entry into the open-label extension due to an administrative delay total study participation may be longer than 400 days.

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

Number of Study Sites:
This study will be conducted at two clinical sites.

Inclusion Criteria:
Subjects must meet all the following criteria to be eligible for participation in the study.

1. Able to comprehend and willing to sign an Informed Consent Form (ICF).
2. Male or non-pregnant, non-nursing female ≥18 years old at the time of informed consent.
3. Have a clinical diagnosis of stable, clinically typical, alopecia universalis or alopecia totalis.
4. Have a duration of the current episode of AU or AT for a minimum of 6 months and a maximum of seven years.
5. Have > 95% total scalp hair loss as measured by the Severity of Alopecia Tool (SALT).
6. If a woman of childbearing potential (WOCBP), must have a negative serum pregnancy test at Screening (Visit 1) and a negative urine pregnancy test (UPT) 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 11).
7. 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.
8. Be willing and able to follow all study instructions and to attend all study visits.
9. 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.
10. 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.
11. 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 including 30 days after the last application of study medication.

2. Patchy alopecia areata, diffuse alopecia areata or a history of an atypical pattern of AA (*e.g.*, ophiasis, sisaipho).
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.).
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 AU or AT 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 of baseline (Visit 2). Subjects on an antibiotic for a non-serious, 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 (HepB surface Ab without the presence of HepB 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 <1,000/mm^3, or platelet count <50,000/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)	Prior or current treatment with a JAK inhibitor is prohibited at any time
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

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 (AA, AU or AT) 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. Screening ECG findings of:
    - QTcF >450msec for males or >470msec for females (use of the ECG algorithm is acceptable for this purpose)
    - Heart rate < 45 or > 100 beats/minutes (inclusive)
    - 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

Duration of Treatment
Double-blind Treatment
Screening period up to 30 days.
Double-blind treatment period is 29 days (maximum of 28 days of study medication application).
Post-treatment Visit 16 is up to 33 days (30 +3 days) following Day 29 for subjects not continuing into the open-label extension.
Total study participation for subjects from Baseline (Visit 2) through Post-treatment Visit 16(Day 59 + 3 days) is up to 62 days (29 + 33).
Double-Blind + Open-Label Extension
The duration of the total study participation from Baseline (Visit 2) to Week 56 (Visit 16) is anticipated to be a maximum of 400 days (393 + 7 days).
The duration of the open-label extension (OLE) should be based on the date of the visit that the subject initiated OLE treatment rather than the days from Baseline (Visit 2). For subjects who entered the open-label extension and had a gap between Visit 7 and entry into the open-label extension due to an administrative delay total study participation may be longer than 400 days.

Endpoints
Pharmacokinetic:
Concentrations of ATI-50002 in the blood and ATI-50002 in the skin will be measured by a bioanalytical lab using fully validated analytical methods for the blood and qualified methods for the skin.

Pharmacodynamic:

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.

Efficacy Assessments:
Early signs of vellus hair regrowth are expected to take a minimum of 3 to 6 months in this population. Measurable terminal hair regrowth may take at least 6 months or longer.

Change from baseline in Severity of Alopecia Tool (SALT) score at Week 28 (Visit 13), Week 40 (Visit 14) and Week 52 (Visit 15).

Change from baseline in Alopecia Density and Extent Score (ALODEX) at Week 28 (Visit 13) Week 40 (Visit 14) and Week 52 (Visit 15).

Subject Satisfaction at Week 28 (Visit 13), Week 40 (Visit 14) and Week 52 (Visit 15).

Descriptive summaries of changes from entry into long-term open-label extension in CEA and SEA at Week 28 (Visit 13), Week 40 (Visit 14) and Week 52 (Visit 15) will be performed for those subjects with eyebrow loss.

Study Medication Administration
Double-blind Period: Subjects will apply a minimum of 1.5 mLs up to a maximum of 4 mLs of topical solution to the entire scalp twice-daily as directed.

Open-label Period: Subjects will apply a minimum of 1.5 mLs up to a maximum of 4 mLs of topical solution to the entire scalp twice-daily as directed. In addition, subject may apply a thin film of study medication to the entire affected eyebrow(s) twice-daily for up to 12 months.

Statistical Methods
Sample Size/ Power Calculations
The planned sample size is 12 subjects.

Statistical Methods
Pharmacokinetic Analyses
Blood Samples
Blood samples for analysis of the concentration of ATI-50002 will be taken from the first 6 subjects enrolled into the study at Day 1 (Visit 2) and Day 28 (Visit 6) at predose (0 hr), 1, 2, 4, 8 and on Day 2 (Visit 3) and Day 29 (Visit 7) at 24 hours post dose. For each subject, standard PK parameters will be calculated, based on the plasma concentrations of ATI-50002.

Scalp Biopsy
The concentration of ATI-50002 will be determined in a 4-mm punch biopsy of the scalp taken 24 hours post study medication application in the office on Day 2 (Visit 3) and Day 29 (Visit 7).

Pharmacodynamic (Biomarker) Analyses
ALADIN Scores

The cytotoxic T lymphocyte infiltration (CTL), Interferon (IFN-gamma), and hair keratin (KRT) ALADIN scores will be calculated for each scalp biopsy sample as previously described in Xing et al, 2014, Jabbari 2016. Change in ALADIN scores within individual subjects will be assessed between Baseline (Visit 2), Day 29 (Visit 7) and Week 28 (Visit 13) for subjects enrolled into the open-label extension.

Histology
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
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.

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. Overall incidence of adverse events will be compared between groups using a chi-square test.

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.

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2. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
AA	Alopecia Areata
AE	Adverse Event
AGA	Androgenic Alopecia
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
AUC	Area Under the Curve
BID, b.i.d.	Twice-daily
BUN	Blood Urea Nitrogen
°C	Degrees Centigrade
CD	Cluster of Differentiation
CEA	Clinician's Eyebrow Assessment
CI	Confidence Interval
C_{max}	Maximum concentration
CMV	Cytomegalovirus
CRA	Clinical Research Associate
eCRF	Electronic Case Report Form
CRO	Contract Research Organization
CS	Clinically Significant
CTL	Cytotoxic T-lymphocytes
DMARDs	Disease Modifying Anti-Rheumatic Drugs
DPCP	Diphenylcyclopropenone
e.g.	for example (Latin; *exempla gratia*)
EC	Ethics Committee
ECG	Electrocardiogram
ERT	eResearchTechnology, Inc.
et al.	and others (Latin; *et alia*)
°F	Degrees Fahrenheit
FACS	Fluorescence Acquisition Cell Sorting
FDA	Food and Drug Administration
G	Gram
GCP	Good Clinical Practice

