CLINICAL STUDY PROTOCOL

Protocol Number: ATI-50002-AAB-201

An Open-Label Pilot Study of the Safety, Tolerability and Efficacy of ATI-50002 Topical Solution Administered Twice-Daily in Adult Subjects with Eyebrow Loss Due to Alopecia Areata, Alopecia Universalis or Alopecia Totalis

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PROTOCOL APPROVAL SIGNATURE PAGE

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Protocol Version: 1.0 06FEB2018

2-6-2018

Christopher Powala
Chief Operating Officer
Aclaris Therapeutics, Inc.

Date

2/6/18

Stuart Shanler
Chief Scientific Officer
Aclaris Therapeutics, Inc.

Date

INVESTIGATOR SIGNATURE PAGE

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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 material will only be provided as necessary to site personnel involved in the conduct of the trial, involved Institutional Review Board (IRB), Ethics Committees, 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 Name (Print)

_____ _____

Investigator Signature Date

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1. SYNOPSIS

Protocol Number:	**ATI-50002-AAB-201**
Protocol Title:	An Open-Label Pilot Study of the Safety, Tolerability and Efficacy of ATI-50002 Topical Solution Administered Twice-Daily in Adult Subjects with Eyebrow Loss due to Alopecia Areata, Alopecia Universalis or Alopecia Totalis
Sponsor:	Aclaris Therapeutics, Inc.
Phase of Development:	Phase 2

Study Drug Description:
ATI-50002 Topical Solution 0.46%

Study Objective:
The main objective of this study is to assess the safety, tolerability and efficacy of ATI-50002 Topical Solution 0.46% in subjects with unilateral or bilateral loss of eyebrow hair due to alopecia areata (AA), alopecia universalis (AU) or alopecia totalis (AT).

Study Design:
This is an open-label study which will be conducted at 1 to 2 sites.

Subjects will be required to have a clinical diagnosis of AA, AU or AT with unilateral or bilateral loss of eyebrow hair with a duration of the current episode of at least 6 months, but not more than seven years. A total of approximately 12 subjects will apply ATI-50002 Topical Solution 0.46%, to the affected eyebrow(s) twice-daily (BID) for 24 weeks.

After completing Visit 1 (start of the screening period), subjects will be assessed for eligibility to receive study medication.

Subjects will apply study medication to the entire affected eyebrow(s), twice-daily for 24 weeks. The 24-week treatment period will be followed by a 4-week no-treatment follow-up period. Assessment of response to treatment will be performed at Week 4, Week 8, Week 12, Week 16, Week 20, Week 24 and Week 28.

Safety and tolerability will be evaluated throughout the study by assessment of electro-cardiograms (ECG), clinical laboratory tests, adverse events and vital signs.

The duration of the study participation is anticipated to be a maximum of 233 days (an up to 28-day screening period, a 169-day treatment period with a 4-day window, a 28-day no-treatment follow-up period with a window of 4 days).

Study visits are:

- Visit 1 (Day -28 to 0): screening; start eligibility assessment period
- Visit 2 (Week 0): initiation of twice-daily study medication application

- Visits 3-10 (Weeks 1-24): treatment period
- Visit 11 (Week 28): no-treatment period follow-up; end of study

Number of Subjects to be Enrolled:
Approximately 12 subjects will be enrolled in this study.

Number of Study Sites:
This study will be conducted at up to 2 sites in the US.

Inclusion Criteria:
To be eligible for the study, subjects must fulfill all the following criteria:

1. Subject is at least 18 years of age.
2. Subject has, based on a subject history and clinical examination, a clinical diagnosis of AA, AU or AT with no eyebrow hair in the affected area(s) and no eyebrow regrowth over the previous 6 months. Affected area is defined as the area(s) of eyebrow hair loss identified at Baseline.
3. Subject has a Clinician Eyebrow Assessment score of 0 (No eyebrow hair in the affected area) for at least one eyebrow.
4. Subject has a Subject Eyebrow Assessment score of 0 (No eyebrow hair in the affected area) for at least one eyebrow.
5. Subject has a duration of the current episode of AA, AU or AT with unilateral or bilateral loss of eyebrow hairs (with at least one distinct patch of $\geq$30% loss of eyebrow hair) for at least 6 months and no more than seven years prior to Visit 1.
6. Subjects who are Women of Childbearing Potential (WOCBP) must have a negative serum pregnancy test at Screening and a negative urine pregnancy test at Baseline and agree to use a highly effective method of birth control for the duration of the study and for 30 days after last study medication application.
7. Subject is non-pregnant and non-lactating and not planning a pregnancy during the duration of the study and for 30 days after the last study medication application.
8. Subject is in good general health and free of any known disease state or physical condition which, in the opinion of the investigator, would interfere with the study requirements or put the subject at undue risk by study participation.
9. Subject is willing and able to follow all study instructions and to attend all study visits.
10. Subjects taking hormonal replacement therapy must have been on the same dose for at least 6 months prior to Visit 1 and must agree to maintain the same dose for the duration of the study and for 90 days after the last study medication application.
11. Subjects taking thyroid replacement therapy must have been on the same dose for at least 6 months prior to Visit 1 and must agree to maintain the same dose for the duration of the study.
12. Subject agrees to refrain from any eyebrow removal (*e.g.*, plucking, threading, etc.) for the duration of the study.