Abbreviation	Term
HCl	Hydrochloride
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
ITT	Intent-to-Treat
IRB	Institutional Review Board
JAK	Janus Kinase
KER	Hair keratin panel
KRT	Hair Keratin
LDH	Lactate dehydrogenase
MeDRA	Medical Dictionary for Regulatory Activities
MHC	Major Histocompatibility Complex
mL	Milliliter
Mm	Millimeter
NCS	Not Clinically Significant
NK/ NKG	Natural Killer/ Natural Killer Group
NMSC	Nonmelanoma Skin Cancer
OLE	Open Label Extension
OTC	Over-The-Counter
PDT	Photodynamic Therapy
PP	Per-protocol
PUVA	Psoralen and Ultraviolet A
SADBE	Squaric Acid Dibutyl Ester
SAE	Serious Adverse Event
SALT	Severity of Alopecia Tool
SEA	Subject's Eyebrow Assessment
SI	Subject identifier
SN	Subject Number
SOP	Standard Operation Procedure
STAT	Signal Transducer and Activator of Transcription
$T_{1/2}$	Elimination half-life

Abbreviation	**Term**
TEAE	Treatment Emergent Adverse Event
T_{max}	Time to Maximum Concentration
TNK	Tumor Necrosis Factor
Tyk2	Tyrosine Kinase 2
UPT	Urine Pregnancy Test
US	United States
UVA	Ultraviolet A
UVB	Ultraviolet B
WOCBP	Women of childbearing potential

3. INTRODUCTION

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

3.1. 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 and is a prevalent autoimmune disease in the United States, 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 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, Craiglow 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, AU and AT.

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 AA. Aclaris is also developing ATI-50001 for the treatment of AA, AU and AT. ATI-50001 is an oral pro-drug that is rapidly converted pre-systemically to ATI-50002, a potent highly selective inhibitor of Janus kinase 1 (JAK1) and Janus kinase 3 (JAK3). In this study, we will be evaluating whether or not the topically applied JAK1/JAK3 inhibitor, ATI-50002, will result in regrowth of scalp hair loss and if applicable, eyebrow hair loss due to underlying AU and AT.

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 (ATI-50002 Topical Solution Investigator Brochure).

4. STUDY RATIONALE

4.1. 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, AU and AT. 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, AU and AT.

The double-blind period of the study will evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of ATI-50002 Topical Solution, 0.46% compared to Vehicle Topical Solution applied twice-daily for 28 days in subjects with AU or AT. On Day 1 (Visit 2) and Day 28 (Visit 6), study medication will only be applied once a day. The study will include the assessment of the scalp hair loss at Baseline (Visit 2) and Day 29 (Visit 7) by both SALT and ALODEX. Early signs of hair regrowth are expected to take a minimum of 3 to 6 months in this population. Measurable hair regrowth may take at least 6 months or longer. Data from SALT and ALODEX will be described but no formal statistical comparisons will be made between Day 1 (Visit 2) and Day 29 (Visit 7) assessments.

Subjects who complete the double-blind period without any clinically significant adverse events or tolerability issues as assessed by the Investigator and who continue to meet the entry criteria will have the option to enroll in the open-label extension. The open-label period will evaluate the safety, tolerability and efficacy of ATI-50002 Topical Solution, 0.46% for the treatment of subjects with AU or AT. Subjects will apply ATI-50002 Topical Solution 0.46% to the entire

scalp, twice daily for 12 months. In addition, subjects with eyebrow loss may apply a thin film of study medication to the affected eyebrow(s) twice-daily for 12 months.

The 12-month open-label treatment period is supported by the results of 26-week mouse and 39-week dog oral toxicity studies with systemic administration and the 9-month minipig study with dermal administration.

ATI-50002-AA-202 Study Update
Eleven subjects have been treated with ATI-50002 Topical Solution. According to data entered into the eCRF as of August 3, 2018, five subjects experienced 7 adverse events; cold sore lesion, skin abrasion (scalp), hypertensive urgency, lower leg swelling, worsening depression, elevated A1C and headache/ migraine. All adverse events were considered unrelated to study medication and all were reported as mild severity except for hypertensive urgency and worsening depression which were moderate in severity. There were no serious treatment-related adverse events reported in the study. One serious adverse event, worsening of depression, was reported in subject 01-005. The subject had a 48-year history of depression and a 3-year history of bipolar disorder. She was treated with electroconvulsive therapy and the event is ongoing and assessed by the investigator as not related to study medication. Levels of ATI-50002 in the blood were all below the limit of quantification (1 ng/mL) at all timepoints on Day 28. Two subjects have discontinued from the study; both subjects withdrew consent.

Early hair growth (reductions in SALT scores) is evident in 3 subjects who are currently on active treatment. In order to continue to assess response to treatment given the favorable safety to date, this amendment will allow subjects to continue with twice-daily active study medication applications up to 12 months.

5. STUDY OBJECTIVE

5.1.1. Primary Objective

The primary objective is to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of ATI-50002 Topical Solution, 0.46% in subjects with AU or AT.

5.1.2. Secondary Objectives

Secondary objectives are:

To assess the efficacy of ATI-50002 Topical Solution, 0.46% for the treatment of scalp hair loss and eyebrow in subjects with AU or AT.

To assess subject satisfaction following treatment with ATI-50002 Topical Solution, 0.46% for the treatment of scalp hair loss in subjects with AU or AT.

6. SELECTION AND DISPOSITION OF STUDY POPULATION

6.1. Number of Subjects

Approximately 12 subjects will be enrolled in this study at two US investigational centers.

6.2. Study Population Characteristics

Male and female subjects, 18 years of age or older, with a clinical diagnosis of stable, clinically typical, alopecia universalis and alopecia totalis of the scalp, who meet all the inclusion criteria and none of the exclusion criteria, will be eligible to enroll in this study.

6.3. 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 Form (ICF).
2. Male or non-pregnant, non-nursing female ≥ 18 years old at the time of informed consent.
3. Have a clinical diagnosis of stable, clinically typical, alopecia universalis or alopecia totalis.
4. Have a duration of the current episode of AU or AT for a minimum of 6 months and a maximum of seven years.
5. Have > 95% total scalp hair loss as measured by the Severity of Alopecia Tool (SALT).
6. If a woman of childbearing potential (WOCBP), must have a negative serum pregnancy test at Screening (Visit 1) and a negative urine pregnancy test (UPT) at Baseline 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 11).
7. 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.
8. Be willing and able to follow all study instructions and to attend all study visits.
9. 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.
10. 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.
11. 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.

6.4. 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 including 30 days after the last application of study medication.
2. Patchy alopecia areata, diffuse alopecia areata or a history of an atypical pattern of AA (*e.g.*, ophiasis, sisaipho).
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.).

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 AU or AT 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 infection (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, history of treated (*e.g.*, pneumonia, septicemia) within 3 months prior to baseline (Visit 2). Subjects on an antibiotic for a non-serious, 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 (HepB surface Ab without the presence of HepB 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 <1,000/mm^3 or platelet count <50,000/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
	Prior or current treatment with a JAK inhibitor is prohibited at any time.

Therapy/Medication	Washout Period
Systemic Therapies	
JAK inhibitors (oral or topical)	
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

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 having participated in a study of an investigational drug, device or biologic agent for alopecia areata (AA, AU, AT) 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. Screening ECG findings of:
    - QTcF >450msec for males or >470msec for females (use of the ECG algorithm is acceptable for this purpose)
    - Heart rate < 45 or > 100 beats/minutes (inclusive)
    - 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

6.5. 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 6.5.2).

6.5.1. Permitted Concomitant Therapies and Over the Counter Products (OTC)

Concomitant therapies are any new or existing therapies received from Screening (Visit 1) until Day 393 (Visit 16). 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 use therapies not restricted by the protocol if 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/scalp products (shampoos, conditioners, other products) should be reviewed by the Investigator and are permitted if, in the Investigator's opinion, they will not affect the safety, pharmacokinetic or pharmacodynamic assessments of the subject during 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 study medication will be documented in the subject's source document and eCRF. In addition, any new permitted medications administered during protocol treatment and through Week 56 (Visit 16) will be documented in the subject's source document and eCRF.

6.5.2. Prohibited and/ or Restricted Medications

Any medication, shampoo or hair/ scalp product known to affect hair growth in AU or AT is prohibited throughout the study period. Subjects who are on a stable dose of finasteride for benign prostatic hypertrophy for greater than 1 year, are eligible for enrollment into 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 and 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)	Prior or current treatment with a JAK inhibitor is prohibited at any time
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

6.6. 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 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.

6.7. Replacement Subjects

Subject enrollment will continue until approximately 12 subjects have been randomized. Subjects who are randomized and do not complete the study will not be replaced.

6.8. 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 or use of a prohibited therapy. The Investigator or designee must inform the Aclaris Therapeutics, Inc. study monitor of any subject discontinuation. (Refer to Section 10.2.4 for study medication discontinuation or termination criteria).

In case of premature discontinuation of study participation, all efforts will be made to perform all Week 56 (Visit 16) 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.

6.9. 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, business, 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.

7. INVESTIGATIONAL PLAN

7.1. Overall Study Design

In the double-blind period, this Phase 2, multicenter, randomized study is designed to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of ATI-50002 Topical Solution in subjects with AU and AT. Subjects will be required to have a clinical diagnosis of stable, clinically typical AU or AT for a duration of at least six months up to and including seven years. A total of 12 subjects will be randomized.

During the screening period, subjects will be assessed for eligibility into the study. Subjects who meet all the entry criteria will be randomized 2:1 to ATI-50002 Topical Solution, 0.46% or Vehicle Topical Solution and will apply a minimum of 1.5 mLs up to a maximum of 4 mLs of study medication to the entire scalp twice-a-day for 28 days. On Day 1 (Visit 2), Day 28 (Visit 6), and Day 196 (1 day prior to Visit 13), subjects will apply study medication once-a-day.

Blood samples for concentrations of ATI-50002 will be collected from the first 6 subjects enrolled into the study on Day 1 (Visit 2), and Day 28 (Visit 6) at pre-dose and 1, 2, 4, 8 hours post dose and on Day 2 (Visit 3) and Day 29 (Visit 7), 24 hours post-dose. Blood samples for immunology will be collected on Day 1 (Visit 2) before study medication application, and on Day 29 (Visit 7), 24 hours after study medication application on Day 28 (Visit 6). A 4-mm punch biopsy of the scalp will be obtained to determine the concentration of ATI-50002 in the scalp skin on Day 2 (Visit 3) 24 hours after study medication application on Day 1 (Visit 2) and on Day 29 (Visit 7), 24 hours after study medication application on Day 28 (Visit 6). A 4-mm punch biopsy will be obtained to determine histology and immunology prior to study medication application on Day 1 (Visit 2). Additional 4-mm punch biopsies to determine histology and immunology will be collected on Day 29 (Visit 7) and Day 197 (Visit 13), 24 hours after study medication application on Day 28 (Visit 6) and Day 196. 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.

On Day 29 (Visit 7) subjects who complete Visit 7, continue to meet the entry criteria and have no clinically significant AEs or tolerability issues will be eligible to enter a 12-month open-label extension. In the open-label extension, safety, tolerability and efficacy will be assessed. All subjects enrolled in the open-label extension will apply ATI-50002 Topical Solution, 0.46% to the entire scalp, twice daily for 52 weeks (12 months). In addition, subjects with eyebrow loss may apply a thin film of study medication to the affected eyebrow(s) twice-daily for 52 weeks (12 months). Subjects will be followed for safety, tolerability and efficacy as detailed in Section 7.3. Subjects who decline or are not eligible for the open-label extension should be seen for suture removal, if applicable per Section 7.3 and Visit 16 (Post-treatment) 30 days ± 3 days following the Day 28 (Visit 6). Subjects enrolled in the initial 6-month Open Label Extension outlined by Protocol Amendment 1 (dated 06Feb2018) must be re-consented under Protocol

Amendment 2 (dated 14AUG2018) in order to continue for an additional 6 months of Open Label treatment.

7.2. STUDY PROCEDURES

The investigator or a designated and appropriately trained staff member should perform the study assessments according to the schedule of assessments. 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.

7.3. Schedule of Assessments

	Screening	Baseline	Double Blind Treatment						
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Call	Visit 6	Visit 7	Optional Visit (Suture Removal)
Week		0	0	1	2	4	4	4	6
Treatment Day	-30 to 0	1	2	8	15	27	28	29	43
Treatment Window(days)	N/A	N/A		± 3	± 3	-3	-3	-3	± 3
Informed consent[1]	X							X[17]	
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
Clinical laboratory sampling[4]: CBC, Chemistry with lipids, Virology, Serum Pregnancy, Urinalysis	X[4]	X			X			X	
Urine pregnancy test (WOCBP)[5]		X					X		
ECG	X	X			X			X	
SALT Score (prior to ALODEX)[6]		X						X	
ALODEX Score (after SALT)[7]		X						X	
Clinician Eyebrow Assessment								X[18]	
Subject Eyebrow Assessment								X[18]	
Subject Global Impression of Treatment Satisfaction (SGIS)									
Photography[8]		X						X	
Subject randomization		X							
Subject instructions[9]		X	X	X	X	X	X	X[17]	
Biopsy for PK[10]			X					X	
Biopsy for PD[11]		X						X	
Blood sample for PK[12]		X	X				X	X	
Blood for immunologic studies[13]		X						X	
Dispense, collect, weigh study medication bottles[14]		X	X	X	X		X	X[17]	
In office study medication application[9,14]		X					X		
Suture removal, if applicable[15]					X				X
Telephone Call[16]						X			
Concomitant therapies		X		X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X