13. Subject agrees to refrain from any cosmetic surgery (*e.g.*, piercing, tattooing, etc.), on the treatment areas, for the duration of the study.
14. Subject can comprehend and is willing to sign an Informed Consent Form (ICF).
15. 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:

Any subject who meets one or more of the following criteria will not be included in this study:

1) Subject has, in the opinion of the investigator, permanent eyebrow loss attributed to causes other than AA, AU or AT such as overgrooming, or scarring hair loss.
2) Subject currently has, or has a history of, skin disease in the eyebrow area (*e.g.*, psoriasis, seborrheic dermatitis, etc.) that, in the opinion of the investigator, would interfere with the study medication application or study assessments.
3) Subject currently has, or has a history of, severe, progressive or uncontrolled autoimmune, metabolic, endocrinologic, renal, hepatic, gastrointestinal, pulmonary, cardiovascular, genitourinary (*i.e.*, renal disease), hematological disease, neurologic or cerebral disorders, infectious disease or coagulation disorders that, in the opinion of the investigator, put the subject at undue risk by study participation.
4) Subject currently has, or has a history of, proven or suspected systemic or cutaneous malignancy and /or lymphoproliferative disease, other than a history of adequately treated, well healed and completely cleared non-melanoma skin cancers (*e.g.* basal or squamous cell carcinoma) treated successfully at least one year prior to Visit 1.
5) Subject currently has evidence of active or latent bacterial infection, including tuberculosis, or viral infections at the time of enrollment or a history of incompletely treated or untreated tuberculosis. Subjects who have completed therapy for latent tuberculosis may participate.
6) Subject has a history of serious local infection (*e.g.*, cellulitis, abscess) or a systemic infection, including but not limited to, pneumonia or septicemia, within 12 weeks prior to Visit 1. Subjects on an antibiotic for a nonserious, acute local infection must complete the antibiotic course prior to enrollment in the study.
7) Subjects positive for HIV, Hepatitis B or C. Subjects with serologic evidence of Hepatitis B vaccination (HepB surface Ab without the presence of HepB surface Ag) will be allowed to participate.
8) Subject currently has or has a known history of herpes zoster or cytomegalovirus (CMV) within 8 weeks prior to Visit 1.
9) Subject has a history of frequent outbreaks of Herpes Simplex Virus defined as 4 or more episodes per year.
10) Subject has any history of eyebrow tattooing, or microblading that in the opinion of the investigator would interfere with the assessment of safety or efficacy.
11) Subject has used any semi-permanent eyebrow coloring (*e.g.*, tinting, dying, etc.) within 6 months prior to Visit 1 that in the opinion of the investigator would interfere with the assessment of safety or efficacy.

12) Clinically significant laboratory abnormalities at Screening that, in the opinion of the Investigator, would make the subject a poor candidate for the study.
13) Subjects with absolute neutrophil count <1,000/mm^3, or platelet count <50,000/ml.
14) Subject used any of the following therapies within the specified period prior to Visit 1:

Systemic therapies:

- Disease Modifying Anti-Rheumatic Drugs (DMARDS), Biologics or immunosuppressants, such as: anakinra, adalimumab, azathioprine, glucocorticosteroids, cyclosporine, etanercept, infliximab, methotrexate, TNF inhibitors, ustekinumab, secukinumab, ixekizumab, certolizumab pegol: 4 weeks or 5 half-lives whichever is longer
- Oral retinoids: 12 weeks
- Plaquenil: 8 weeks
- JAK inhibitors (oral or topical): 1 year
- Intralesional steroids on the eyebrow area: 4 weeks

Topical therapies on the eyebrow area:

- Phototherapy, Laser Therapy: 12 weeks
- Anthralin, bimatoprost, glucocorticosteroids, diphencyprone, diphenylcycloprophenone (DPCP), Squaric acid dibutyl ester (SADBE), minoxidil, pimecrolimus, tacrolimus: 4 weeks
- Topical treatments (prescription and over-the-counter) that contain retinoids, retinol, alpha hydroxy acids (e.g. glycolic, lactic acids) and beta hydroxy acids (e.g. salicylic acid) on and around the eyebrow area: 4 weeks

15) Subject has a history of sensitivity to any of the ingredients in the study medication.
16) Subject has participated 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) prior to 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) History of or current alcohol or drug abuse within 2 years of assessment for study enrollment.
18) 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

Criteria for Evaluation:

Efficacy:

The investigator will evaluate the severity of eyebrow loss using the Clinician's Eyebrow Assessment. Subjects will evaluate the severity of eyebrow loss using the Subject's Eyebrow Assessment.

Safety:

The investigator will assess clinical laboratory safety tests, vital sign readings, concomitant therapies, urine pregnancy test results, and adverse event (AE) information throughout the study.

Other:

Subjects will assess their satisfaction with the status of each eyebrow using the Subject Eyebrow Satisfaction scale. Standardized color photographs to assist with documenting the status of the subject's eyebrows will be taken throughout the study.

Study Drug Administration:

Subjects will apply ATI-50002 Topical Solution 0.46% BID for 24 weeks to the affected eyebrow(s). At Visit 2 (Baseline), after confirmation of the inclusion and exclusion criteria, an investigational staff member will instruct the subject on the proper study medication application technique, observe the first study medication application and monitor the subject for at least 20 minutes after the application. The subject will then start a 24-week treatment period.

Statistical Methods:

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

Mean change from baseline in eyebrow growth will be calculated as the mean change in the Clinician's Eyebrow Assessment (CEA) from Visit 2 (Baseline) compared to end of treatment (Visit 10). 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 Visit 2 (Baseline) to end of treatment (Visit 10).

Mean change from baseline in the Subject's Eyebrow Satisfaction (SES) will be calculated from Visit 2 (Baseline) to end of treatment (Visit 10).

All efficacy summaries will be based on the per-protocol population including all subjects who have Baseline (Visit 2) and Visit 10 efficacy data and no major protocol violations.

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 Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class. Vital signs and clinically significant abnormal laboratory results will also be

tabulated and presented. Change from Baseline in the Local Tolerability Assessments (LTA) to end of treatment (Visit 10) will be summarized.

Data from all enrolled and treated subjects will be presented and summarized. Safety summaries will include listings 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.

2. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
AA	Alopecia Areata
AE	Adverse Event
ALT	Alanine aminotransferase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
AT	Alopecia Totalis
AU	Alopecia Universalis
BID	Twice-daily
BUN	Blood Urea Nitrogen
°C	Degrees Centigrade
CD	Cluster of Differentiation
CEA	Clinician's Eyebrow Assessment
CMV	Cytomegalovirus
CRA	Clinical Research Associate
CRF	Case Report Form
CS	Clinically Significant
DMARDS	Disease modifying anti-rheumatic drugs
DPCP	Diphenylcycloprophenone
e.g.	for example (Latin; exempla gratia)
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EHL	Eyebrow Hair Loss
°F	Degrees Fahrenheit
FDA	Food and Drug Administration
G	Gram
GCP	Good Clinical Practice
HEENT	Head, Eye, Ear, Nose and Throat
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form

Abbreviation	Term
ICH	International Conference on Harmonization
i.e.	that is (Latin; id est)
IFN	Interferon
IL	Interleukin
IRB	Institutional Review Board
IUD	Intrauterine Device
IUS	Intrauterine Hormone Releasing System
JAK	Janus Kinase
LDH	Lactate Dehydrogenase
LTA	Local Tolerability Assessment
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
MHC	Major Histocompatibility Complex
ml	Milliliter
Mm	Millimeter
NCS	Not Clinically Significant
NK/ NKG	Natural Killer/ Natural Killer Group
OTC	Over-The-Counter
PDT	Photodynamic Therapy
PUVA	Psoralen and Ultraviolet A
SADBE	Squaric acid dibutyl ester
SAE	Serious Adverse Event
SEA	Subject's Eyebrow Assessment
SES	Subject's Eyebrow Satisfaction
SI	Subject Identifier
SOP	Standard Operation Procedure
STAT	Signal Transducer and Activator of Transcription
TEAE	Treatment Emergent Adverse Event
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 (JAK 1) and Janus kinase 3 (JAK 3).

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 the most 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 assessment (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 subjects.

Immunopathology & Pathophysiology of AA

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 more sparse 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 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 hair follicle 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, Gupta 2016, Scheinberg 2016). As ATI-50002 is a potent inhibitor at JAK 1 and JAK 3, it is strongly suggested that ATI-50002 may be effective in the treatment of AA.

Among patients with AA, patients with higher disease burdens are unlikely to have satisfactory outcomes with current therapies. Aclaris Therapeutics, Inc. is developing ATI-50002 as a topical treatment for stable AA. Aclaris is also developing ATI-50001 for the treatment of AU and AT. ATI-50001 is a prodrug 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 looking to see if topical application of the JAK1/JAK3 inhibitor, ATI-50002, will result in regrowth of eyebrows in areas of loss due to AA, AU or AT.

In a previous Phase 1 study in healthy volunteers, single ascending oral doses of ATI-50001, 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 >5%

subjects were abdominal pain (10%), flatulence (7%), diarrhea (6%) and headache (6%). Three subjects showed mildly elevated ALT or AST concentrations that were considered not clinically significant. 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 August 2017).

3.2. Study Rationale

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

The purpose of this proof of concept study is to demonstrate the safety, tolerability and efficacy of ATI-50002 Topical Solution, a topical Janus kinase (JAK 1/3) inhibitor in the treatment of unilateral or bilateral loss of eyebrow hair in subjects with AA, AT and AU.

4. STUDY OBJECTIVES

The main objective of this study is to assess the safety, tolerability and efficacy of ATI-50002 Topical Solution 0.46% in subjects with unilateral or bilateral loss of eyebrow hair due to AA, AU or AT.

5. SELECTION AND DISPOSITION OF STUDY POPULATION

5.1. Number of Subjects

Approximately 12 subjects will be enrolled at 1 to 2 sites in the United States.

5.2. Study Population Characteristics

Male and female subjects, 18 years of age or older, with a clinical diagnosis of unilateral or bilateral loss of eyebrow hair due to AA, AU or AT, who meet all the inclusion criteria and none of the exclusion criteria will be eligible to enroll in this study.

5.3. Inclusion Criteria

To be eligible for the study, subjects must fulfill all the following criteria:

1. Subject is at least 18 years of age.
2. Subject has, based on a subject history and clinical examination, a clinical diagnosis of AA, AU or AT with no eyebrow hair in the affected area(s) and no eyebrow regrowth over the previous 6 months. Affected area is defined as the area(s) of eyebrow hair loss identified at Baseline.
3. Subject has a Clinician Eyebrow Assessment score of 0 (No eyebrow hair in the affected area) for at least one eyebrow.
4. Subject has a Subject Eyebrow Assessment score of 0 (No eyebrow hair in the affected area) for a least one eyebrow.
5. Subject has a duration of the current episode of AA, AU or AT with unilateral or bilateral loss of eyebrow hairs (with at least one distinct patch of $\geq$30% loss of eyebrow hair) for at least 6 months and no more than seven years prior to Visit 1.
6. Subjects who are Women of Childbearing Potential (WOCBP) must have a negative serum pregnancy test at Screening and a negative urine pregnancy test at Baseline and agree to use a highly effective method of birth control for the duration of the study and for 30 days after last study medication application.
7. Subject is non-pregnant and non-lactating and not planning a pregnancy during the duration of the study and for 30 days after the last study medication application.
8. Subject is in good general health and free of any known disease state or physical condition which, in the opinion of the investigator, would interfere with the study requirements or put the subject at undue risk by study participation.
9. Subject is willing and able to follow all study instructions and to attend all study visits.
10. Subjects taking hormonal replacement therapy must have been on the same dose for at least 6 months prior to Visit 1 and must agree to maintain the same dose for the duration of the study and for 90 days after the last study medication application.
11. Subjects taking thyroid replacement therapy must have been on the same dose for at least 6 months prior to Visit 1 and must agree to maintain the same dose for the duration of the study.
12. Subject agrees to refrain from any eyebrow removal (*e.g.*, plucking, threading, etc.) for the duration of the study.
13. Subject agrees to refrain from any cosmetic surgery (*e.g.*, piercing, tattooing, etc.), on the treatment areas, for the duration of the study.
14. Subject can comprehend and is willing to sign an Informed Consent Form (ICF).
15. Sexually active male subjects must agree to use a barrier method of contraception from the first application of study medication to at least 30 days after the last application of study medication.