	Open-Label Treatment										Post-Treatment[17]
	Visit 8	**Visit 9**	**Visit 10**	**Visit 11**	**Visit 12**	**Call**	**Visit 13**	**Optional Visit (Suture Removal)**	**Visit 14**	**Visit 15**	**Visit** 16
Week	8	12	16	20	24	28	28	30	40	52	56
Treatment Day[17]	57 (OLE 28)	85 (OLE 56)	113 (OLE 84)	141 (OLE 112)	169 (OLE 140)	196 (OLE 167)	197 (OLE 168)	211 (OLE 182)	281 (OLE 252)	365 (OLE 336)	59/ 393 (OLE 364)
Treatment Window(days)	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 7	+7	± 3/± 7
Informed consent[1]											
Inclusion/exclusion criteria											
Physical exam[2]											X
Demographics & medical history											
Alopecia Areata History											
Vital signs[3]	X	X	X	X	X		X	X	X	X	X
Clinical laboratory sampling[4]: CBC, Chemistry with lipids, Virology, Serum Pregnancy, Urinalysis		X					X		X	X	X
Urine pregnancy test (WOCBP)[5]	X	X	X	X	X		X		X	X	X
ECG		X					X				X
SALT Score (prior to ALODEX)[6]	X	X	X	X	X		X		X	X	X
ALODEX Score (after SALT)[7]	X	X	X	X	X		X		X	X	X
Clinician Eyebrow Assessment	X	X	X	X	X		X		X	X	X
Subject Eyebrow Assessment	X	X	X	X	X		X		X	X	X
Subject Global Impression of Treatment Satisfaction (SGIS)	X	X	X	X	X		X		X	X	
Photography[8]	X	X	X	X	X		X		X	X	
Subject randomization											
Subject instructions[9]	X	X	X	X	X		X		X	X	
Biopsy for PD[11]							X				
Blood for immunologic studies[13]							X				

	Open-Label Treatment										Post-Treatment[17]
	Visit 8	**Visit 9**	**Visit 10**	**Visit 11**	**Visit 12**	**Call**	**Visit 13**	**Optional Visit (Suture Removal)**	**Visit 14**	**Visit 15**	**Visit** 16
Week	8	12	16	20	24	28	28	30	40	52	56
Treatment Day[17]	57 (OLE 28)	85 (OLE 56)	113 (OLE 84)	141 (OLE 112)	169 (OLE 140)	196 (OLE 167)	197 (OLE 168)	211 (OLE 182)	281 (OLE 252)	365 (OLE 336)	59/ 393 (OLE 364)
Treatment Window(days)	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 7	+7	± 3/± 7
Dispense, collect, weigh study medication bottles[14]		X	X	X	X	X	X		X	X	
In office study medication application[9,14]		X									
Suture removal, if applicable[15]								X		X	
Telephone Call[16]						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

[1]A written signed ICF 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 UPT).

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

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

[4] Clinical laboratory sampling includes: CBC, Chemistry with lipids, Urinalysis, at each visit and **At Screening only:** Quantiferon Gold, TIBC, Serum iron, Serum Ferritin, Serum pregnancy, T3, T4, and TSH.

[5] UPT must be performed prior to randomization at Visit 2 and prior to study medication application at Day 28 (Visit 6) and at each OLE visit. UPT must be negative for the subject to continue in the study. WOCBP must have a negative serum pregnancy test at screening and a negative UPT at baseline prior to randomization.

[6]SALT is performed prior to ALODEX score using device provided.

[7] ALODEX is performed after SALT score using device provided.

[8] Photography should be performed on Day 1 (prior to application of study medication application) and on Day 29 & Day 197 prior to the scalp biopsy.

[9]Subjects must be instructed to apply the study medication according to the instructions in Appendix 1. Subjects will apply the first dose of study medication on the morning of Day 1 (Visit 2) and the last application of study medication on the morning of Day 28 (Visit 6), under the instruction and supervision of the study staff. Study medication at these visits should be applied in the morning to allow the 8-hour post dose assessments to occur at a reasonable time. On Day 1 (Visit 2) and Day 29 (Visit 7) subjects will only apply study medication once-a-day in the office.

[10] PK: A 4mm punch biopsy must be obtained on Day 2 (Visit 3) and Day 29 (Visit 7) 24 hours (± 30 minutes) after study medication application on Day 1 (Visit 2) and Day 28 (Visit 6).

[11] PD: A 4mm punch biopsy must be obtained on Day 1 (Visit 2) before application of first dose of study medication and Day 29 (Visit 7) 24 hours (± 30 minutes) after study medication application. Subjects that choose to continue in to open-label extension treatment will also have a 4mm punch biopsy collected on Day 197 (Visit 13).

[12]PK: The first 6 subjects enrolled in the study will have the blood PK testing performed. Blood for PK sampling will be obtained on Day 1 (Visit 2) and Day 28 (Visit 6), just prior to study medication application (predose), and 1, 2, 4, 8 hours post dose and on Day 2 (Visit 3) and Day 29 (Visit 7), 24 hours after study mediation application (on Day 1 (Visit 2) and Day 28 (Visit 6). See Section 9.3.2 for PK sample draw time windows.

	Open-Label Treatment										Post-Treatment[17]
	Visit 8	**Visit 9**	**Visit 10**	**Visit 11**	**Visit 12**	**Call**	**Visit 13**	**Optional Visit (Suture Removal)**	**Visit 14**	**Visit 15**	**Visit** 16
Week	8	12	16	20	24	28	28	30	40	52	56
Treatment Day[17]	57 (OLE 28)	85 (OLE 56)	113 (OLE 84)	141 (OLE 112)	169 (OLE 140)	196 (OLE 167)	197 (OLE 168)	211 (OLE 182)	281 (OLE 252)	365 (OLE 336)	59/ 393 (OLE 364)
Treatment Window(days)	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 3	± 7	+7	± 3/± 7

[13] Blood for immunology will be obtained prior to administration of study medication on Day 1 (Visit 2) and on Day 29 (Visit 7) 24 hours (± 30 min) after study medication application on Day 28 (Visit 6). Subjects that choose to continue in to open-label extension treatment will also have blood for immunology collected on Day 197 (Visit 13).

[14] On Day 1 (Visit 2) and Day 28 (Visit 6) the study medication bottle should be weighed and then dispensed to the subject for the initial application under the supervision of study staff in the office. After the initial application on Day 1, the study medication bottle should be collected, weighed and dispensed to the subject after the 24-hour post-dose blood draw on Day 2 (Visit 3). For Visits 3 -6, each study medication bottle (with the cap) must be weighed before dispensing to the subject and at the time of return using the scale provided by Aclaris. On Day 28 (Visit 6), the study medication bottle should be weighed after subject applies the study medication in the office and not redispensed.

[15] The investigator or designee will remove the sutures approximately 14 days after scalp biopsy, unless an absorbable suture is used.

[16] On Day 27 or the day prior to Day 28 (Visit 6), the study staff will call the subject to remind them not to apply study medication on the morning of Day 28 (Visit 6) and to bring both used and unused study medication bottles to the visit. Study medication will be applied in the office under the supervision of the study staff. On Day 196 or the day prior to Week 28 (Visit 13) the study staff will call the subject to remind them not to apply study medication in that evening and on the morning of Week 28 (Visit 13), and to bring both used and unused study medication bottles to the visit. The scalp biopsy at Week 38 (Visit 13) should be performed 24 hours (±30 minutes) post the last study medication application.

[17]On Day 29 (Visit 7) subject may be given the option to move into open-label extension treatment.

- If the subject declines the open-label extension, she/he should be seen for the Visit 16 (Post-treatment) assessments 30 days following the date of Day 29 (Visit 7).
- If the subject chooses to continue in to the open-label extension, she/he must sign an ICF prior to being dispensed open-label drug and receiving continued study medication application instructions.
- If the subject enrolls in open-label treatment after Day 29 (Visit 7), an unscheduled visit will be conducted in order to obtain consent, review drug application instructions, and dispense drug. This will be considered the official date that the subject initiated open-label extension treatment and the scheduling of subsequent visits 8-16 should be based off of this date as referenced by (OLE xxx) in the treatment day schedule header above.

[18]Subjects who are eligible and consent to enter the open-label extension will have a baseline CEA and SEA completed at Visit 7 or the unscheduled Visit.

7.4. Subject Nourishment

The site should provide meals, snacks and beverages to the subject during the Day 1 (Visit 2) and Day 28 (Visit 6) pharmacokinetic and pharmacodynamic sampling times.

7.5. Duration of the Study

The anticipated time for study enrollment is 3 to 6 months. The duration of the screening period is up to 30 days. The expected double-blind treatment period for each subject is 29 days (maximum of 28 days of study medication application). The post-treatment follow-up period is up to 33 days (30 + 3-day window). The maximum total study duration for the double-blind period from the subject's first visit (Visit 1) through the subject's last visit (Visit 8) is 92 days. On Day 1 (Visit 2) and Day 28 (Visit 6), subjects are required to be confined at the investigational center until all study procedures are completed. The confinement period may last approximately 10 hours. The duration of the total study participation from Baseline (Visit 2) to the open-label post-treatment follow up visit, Week 56 (Visit 16) is anticipated to be a maximum of 400 days. The duration of the open-label extension (OLE) should be based on the date of the visit that the subject initiated OLE treatment rather than the days from Baseline (Visit 2). For subjects who entered the open-label extension and had a gap between Visit 7 and entry into the open-label extension due to an administrative delay total study participation may be longer than 400 days.

8. STUDY TREATMENT

8.1. Investigational Study Medication

The study medications for the double-blind period of this study are ATI-50002 Topical Solution, 0.46% and Vehicle Topical Solution. The study medication for the open-label period is ATI-50002-Topical Solution, 0.46%. The study medications are thin clear solutions that are indistinguishable as packaged and labeled. The inactive ingredients include: water, Transcutol P, propylene glycol, polyethylene glycol 400, dimethyl sulfoxide (DMSO), Kolliphor CS 20, benzyl alcohol, poloxamer 188, and povidone K30.

STUDY MEDICATION INFORMATION			
	Double-Blind Period		**Open-Label Period**
Study medication name	**ATI-50002**	**Vehicle**	**ATI-50002**
Dosage Strength	0.46%	-	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 (Except for Day 1/Visit 2 and Day 28/Visit 6)		
Duration of administration	28 days		52 weeks

STUDY MEDICATION INFORMATION		
	Double-Blind Period	**Open-Label Period**
Other supplies	Disposable, single-use droppers will be provided. Applicators for eyebrow application will be provided (open-label)	

8.2. 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.

For the double-blind period, subjects will be assigned to 1 of the 2 treatment groups in a random manner and at a 2:1 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 label page and record the Subject Kit number in the subject's eCRF.

The 52-week (12 month) extension is open-label, all eligible subjects will receive ATI-50002 Topical Solution, 0.46%.

8.3. Application of Study Medication

At Visit 2 (Day 1), the investigational center staff member will instruct each subject to apply a minimum of 1.5 mL up to a maximum of 4-mL dose of ATI-50002 Topical Solution, 0.46% or Vehicle Topical Solution, twice-daily to the entire scalp; once in the morning and approximately 12 hours later following the instructions in APPENDIX 1. On Day 1 (Visit 2) and Day 28 (Visit 6), study medication will be applied once-a-day due to the 24-hour PK and PD assessments. The subject must wash his/her hands thoroughly before and after each study medication application. The predose PK sample should be drawn and the baseline PD scalp biopsy should be obtained prior to study medication application. The subject should apply a thin film of the assigned study medication to his/her entire scalp and gently rub in the study medication.

On Day 1/ Baseline (Visit 2) the investigational staff member will:

- Dispense the appropriate study medication bottle.
- Weigh the bottle with the cap prior to the first study medication application and record in the subject's source document.
- Instruct the subject on the appropriate application technique (APPENDIX 1).

- Instruct the subject not to apply study medication to the biopsy site and to avoid contact with the eyes.
- Observe the subject's first study medication application to ensure proper coverage and monitor the subject for at least 20 minutes. Collect the study medication bottle.
- Record the first application completion time.
- Weigh the bottle with the cap after study medication application and record the weight in the subject's source document.
- Redispense study medication after the 24-hour PK and PD blood and biopsy sample collections on Day 2 (Visit 3).

On Day 28 (Visit 6) the investigational staff member will:

- Collect and weigh all study medication bottles (with the cap on the bottle) returned from the subject and record weight(s) in the subject's source document.
- Select one study medication bottle for the Day 28 (Visit 6) application.
- Weigh the bottle and cap prior to study medication application and record weight in the subject's source document.
- Review the application instructions with the subject (APPENDIX 1).
- Instruct the subject to avoid contact with the eyes.
- Observe the subject's study medication application to ensure proper coverage. Collect the study medication bottle after application.
- Record the study medication application completion time in the subject's source document.
- Weigh the bottle and cap after study medication application.
- Do not return the study medication bottle to the subject.

8.4. Study Medication Weights

On Day 1 (Visit 2) and Day 28 (Visit 6), a study staff member will weigh the study medication bottles with the cap prior to and after study medication applications. At the other study visits (Visit 3-15), the staff will weigh the bottles with the cap prior to dispensing new bottle(s) to the subject and after collection of used bottles from the subject. The weights of the study medication bottles must be recorded in the subject's source documentation. Bottles that are not dispensed to the subject do not need to be weighed.

8.5. Treatment Compliance

The investigator or designee will be responsible for monitoring subject compliance by reviewing the amount of study medication used based on the weight of the used study medication bottles and by querying the subject on usage at each visit. At Visits 4-15, an investigational center staff member must query each subject to determine compliance with the study medication application procedure and frequency. The staff member will document the study medication usage in the source document and eCRF. Study staff will counsel the subjects, as required to make sure subjects are compliant with study medication applications.

8.6. Dose Modification

Subjects should be instructed not to modify the study medication application procedure or frequency. All application modifications must be reported on the appropriate source document

and eCRF. If the subject experiences any significant application site AE(s), after discussion with the Sponsor, the investigator or designee may direct the subject to reduce study medication application frequency to once a day. Every effort should be made to resume twice daily applications for the week prior to Day 28 (Visit 6). If a subject cannot resume twice-daily applications to the scalp for more than 4 consecutive days, the subject must be removed from the study. Refer to Section 10.2.4 for Study Medication Interruption or Discontinuation due to related SAEs or specific abnormal laboratory values.

8.7. Study Medication Accountability

The Investigator or designee is responsible for ensuring accountability for the investigational study medication, including reconciliation of study medications and maintenance of study 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 up to date, specifying the amount dispensed for each subject and the date of dispensing. This inventory record will be available for inspection at any time. The original inventory record will be available for review by Aclaris Therapeutics, Inc. upon request. At the completion of the study, all used and unused study medication bottles will be returned to Aclaris Therapeutics, Inc. (or designee) for disposal per Aclaris Therapeutics, Inc. (or designee) written instructions. Alternatively, drug disposal may be completed by the investigative site (following appropriate monitor review of inventory) if the site has a written procedure in place dealing with destruction/disposal of investigational products.