5.4. Exclusion Criteria

Any subject who meets one or more of the following criteria will not be included in this study:

1. Subject has, in the opinion of the investigator, permanent eyebrow loss attributed to causes other than AA, AU or AT such as overgrooming, or scarring hair loss.
2. Subject currently has, or has a history of, skin disease in the eyebrow area (*e.g.*, psoriasis, seborrheic dermatitis, etc.) that, in the opinion of the investigator, would interfere with the study medication application or study assessments.
3. Subject has, or has a history of, severe, progressive or uncontrolled autoimmune, metabolic, endocrinologic, renal, hepatic, gastrointestinal, pulmonary, cardiovascular, genitourinary (*i.e.*, renal disease), hematological disease, neurologic or cerebral disorders, infectious disease or coagulation disorders that, in the opinion of the investigator, would put the subject at undue risk by study participation.
4. Subject currently has, or has a history of, proven or suspected systemic or cutaneous malignancy and /or lymphoproliferative disease, other than a history of adequately treated, well healed and completely cleared non-melanoma skin cancers (basal or squamous cell carcinoma) treated successfully at least one year prior to Visit 1.
5. Subject currently has evidence of active or latent bacterial infection, including tuberculosis, or viral infections at the time of enrollment or a history of incompletely treated or untreated tuberculosis. Subjects who have completed therapy for latent tuberculosis may participate.
6. Subject has a history of serious local infection (*e.g.*, cellulitis, abscess) or a systemic infection including but not limited to, pneumonia or septicemia, within 12 weeks prior to Visit 1. Subjects on an antibiotic for a nonserious, acute local infection must complete the antibiotic course prior to enrollment in the study.
7. Subjects positive for HIV, Hepatitis B or C. Subjects with serologic evidence of Hepatitis B vaccination (HepB surface Ab without the presence of HepB surface Ag) will be allowed to participate.
8. Subject currently has or has a known history of herpes zoster or cytomegalovirus (CMV) within 8 weeks prior to Visit 1.
9. Subject has a history of frequent outbreaks of Herpes Simplex Virus, defined as 4 or more episodes per year.
10. Subject has any history of eyebrow tattooing, microblading that in the opinion of the investigator would interfere with the assessment of safety or efficacy.
11. Subject has used any semi-permanent eyebrow coloring (*e.g.*, tinting, dying, etc.) within 6 months prior to Visit 1 that in the opinion of the investigator would interfere with the assessment of safety or efficacy.
12. Clinically significant laboratory abnormalities at Screening that, in the opinion of the Investigator, would make the subject a poor candidate for the study.
13. Subjects with absolute neutrophil count <1,000/mm^3, or platelet count <50,000/ml.
14. Subject used any of the following therapies within the specified period prior to Visit 1:

    <u>Systemic therapies:</u>

- Disease Modifying Anti-Rheumatic Drugs (DMARDS), Biologics or immunosuppressants, such as: anakinra, adalimumab, azathioprine, glucocorticosteroids, cyclosporine, etanercept, infliximab, methotrexate, TNF inhibitors, ustekinumab, secukinumab, ixekizumab, certolizumab pegol: 4 weeks or 5 half-lives whichever is longer
- Oral retinoids: 12 weeks
- Plaquenil: 8 weeks
- JAK inhibitors (oral or topical): 1 year
- Intralesional steroids on the eyebrow area: 4 weeks

Topical therapies on the eyebrow area:

- Phototherapy, Laser Therapy: 12 weeks
- Anthralin, bimatoprost, glucocorticosteroids, diphencyprone, diphenylcycloprophenone (DPCP), Squaric acid dibutyl ester (SADBE), minoxidil, pimecrolimus, tacrolimus: 4 weeks
- Topical treatments (prescription and over-the-counter) that contain retinoids, retinol, alpha hydroxy acids (e.g. glycolic, lactic acids) and beta hydroxy acids (e.g. salicylic acid) on and around the eyebrow area: 4 weeks

15. Subject has history of sensitivity to any of the ingredients in the study medication.
16. Subject has participated 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) prior to 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. History of or current alcohol or drug abuse within 2 years of assessment for study enrollment.
18. 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

5.5. Concomitant Therapies

During Visit 1 (Screening), the investigator or designee will question the subject to ensure they have not used any excluded therapies. Concomitant therapies are any new or existing therapies used from Screening (Visit 1) until Day 197 (Visit 11). Concomitant therapies include drug (e.g.

prescription and over the counter [OTC]), and non-drug modalities (e.g., chiropractic, physical therapy).

5.5.1. Permitted Concomitant Therapies

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 products such as make up and moisturizers used consistently prior to study enrollment on the face and eyebrow area should be reviewed by the Investigator and are permitted if, in the Investigator's opinion, they will not affect the assessments of the subject during the study.

Topical therapies such as topical corticosteroids are permitted if they are not applied on or near the eyebrow area. 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 Day 197 (Visit 11) will be documented in the subject's source document and eCRF.

5.5.2. Prohibited therapies

Any medication or over-the-counter product known to affect hair growth is prohibited throughout the study period. Any prescription topical retinoids, over-the-counter topical products containing retinol, alpha hydroxy acids (e.g. glycolic, lactic acids) and beta hydroxy acids (e.g. salicylic acid) or cosmetic procedures applied to or in close proximity around the eyebrow area that in the opinion of the investigator may affect the safety or efficacy of the subject are prohibited throughout the study. New eyebrow tattooing, microblading, or piercing is not allowed during the study. During this study, subjects are prohibited from using therapies listed in the exclusion criteria. The investigator should notify the Medical Monitor, if any prohibited therapies are required to ensure subject safety.

5.6. Subject Discontinuation from the Study

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. Notification of discontinuation will immediately (within 24 hours) be made to the Aclaris Therapeutics, Inc. study monitor.

In case of premature discontinuation of study participation, efforts will be made to perform all final study day assessments. The date the subject is withdrawn from the study and the reason for discontinuation will be recorded in the subject's source documents and on his/her case report forms (eCRFs). All withdrawn subjects with ongoing AEs will be followed as specified in section 8.2.1 of this protocol.

The study may be discontinued at the discretion of Aclaris Therapeutics, Inc. Some examples of reasons for discontinuation are the occurrence of the following:

- Increased frequency, severity or duration of known AEs
- Medical, business, regulatory or ethical reasons affecting the continued performance of the study
- Difficulties in the recruitment of subjects

5.7. Subject Identifier (SI)

The investigator or designee will assign a unique five-digit subject identifier (SI) to each subject at Visit 1.