8.8. Other Study Supplies

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

- Equipment, supplies and training for taking standardized photographs
- Equipment and training for SALT and ALODEX assessments
- Equipment, supplies and training for capture of ECGs for central reading
- Scales for weighing the study medication bottles
- Supplies and shipment instructions of PK and PD samples
- UPT kits

8.9. Blinding

Blinding of study medication is important for validity of this study. This study uses a double-blind design for the initial 28-day double-blind treatment period. The study medications used during the double-blind period are indistinguishable in appearance, as packaged and labeled.

8.9.1. Unblinding the Study Medication (Double-blind Period)

The blinding may be broken 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 concurrence. 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 discharged from the study (Section 6.8). Subject must complete the double-blind treatment period to be eligible for entry into the open-label extension.

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

8.10. 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. During the open-label portion of the study, applicators will also be provided for application of the ATI-50002 Topical Solution, 0.46% to the eyebrow(s).

For the double-blind period, one Subject Kit box will contain 6 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 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 1-3 bottles of ATI-50002 Topical Solution, 0.46% per month. Each bottle is packaged in a carton and both the bottle and carton will be labelled with a single-panel label.

9. STUDY ASSESSMENTS

The study assessments will be performed according to the schedules noted below by the investigator, an appropriately trained study staff member or the subject as noted for each assessment. The SALT and ALODEX assessments should be performed by the same staff member for a given subject throughout the study. If this is not possible, an appropriate designee with overlapping experience with the subject and study should perform the assessments. Hair regrowth in subjects is not anticipated during the 28-day duration of the double-blind study medication application.

9.1. Efficacy Assessments

9.1.1. Severity Alopecia Tool (SALT) Score

The SALT score is a measurement of the amount of scalp without any hair. The Investigator will assess the SALT score using an iPAD provided by Aclaris at Baseline (Visit 2), Day 29 (Visit 7), and as detailed in Section 7.3 for the subjects who continue in the open-label extension. Equipment, supplies, training and a detailed Reference Guide will be provided to the investigational site prior to the initiation of subject enrollment.

9.1.2. Alopecia Density and Extent Score (ALODEX)

The ALODEX score is a measurement of the amount of scalp with hair loss (Olsen, submitted). The Investigator will calculate the ALODEX score using the iPAD provided by Aclaris at Baseline (Visit 2), Day 29 (Visit 7) and as detailed in Section 7.3 for subjects who continue in the open-label extension. Equipment, supplies, training and a detailed Reference Guide will be provided to the investigational site prior to the initiation of subject enrollment.

9.2. Pharmacodynamic (Biomarker) Assessment

9.2.1. Scalp Biopsy (PD)

A 4-mm punch biopsy of the scalp will be obtained on Baseline (Visit 2) just prior to the first application of study medication and at Day 29 (Visit 7) 24 hours (± 30 minutes) after the study medication application for histology and RNA sequencing (APPENDIX 5). At Visit 13, a 4-mm biopsy will be obtained approximately 24 hours ± 30 minutes after the morning dose on the day prior to the visit.

9.2.2. Blood for Immunologic Evaluation (T-cells)

The blood samples will be obtained on Day 1 (Visit 2) predose and on Day 29 (Visit 7) 24 hours (± 30 minutes) after the study medication application 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

At Visit 13, blood samples will be obtained approximately 24 hours ± 30 minutes after the morning dose on the day prior to the visit.

9.3. Pharmacokinetic Assessment

9.3.1. Scalp Biopsy (PK)

A 4-mm punch biopsy of the scalp will be obtained on Day 2 (Visit 3) and on Day 29 (Visit 7) 24 hours (± 30 minutes) after the study medication application (on Day 1/Visit 2 and Day 28/Visit 6) to determine the concentration of ATI-50002 in the skin (APPENDIX 5). The scalp biopsy should be obtained after the area of the scalp to be biopsied is tape stripped using 5 D-Squame circular discs.

The bioanalytical lab will provide instructions on the collection and shipment of samples for analysis.

9.3.2. Blood Samples (Double-blind Period)

For the first six subjects enrolled into the study, blood samples for PK analysis of ATI-50002 will be collected by a qualified staff member. On Baseline/ Day 1 (Visit 2) and Day 28 (Visit 6) blood samples will be collected at predose (0-hour) (just prior to study medication application in the morning) and then at the following times **after study medication application**:

- 1 hour (±5 minutes)
- 2 hours (±10 minutes)
- 4 hours (±10 minutes)
- 8 hours (±10 minutes)

On Day 2 (Visit 3) and Day 29 (Visit 7)

- 24 hours (±30 minutes)

The bioanalytical laboratory will provide instructions on the collection and shipment of samples.

9.4. Eyebrow Assessments (Open-Label Extension)

Subjects with eyebrow loss in one or both eyebrows will be allowed to treat their affected eyebrow(s) during the open-label period of the study. Both the investigator and subject will assess eyebrow loss at entry into the open-label extension and then monthly for the first 6 months, then at 9 and 12 months during the open-label treatment period. The Investigator will document the affected areas of the eyebrow or brow(s) on the source document and eCRF.

9.4.1. Clinician's Eyebrow Assessment (CEA) (Open-Label Period)

The CEA is the Investigator's assessment of the affected eyebrow(s) at a particular point in time. The Investigator should NOT refer to any other assessments to assist with these assessments.

At Visits 8 - 16, the Investigator will assess the affected eyebrow(s) using the scale below and report the one integer that best describes the amount of eyebrow hair present. **If both eyebrows are affected, each eyebrow will be assessed separately.**

Clinician's Eyebrow Assessment

Grade	Descriptor
0	**No eyebrow hair:** No terminal hairs are visible in the affected area(s)
1	**A little eyebrow hair:** Occasional terminal hairs are visible in the affected area(s)
2	**Some eyebrow hair:** Numerous terminal hairs are visible in the affected area(s)
3	**Most eyebrow hair:** Mostly complete eyebrow regrowth with terminal hair in the affected area(s)
4	**Full eyebrow hair:** Complete eyebrow regrowth with terminal hair in the affected area (s)

9.4.2. Subject's Eyebrow Assessment (SEA) (Open-Label Period):

The SEA is the subject's assessment of the appearance of eyebrow hair present on the affected eyebrow(s) at a particular point in time. The subject should NOT refer to any other assessments to assist with these assessments.

At Visits 8-16, the subject will assess the affected eyebrow(s) using the scale below and report the one integer that best describes the amount of eyebrow hair present. **If both eyebrows are affected, each eyebrow will be assessed separately.**

Subject's Eyebrow Assessment

Grade	Descriptor
0	**No eyebrow hair:** No thick, coarse hairs are visible in the affected area(s)
1	**A little eyebrow hair:** A few thick, coarse hairs are visible in the affected area(s)
2	**Some eyebrow hair:** Numerous thick, coarse hairs are visible in the affected area(s)
3	**Most eyebrow hair:** The majority of the affected area(s) of the eyebrow is covered in thick, coarse hairs
4	**Full eyebrow hair:** The affected area(s) of the eyebrow is fully covered in thick, coarse hairs

An investigational staff member will identify the eyebrow to be evaluated by the subject, educate the subject on the SEA scale before each evaluation and direct the subject to assess the eyebrow, one eyebrow at a time. The staff member should not influence the subject's assessment.

The study staff member will report the SEA grade the subject indicates in the source document for the affected eyebrow(s). Both the subject and the study staff member must sign/initial the source document to indicate the subject performed the SEA as instructed.

9.5. Assessment of Safety

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

9.5.1. Vital signs

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

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

Any measure that is, in the opinion of the investigator, abnormal AND clinically significant (CS) must be recorded as medical history if found prior to the first study medication application, or as an AE if found after the first study medication treatment 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) on the eCRF. A weight >300 lbs. is considered abnormal and therefore must be defined as CS or NCS on the eCRF.

9.5.2. Physical Examination

The investigator or designee will perform a physical examination for all body systems (general appearance, examination of head, eyes, ears, nose and throat, respiratory, cardiovascular, abdominal, extremities, neurological, musculoskeletal, lymphatic and skin assessment) at Screening (Visit 1) and Day 29 (Visit 7). For subjects who are enrolled in the Open-label extension, a physical examination will also be performed at Week 56 (Visit 16).

9.5.3. Clinical Laboratory Assessments

A qualified staff member will collect non-fasting samples for clinical laboratory analysis at the times in the visit schedule in Section 7.3. Samples will be sent to a central laboratory 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**

Chemistry Panel	Complete Blood Count
Total protein	
Uric acid	
HDL, LDL, Total cholesterol, Triglycerides	

Screening Tests Only
Hep B, HCV, HIV
Quantiferon Gold
Total Iron Binding Capacity (TIBC)
Serum iron
Serum Ferritin
T3/T4, TSH
Serum Pregnancy

The results of the clinical laboratory tests will be reported on the central 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. The investigator must review all laboratory reports in a timely manner.

9.5.4. Pregnancy tests

Subjects who are WOCBP must have a negative serum pregnancy test result at Screening (Visit 1) to continue in the study, a negative UPT at Baseline (Visit 2) **prior to randomization** and **prior to study medication application** at Day 28 (Visit 6). WOCBP who are enrolled in the open label period will have UPT at the visits detailed in Section 7.3 during the treatment period. The UPT kits provided by Aclaris Therapeutics, Inc. have a minimum sensitivity of 25-mIU ß-HCG/milliliter (mL) of urine. If the result of any post-treatment UPT is positive, the subject will be withdrawn from the study and the subject's pregnancy documented and followed.

9.5.5. ECGs

Standard 12-lead ECGs will be performed by a qualified staff member at Screening (Visit 1), Baseline (Visit 2), Day 15 (Visit 5), Day 29 (Visit 7) and the visits outlined in Section 7.3. The ECGs must be obtained using a standard 12-lead ECG with a 10mm/mV amplitude, at 25mm/sec and a 5-10-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, eResearchTechnology, Inc. (ERT), will provide 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 medical 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 10.1.1).

9.6. Other Assessments

9.6.1. 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 (Visit 1): 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 extensive AA history at Screening (Visit 1) (APPENDIX 3).

9.6.2. Photographic Assessment

A qualified investigational staff member will take standardized photographs of the scalp at Baseline (Visit 2), prior to study medication application, Visit 7 (Day 29) prior to the scalp biopsy and if enrolled in the open-label extension at the visits detailed in Section 7.3. The photographs are to document the baseline hair loss and any early signs of hair growth during treatment. Equipment, supplies, training and detailed instructions for obtaining and managing photographs will be provided to the investigational center prior to the initiation of subject enrollment.

10. ADVERSE EVENTS

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

10.1. Definitions

10.1.1. Adverse Events (AE)

An AE is any untoward medical occurrence in a subject or clinical investigation 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.

10.1.2. 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.

10.1.3. 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.

10.1.4. 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.

10.1.5. 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.

10.1.6. 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).

10.2. Reporting Procedures

10.2.1. 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.

10.2.2. 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.
   Immediately (within 24 hours) inform the Medical Monitor of the SAE by telephone:
   Terrence Chew, MD, RAC
   Aclaris Therapeutics, Inc.
   640 Lee Rd
   Suite 200
   Wayne, PA 19087
   Office Telephone: 858-922-6307
   SAE facsimile 484-324-2359
   E-mail: tgchew@icloud.com
2. Within 24-hours complete, as fully as possible, an AE eCRF and an SAE form; fax or e-mail the forms and any other relevant information (*e.g.*, concomitant medication eCRF, medical history eCRF, laboratory test results) to the Aclaris Therapeutics, Inc. Medical Monitor.
3. 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. Medical Monitor agree that the SAE is satisfactorily resolved.
4. Inform the Aclaris Therapeutics, Inc. Medical Monitor of SAE updates by telephone followed by an SAE form update sent by e-mail.
5. 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).

10.2.3. Safety Monitoring ECG Discontinuation Criteria

Any subject who develops any of the following ECG criteria 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
- New QTcF > 500 ms
- Acute signs of ischemia or infarction
- Any ECG abnormality which may, in the opinion of the investigator, represent a new medical issue of concern

10.2.4. Study Medication Interruption and Discontinuation

10.2.4.1 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 decrease > 2 g/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 interrupt 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 interruption should continue, 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. Study medication can be resumed after the abnormal lab value returns to normal or baseline, if in the opinion of the Investigator, it is in the best interest of the subject to continue.

10.2.4.2 Study Medication Discontinuation

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

- Severe infection requiring parenteral antimicrobial therapy or hospitalization
- Symptomatic herpes zoster
- Malignancy – except for non-melanoma skin cancer (*e.g.* squamous or basal cell carcinoma) 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 1 week 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.

11. PREGNANCY

11.1. 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 (Visit 1) and a negative UPT at Baseline (Visit 2) prior to randomization.

11.2. 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)
- Obstruction of fallopian tubes via medical device (Essure™)
- 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 (study duration and 30 days after the last study medication application). 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 ≥90 days)
- Injectables (on a stable dose for ≥90 days)
- Patches (on a stable dose for ≥90 days)
- Combined oral contraceptives (on a stable dose for ≥90 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 study. The subject must sign an informed consent 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.

12. STATISTICAL ANALYSES

12.1. Sample Size and Power Calculations

The planned sample size is 12 subjects. This is the first multicenter study evaluating the effect of ATI-50002 Topical Solution in subjects with stable alopecia universalis and alopecia totalis. No formal power calculations will be performed.

12.2. Analysis Populations

Pharmacokinetic and Pharmacodynamic study populations will be described in a Statistical Analysis Plan and finalized prior to database lock.

12.3. 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.

12.4. Pharmacokinetic Analyses

Blood Samples

Blood samples for analysis of the concentration of ATI-50002will be taken from the first 6 subjects enrolled into the study on Baseline/Day 1 (Visit 2) and Day 29 (Visit 7) at predose (0 hr), and 1, 2, 4, 8 hours after study medication application and on Day 2 (Visit 3) and Day 29 (Visit 7), 24 hours post dose. For each subject, standard PK parameters (C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$) will be calculated, as a minimum and whenever possible, based on the plasma concentrations of ATI-50002.