The SI format will be NN-NNN where the first 2 digits are the investigational center site number (using leading zeroes as appropriate). The final 3 digits are the subject number and must be assigned in ascending numerical order, without omitting or repeating any number, starting with 001. For example, the SI for the second subject that signs an informed consent at site number 01 would be 01-002.

The subject will be identified using the SI in all study documentation for the duration of the study.

5.8. Replacement Subjects

Subject enrollment will continue until approximately 12 subjects meet all the entry criteria and apply study medication at Visit 2 (Baseline). Subjects who are enrolled and do not complete the study will not be replaced.

6. INVESTIGATIONAL PLAN

6.1. Study Design

This is an open-label proof of concept study in subjects with unilateral or bilateral loss of some or all eyebrow hair due to AA, AU or AT. Subjects will treat affected eyebrow(s) with ATI-50002 Topical Solution 0.46% twice-daily for 24 weeks and will have a 4-week post-treatment follow up visit.

6.2. Study Flow Chart

Visit Number	1 Screening	2 Baseline	3	4	5	6	7	8	9	10	11	Protocol Section
Week	-4 to -1	0	1	2	4	8	12	16	20	24	28	
Day	-28 to 0	1	8	15	29	57	85	112	141	169	197	
Informed consent	X											12.3
Subject identifier	X											5.7
Inclusion/exclusion criteria	X	X										5.3/5.4
Demographics & Medical History (including Alopecia History)	X											7.3.1
Brief physical examination	X										X	7.2.2
Vital signs (Height and Weight at Visit 1 only)	X	X	X	X	X	X	X	X	X	X	X	7.2.3
ECG	X	X								X	X	7.2.1
Clinical laboratory sampling	X	X					X			X	X	7.2.4
Serum Pregnancy Test for WOCBP	X											7.2.4
Urine pregnancy test		X			X	X	X	X	X	X	X	7.2.6
Clinician Eyebrow Assessment	X	X			X	X	X	X	X	X	X	7.1.1
Subject Eyebrow Assessment	X	X			X	X	X	X	X	X	X	7.1.2
Local Tolerability Assessment (Investigator and Subject)		X Pre & Post Dose	X	X	X	X	X	X	X	X	X	7.2.5
Subject Eyebrow Satisfaction		X				X	X	X	X	X	X	7.1.3
Standardized photography		X			X	X	X	X	X	X	X	7.3.2
Confirm eligibility		X										6.6.4
Dispense study medication		X	X	X	X	X	X	X	X			6.6.5
Collect Study Medication			X	X	X	X	X	X	X	X		6.6.5
Observe first study medication application		X										6.6.6
Study medication applications		◆	◆	◆	◆	◆	◆	◆	◆	◆		6.6.6

Visit Number	1 Screening	2 Baseline	3	4	5	6	7	8	9	10	11	Protocol Section
Week	-4 to -1	0	1	2	4	8	12	16	20	24	28	
Day	-28 to 0	1	8	15	29	57	85	112	141	169	197	
No-treatment follow-up											⟷	6.6.6
Subject instructions	X	X	X	X	X	X	X	X	X	X		6.4
Concomitant therapies	X	X	X	X	X	X	X	X	X	X	X	5.5.1
Adverse events		X	X	X	X	X	X	X	X	X	X	8

6.3. Study Visits Description and Procedures

A written, signed informed consent form (ICF) must be obtained from each subject prior to performing any study related procedure (*i.e.*, physical examination, clinical laboratory sampling, urine pregnancy test, ECG, or photography).

6.3.1. Visit 1/ Screening (Week -4 to 0)

At this visit, the investigator or designee will:

1. Review and explain the nature of the study to the subject, obtain the subject's signature on the appropriate approved ICF and provide a signed and dated copy to the subject.
2. Assign a SI to the subject.
3. Confirm the subject meets all inclusion criteria and no exclusion criteria.
4. Collect demographic and medical history information (including alopecia history).
5. Collect concomitant therapies information.
6. Perform a brief physical examination.
7. Measure vital signs (including Height & Weight at this visit only).
8. Conduct an ECG.
9. Send patient for clinical laboratory tests including serum pregnancy test (if subject is WOCBP).
10. Identify the affected areas of eyebrow(s). Affected area is defined as the area of the eyebrow hair loss identified at baseline.
11. Perform a Clinician Eyebrow Assessment (CEA); a grade of 0 is required for at least one eyebrow with at least 30% eyebrow loss for the subject to continue in the study. Note: the subject may have multiple patches of eyebrow hair loss, but at least one distinct patch must demonstrate a minimum hair loss of at least 30% (independent of any other patches).
12. Have the subject complete a Subject Eyebrow Assessment (SEA); a grade of 0 is required for at least one eyebrow with at least 30% eyebrow loss for the subject to continue in the study. Note: the subject may have multiple patches of eyebrow hair loss, but at least one distinct patch must demonstrate a minimum hair loss of at least 30% (independent of any other patches).
13. Review the study instructions with the subject.
14. Schedule Visit 2 as appropriate.

6.3.2. Visit 2/ Baseline (Day 1)

This visit must occur within 28 days after Visit 1.

Subsequent study visit dates must be scheduled based on the date of Visit 2.

This visit may not occur before the investigator reviews the Visit 1 clinical laboratory test results for all the measured analytes. For the subject to continue in the study all clinical laboratory test results must be within the range of normal for the laboratory or, if there are any abnormal results, they must be defined as not clinically significant (NCS) by the investigator. For WOCBP, the serum pregnancy test from Visit 1 must be negative for a subject to continue in the study.

The investigator must review the evaluator's interpretation of each subject's Visit 1 ECG prior to Baseline/Visit 2 and comment on the clinical significance of any result that is defined by the evaluator as abnormal. The subject must not continue in the study and apply study medication, if the Visit 1 ECG meets any of the Criteria in Exclusion Criterion 18).