Scalp Biopsy

The concentration of ATI-50002 in skin will be determined in a 4-mm punch biopsy of the scalp taken on Day 2 (Visit 3) and Day 29 (Visit 7) 24 hours post study medication application in the office on Day 1(Visit 2) and Day 28 (Visit 6).

12.5. Pharmacodynamic (Biomarker) Analyses

12.5.1. ALADIN Scores

The cytotoxic T-lymphocyte infiltration (CTL), Interferon (IFN-gamma), and hair keratin (KRT) ALADIN scores will be calculated for each scalp biopsy sample as previously described in (Xing

et al, 2014, Jabbari 2016). Change in ALADIN scores within individual subjects will be assessed between Baseline/ Day 1 (Visit 2), Day 29 (Visit 7) and Week 28 (Visit 13) for subjects enrolled in the open-label extension.

12.5.2. Histology

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.

12.5.3. Blood for Immunology

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.

12.5.4. Secondary Analyses

Summary descriptive statistics (N, mean, median, SD) by visit will be provided for all efficacy parameters. Secondary efficacy parameters are described below:

The secondary efficacy endpoints include mean change from baseline in the SALT score at Week 28 (Visit 13), Week 40 (Visit 14) and Week 52 (Visit 15).

The percentage hair growth will be calculated as the mean change from Baseline (Visit 2) scores compared to end of treatment Week 28 (Visit 13), Week 40 (Visit 14) and Week 52 (Visit 15).

Mean change from baseline in the ALODEX score, proportion of subjects achieving a $\geq$ 50% hair growth compared with baseline.

Treatment satisfaction will be summarized.

The ALODEX score analysis will use the same methodology as specified for SALT score analysis. Other parameters will be analyzed as detailed in the statistical analysis plan.

Mean change from baseline in eyebrow growth will be calculated as the mean change in the Clinician's Eyebrow Assessment (CEA) from entry into the long-term, open-label extension compared to Week 28(Visit 13), Week 40 (Visit 14) and Week 52 (Visit 15). The CEA evaluates eyebrows on a scale from "No eyebrow hair" (Grade 0) to "Full eyebrow hair" (Grade 4).

Mean change from baseline in the Subject's Eyebrow Assessment (SEA) will be calculated from entry into the long-term open label extension to Week 28 (Visit 13), Week 40 (Visit 14) and Week 52 (Visit 15). The SEA evaluates eyebrows on a scale from "No eyebrow hair" (Grade 0) to "Full eyebrow hair" (Grade 4).

12.6. 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. Overall incidence of adverse events will be compared between groups using a chi-square test.
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.

13. TRAINING, MONITORING, DATA MANAGEMENT AND QUALITY ASSURANCE

13.1. 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.

13.2. 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.

13.3. 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.

13.4. 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.

14. ETHICS AND GENERAL STUDY CONDUCT CONSIDERATIONS

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

This protocol, informed consent 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.

14.2. 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 current 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.

Subjects will provide voluntary informed consent prior to initiation of any study related procedures.

14.3. Subject Information and Consent

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

14.4. 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.

The investigator should document and explain any deviation from the protocol. 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 result 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.

14.5. 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.

14.6. 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.

14.7. Data Collection and Archiving

14.7.1. 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.

14.7.2. 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. Any AEs must be completely documented. Source documentation includes results of any diagnostic tests conducted during the study.

14.7.3. 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.

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

Preparation and General Instructions:

1. Gather a clean, dry washcloth and towel, the study medication bottle and disposable droppers.
2. Scalp should be clean (free of any products applied to the scalp), and dry before applying study medication. A clean scalp will allow the study medication to penetrate down into the scalp to ensure you are getting the best application. You should wash your scalp at least an hour prior to study medication application, at least once a day to prevent accumulation of study medication on your skin.
3. Wash your hands thoroughly with soap and water before and after using this study medication.
4. You will apply a thin layer (a minimum of 1.5 up to a maximum of 4 mL) of study medication to the entire scalp twice-a day approximately every 12 hours, as instructed by the study doctor or the study staff. On the first and last days of study medication dosing, you will apply study medication once-a-day only. Once you apply study medication, do not wash your scalp or participate in strenuous exercise that would cause profuse sweating for at least 6 hours.
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. Avoid exposing your scalp to excessive natural or artificial ultraviolet radiation (e.g., sunlight, tanning beds) and wear a hat or use sunscreen on your scalp, if excessive sun exposure cannot be avoided.
7. Remember to bring your study medication bottles (both used and unused) to each study visit.
8. Apply your study medication in the evening before Day 28 (Visit 6) and note the time. Do not apply your morning dose of study medication on your Day 28 (Visit 6) 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. Remove the cap from the bottle. Squeeze the rubber bulb of the dropper and insert the dropper into the bottle. Release the bulb, allowing the dropper to fill with the solution to the 1 mL line. If the level of the solution is above the 1 mL line, squeeze the extra amount back into the bottle. The study medication level should be in line with the 1mL mark on the dropper.
2. During study medication application, keep your head tilted back to avoid any study medication running into your eyes.
3. Start to slowly deposit drops of study medication on the left side of your scalp from the front to the back. Gently rub the solution into your scalp as you go, to avoid the medication from running off of your scalp.
4. Draw up the next dropper of study medication and repeat the process. Work from the left to the right side of your scalp until your entire scalp is covered with a thin film of study medication. The total amount of study medication you should apply per application is _____ mL.
5. Replace the screw top cap and make sure it is closed tightly. Dispose of the used dropper.

6. Wash your hands thoroughly after using the study medication to prevent any residue being left on your hands.
7. Allow the study medication to dry for at least 20 minutes (or until completely dry) before you cover your head with a hat, wig, or scarf to avoid transfer of study medication.
8. If you missed any applications of study medication, be sure to inform the study staff at your next visit.
9. Do not wash your scalp or participate in strenuous exercise that would cause profuse sweating 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 20 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 applied 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, note this missed dose, inform the study staff at your next visit 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 out of the reach of children.

APPENDIX 2: Subject Instructions for Study Medication Application to the Eyebrow(s)

General Instructions:

1. Before application of study medication, your face and 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 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 14 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 face, 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 a 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 3: Alopecia Areata History

The following AA history will be obtained:

1. Onset date of alopecia
2. Onset date of current episode of AU or AT
3. Did the subject use previous therapies for Patchy AU or AT?
    a. If Yes, indicate which therapies
        1. Topical immunotherapy
        2. Glucocorticosteroids
        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 4: 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 5: Scalp Biopsy

All scalp punch biopsies will be performed by the investigator according to the Schedule of Events in Section 7.3. **Note: The scalp biopsy for pharmacokinetic analysis performed on Day 2 and Day 29 should be obtained after the area of the scalp to be biopsied is tape stripped using 5 D-Squame circular discs.**

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, if necessary, and examine the healing process of the biopsy site to ensure it is healing appropriately.