At this visit, the investigator or designee will perform the following procedures PRIOR TO APPLICATION OF STUDY MEDICATION:

1. Subject should have clinical laboratory tests performed at the local laboratory on the same day as the visit.
2. Query the subject in a non-directive manner about any changes in her/his health since the previous visit and report changes on the appropriate form.
3. Query the subject about any changes in her/his concomitant therapies since the previous visit and report changes on the appropriate form.
4. Confirm the subject meets all inclusion criteria and no exclusion criteria.
5. Confirm the subject continues to comply with all study restrictions, is eligible to continue in the study and has followed all study instructions.
6. Measure vital signs.
7. Perform a urine pregnancy test for WOCBP; results must be negative for the subject to apply the first dose of study medication.
8. The investigator or designee will perform a CEA; a CEA grade of 0 in a distinct patch of at least 30% total unilateral eyebrow hair loss is required for the subject to apply the first dose of study medication. Note: the subject may have multiple patches of eyebrow hair loss, but at least one distinct patch must demonstrate a minimum hair loss of at least 30% (independent of any other patches).
9. Confirm subject is eligible to apply the first dose of study medication.
10. Discharge from the study subjects who are not eligible to apply the first dose of study medication.

For subjects who are eligible to apply the first dose of study medication the investigator or designee will perform the following procedures:

1. Have the subject complete a SEA; a SEA grade of 0 in a distinct patch with at least 30% total unilateral eyebrow hair loss is required for the subject to apply the first dose of study medication. Note: Subject may have multiple patches of eyebrow hair loss, but at least one distinct patch must demonstrate a minimum hair loss of at least 30% (independent of any other patches).
2. Have the subject complete the SES question.
3. Have the subject perform a pre-application Local Tolerability Assessment (LTA).
4. The Investigator or designee will perform a pre-application LTA.
5. Take standardized color photographs.
6. Conduct an ECG.
7. Dispense study medication to the subject.
8. An investigational staff member will instruct the subject on the proper study medication application technique and will observe the subject's first study medication application.
9. A staff member will monitor the subject for at least 20 minutes after the first study medication application completion time to detect any adverse events.
10. Have the subject perform a post-application LTA 10 (±4) minutes after the application completion time.
11. The investigator or designee will perform a post-application LTA 20 (±4) minutes after the application completion time.
12. Review the study instructions with the subject.
13. Schedule Visit 3 as appropriate.

6.3.3. Visits 3-9 (Weeks 1, 2, 4, 8, 12, 16 and 20)

These visits must occur on the following weeks after Visit 2:

- Visit 3, 1 week (±4 days)
- Visit 4, 2 weeks (±4 days)
- Visit 5, 4 weeks (±4 days)
- Visit 6, 8 weeks (±4 days)
- Visit 7, 12 weeks (±4 days)
- Visit 8, 16 weeks (±4 days)
- Visit 9, 20 weeks (±4 days)

At these visits, the investigator or designee will perform the following procedures:

1. Query the subject in a non-directive manner about any changes in her/his health since the previous visit and report all AEs on the appropriate form.
2. Query the subject about any changes in her/his concomitant therapies since the previous visit and report changes on the appropriate form.
3. Confirm the subject continues to comply with all study restrictions, is eligible to continue in the study and has followed all study instructions.
4. Measure vital signs.
5. **AT VISIT 7 ONLY,** send subject for clinical laboratory tests prior to the study visit (on the same day).
6. Have the subject perform a LTA.
7. The investigator or designee will perform a LTA.

8. **AT VISITS 5-9 ONLY**, the investigator or designee will perform the CEA.
9. **AT VISITS 5-9 ONLY**, have the subject complete the SEA.
10. **AT VISITS 5-9**, perform a urine pregnancy test for WOCBP; results must be negative for the subject to continue in the study.
11. **AT VISIT 7 ONLY**, have the subject complete the SES.
12. **AT VISITS 5-9 ONLY**, take standardized photographs.
13. Dispense and/or collect study medication as appropriate.
14. Review the study instructions with the subject.
15. Schedule the next visit as appropriate.

6.3.4. Visit 10 (Week 24)

This visit must occur 24 weeks (±4 days) after Visit 2.

At this visit, the investigator or designee will perform the following procedures:

1. Query the subject in a non-directive manner about any changes in her/his health since the previous visit and report all AEs on the appropriate form.
2. Query the subject about any changes in her/his concomitant therapies since the previous visit and report changes on the appropriate form.
3. Confirm the subject continues to comply with all study restrictions and has followed all study instructions.
4. Measure vital signs.
5. Conduct an ECG.
6. Confirm subject had clinical laboratory tests prior to the visit (on the same day).
7. Perform a urine pregnancy test for WOCBP.
8. Have the subject perform a LTA.
9. The investigator or designee will perform a LTA.
10. The investigator or designee will perform the CEA.
11. Have the subject complete the SEA.
12. Have the subject complete the SES.
13. Collect all study medication.
14. Take standardized photographs.
15. Review the study instructions with the subject.
16. Schedule the next visit as appropriate.

6.3.5. Visit 11 (Week 28)

This visit must occur 28 weeks (±4 days) after Visit 2.

At this visit, the investigator or designee will perform the following procedures:

1. Query the subject in a non-directive manner about any changes in her/his health since the previous visit and report all AEs on the appropriate form.
2. Query the subject about any changes in her/his concomitant therapies since the previous visit and report changes on the appropriate form.
3. Confirm the subject continues to comply with all study restrictions and has followed all study instructions.

4. Conduct a brief physical examination.
5. Measure vital signs.
6. Conduct an ECG.
7. Confirm subject had clinical laboratory tests prior to visit (same day).
8. Perform a urine pregnancy test for WOCBP.
9. Have the subject perform a LTA.
10. The investigator or designee will perform a LTA.
11. The investigator or designee will perform the CEA.
12. Have the subject complete the SEA.
13. Have the subject complete the SES.
14. Take standardized photographs.
15. Discharge the subject from the study.

6.4. Subject Instructions

An investigational center staff member will dispense and review the Subject Instruction Sheet to each subject at Visit 2 through Visit 10 (see Section 14, Appendix A).

6.5. STUDY DURATION

The duration of study participation is anticipated to be a maximum of 233 days per subject. This includes the up to 28-day screening period, a 169-day treatment period and a 28 day no-treatment follow-up period. The final study visit (Visit 11) has a maximum allowable visit window of +4 days.

The study end date is the date of the last subject's last visit.

6.6. STUDY MEDICATIONS

6.6.1. Study medication identity

The study medication for this study is ATI-50002 Topical Solution 0.46%. The study medication is a clear, colorless to light pink solution. The inactive ingredients include: water, Transcutol P, propylene glycol, PEG400, dimethyl sulfoxide (DMSO), Kolliphor CS 20, benzyl alcohol, poloxamer 188, and povidone K30. The study medications must be stored in a secure area with limited access under appropriately controlled and monitored storage conditions.

Study Medication Information	
Study medication name	ATI-50002 Topical Solution 0.46%
Manufacturer	PMRS, Inc., Horsham, PA, USA
ATI-50002 concentration (%)	0.46
Pharmaceutical Form	Topical Solution
Container	Amber Glass Bottles, 30 ml with screw cap
Storage Conditions	59°F to 77°F (15°C to 25°C) protected from light, excessive heat, open flame and combustibles, out of direct sunlight and in a well-ventilated, dry area
Dose regimen	
Route	Topical
Frequency	Twice-daily application
Duration of administration	24 weeks
Other Supplies	Disposable applicators

6.6.2. Study medication packaging and labeling

The study medication will be packaged in 30 ml amber glass bottles with tamper-resistant caps.

Study medication packaged in individual cartons, each with a unique number, will be provided to the investigational center for each subject. An adequate supply of study medication applicators will also be supplied. One bottle of study medication is sufficient for approximately 2 weeks of twice-daily applications.

Each study medication bottle will be labeled with a one-part label that remains attached to the bottle and shows at least the following:

- Protocol number
- ATI-50002 Topical Solution 0.46%
- Storage conditions
- Sponsor information
- Investigational drug warning
- Space to enter Subject Identifier

6.6.3. Method of treatment assignment

This study is an open-label study and all subjects will receive ATI-50002 Topical Solution 0.46%.

6.6.4. Subject Eligibility to Receive Study Medication

At Visit 2 the investigator or designee will determine if the subject is eligible to apply study medication. Eligibility requirements include, but are not limited to:

- The subject must not apply the first dose of study medication unless all the required clinical laboratory test results are within the range of normal for the laboratory, or, if there are any abnormal results, they must be defined as not clinically significant (NCS) by the investigator.
- For WOCBP, the serum pregnancy test from Visit 1 must be negative for the subject to continue in the study. Additionally, a urine pregnancy test at Visit 2 must be performed and found to be negative prior to application of the first dose of study medication.
- The subject must not apply study medication if the Visit 1 ECG meets any of the Criteria in Exclusion Criterion 18).

6.6.5. Dispensing and collecting study medication

The study medications must be dispensed only to study subjects, only at the investigational center noted on the FDA Form-1572 and only by authorized personnel as required by applicable regulations, guidelines and protocol.

At Visit 2, after a subject is confirmed to be eligible to apply study medication, research center staff will assign an initial supply of three study medication bottles. Research center staff will record the SI on each bottle label.

At Visits 3-9, examine the subject's dispensed study medication bottles, collect bottles that are empty or do not contain sufficient volume of study medication available for use with the applicator, and dispense additional bottles as appropriate following the instructions above.

At Visit 10, collect all dispensed study medication bottles.

Dispense a packet of study medication applicators to the subject each time a study medication bottle is dispensed. It is not necessary to collect unused applicators from the subject.

The investigational center staff should make every effort to obtain all dispensed study medication from each subject. Two documented telephone contacts with the subject followed by a registered letter to the subject constitute adequate follow-up efforts. If these efforts fail, the reason for the failure must be noted on the appropriate study medication inventory eCRF and in the subject's source documentation.

6.6.6. Study medication application

The study medications are for external, topical use on the eyebrows on the appropriate study subject only.

At Visit 2, the staff member will instruct the subject on the proper application technique as detailed in Section 14 and observe the subject's first study medication application. The staff member should record the time that the subject completes the first study medication application. Monitor the subject for adverse events for at least 20 minutes after the application completion time.

6.6.7. Dose compliance record

The subject will be given an application calendar to record study medication applications. At every post-baseline study visit (Visit 3 and higher), an investigational center staff member will document the study medication compliance in the eCRF.

6.6.8. Dose modification

Subjects should not modify the study medication application procedure or frequency without the investigator's prior approval. All application modifications must be reported on the appropriate eCRF. If any meaningful study medication intolerance or safety issue occurs, after consulting with the Aclaris Therapeutics, Inc. Medical Monitor (see page 1), the investigator or designee may direct the subject to modify the study medication application frequency. If the subject cannot perform the twice-daily application frequency to the affected eyebrow(s) for more than 4 consecutive days, the subject must be removed from the study.

6.7. Study Medication Management

6.7.1. Accountability

The investigator or designee will maintain an accurate record of the receipt of the study medication as shipped by Aclaris Therapeutics, Inc. (or designee), including the date received and the condition of the study medications. One copy of this receipt will be returned to Aclaris Therapeutics, Inc. (or designee) when the contents of the study medication shipment have been verified and one copy maintained in the study file. In addition, an accurate study medication disposition record will be kept, specifying the amount dispensed for each subject and the date of dispensing. This inventory record will be available for inspection at any time. At the completion of the study, the original inventory record will be available for review by Aclaris Therapeutics, Inc. upon request.

6.7.2. Return and disposition of study supplies

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's) written instructions.

6.8. Other Study Supplies

Aclaris Therapeutics, Inc. will provide:

- Supplies for urine pregnancy tests
- Equipment, supplies and training for taking standardized photographs
- Equipment, supplies and training for capturing ECGs for central reading
- Disposable applicators

6.9. Blinding

6.9.1. Verification of blinding

This study uses an open-label design.

6.9.2. Unblinding the study medication

This is an open-label study.

7. STUDY ASSESSMENTS

The investigator, a designated and appropriately trained staff member (*e.g.*, subinvestigator) or the subject will perform the study assessments according to the schedules noted below.

The same individual 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 the study should perform the assessments.

Similar lighting conditions and subject positioning should be used for all evaluations for a given subject.

7.1. Effectiveness Evaluations

The investigator will identify the affected area(s) at baseline in the source document and eCRF. The affected area is defined as the area of eyebrow hair loss identified at baseline.

7.1.1. Clinician's Eyebrow Assessment (CEA)

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

At Visits 1, 2 and 5-11, the investigator or designee will assess the affected area of the 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 should be evaluated 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)

At Visit 1 for the subject to be enrolled and at Visit 2 for the subject to continue in the study and to apply study medication to one or both eyebrow(s), he or she must have a CEA grade of 0 in a distinct patch of at least 30% total hair loss in one or both eyebrows. Note: the subject may have multiple patches of eyebrow hair loss, but at least one distinct patch must demonstrate a minimum hair loss of at least 30% (independent of any other patches).

The investigator will record if vellus hair is present (Y/N) in the affected area at Visits 1, 2 and 5-11.

7.1.2. Subject's Eyebrow Assessment (SEA)

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

At Visits 1, 2 and 5-11, the subject will assess the affected area of the eyebrow(s) using the scale below and report the one integer that best describes the amount of eyebrow hair present. If applicable, each eyebrow should be evaluated 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 to the subject, educate the subject on the SEA scale before each evaluation and direct the subject to assess the affected area of the eyebrow. The staff member should not influence the subject's assessment.

At Visit 1 for the subject to be enrolled and at Visit 2 for the subject to continue in the study and to apply study medication to one or both eyebrows, he or she must have a SEA grade of 0 in at least one eyebrow in a distinct patch of at least 30% total hair loss in one or both eyebrows. Note: the subject may have multiple patches of eyebrow hair loss, but at least one distinct patch must demonstrate a minimum hair loss of at least 30% (independent of any other patches).

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

7.1.3. Subject Eyebrow Satisfaction (SES)

The SES is the subject's assessment of her/his satisfaction with the status of the affected eyebrow(s), relative to the amount of hair present, at a particular point in time. The subject should NOT refer to any other assessments to assist with these assessments.

At Visits 2, 7, 10 and 11, the subject will assess the affected eyebrow(s) using the scale below and report the one integer that best describes her/his satisfaction with the status of the affected eyebrow(s). If both eyebrows are affected, each eyebrow should be evaluated separately:

Subject's Eyebrow Satisfaction

Grade	Descriptor
1	Extremely satisfied
2	Moderately satisfied
3	A little satisfied
4	Neither satisfied or dissatisfied
5	A little dissatisfied
6	Moderately dissatisfied
7	Extremely dissatisfied

An investigational staff member will identify the affected eyebrow to be evaluated to the subject, educate the subject on the SES scale before each evaluation and direct the subject to assess the eyebrow. The staff member should not influence the subject's assessment.

The study staff member will report the SES grade the subject indicates for each eyebrow in the source document. Both the subject and the study staff member must sign/initial the source document to indicate the subject performed the SES as instructed.

7.2. Safety Evaluations

The safety in this study will be monitored on a frequent and regular basis and will be formally reviewed on a monthly basis by the study investigators, who are experienced in the treatment of alopecia, the Sponsor's medical monitor, and the clinical director. In addition to reporting adverse events throughout the study, the investigator, a designated and appropriately trained staff member or the subject, will perform study specific safety assessments according to the schedules noted below.

An independent safety monitor will review the safety data monthly or more frequently, if needed.

7.2.1. ECGs

At Visits 1, 2, 10 and 11 a standard 12-lead ECG will be performed by a qualified staff member. 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 will provide equipment, supplies and site training. In addition, the central lab will process the 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 Visit 1 ECG prior to Visit 2. The investigator will review the evaluator's interpretation of all ECG reports in a timely manner and comment on the clinical significance of any result that is defined by the evaluator as abnormal.

Any abnormalities that are, in the opinion of the investigator, CS 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.

7.2.2. Brief physical examination

At Visits 1 and 11 the investigator or designee will perform a brief physical examination. The examination will include an assessment of the following:

- General appearance
- Vital signs (Section 7.2.3)
- Head, eye, ear, nose and throat (HEENT)
- Respiratory
- Cardiovascular
- Abdominal
- Extremities
- Musculoskeletal
- Lymphatic
- Skin
- Neurological

Any measure that is, in the opinion of the investigator, abnormal AND 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 application begins.

7.2.3. Vital signs

At Visits 1-11 a qualified staff member will measure vital signs. 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 (at Visit 1 only)
- Weight (at Visit 1 only)

Any measure that is, in the opinion of the investigator, abnormal AND 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 application begins.

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

7.2.4. Clinical laboratory sampling

Subjects will go to a local laboratory (to be determined) to have the clinical laboratory tests at Visits 1, 2, 7, 10 and 11. WOCBP will have urine pregnancy tests performed by the study staff at Visit 2, 5, 6, 7, 8, 9, 10 and 11. Approximately 22.5ml of blood will be collected at Visit 1 and 10.5ml for each sample at Visits 2, 7, 10 and 11, a total of approximately 64.5ml per subject for the entire study. The following tests, at a minimum, will be conducted:

Chemistry Panel
Albumin
Alkaline phosphatase
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Blood urea nitrogen (BUN)
Bicarbonate
Calcium
Chloride
Creatinine
Glucose
Lactate dehydrogenase (LDH)
Phosphorus
Potassium
Sodium
Total bilirubin
Total protein
Uric acid
Total cholesterol, LDL, HDL
Triglycerides

Screening Tests Only
Virology (HepB, HCV, HIV)
Total Iron Binding Capacity (TIBC)
Serum Iron

Complete Blood Count
Hematocrit
Hemoglobin
Platelet count
Red blood cell morphology
Red blood cell count
White blood cell count
White blood cell differential
% & absolute
Basophils
Eosinophils
Lymphocytes
Monocytes
Neutrophils

Complete Urinalysis

Serum Ferritin
T3/T4, TSH
Quantiferon
Serum Pregnancy (Visit 1 only)

The results of the clinical laboratory tests will be reported on the laboratory's standard reports. The investigator must review all laboratory reports in a timely manner and note NCS or CS to define the clinical significance of any result that is outside the normal range for the laboratory. The investigator must date and initial every laboratory report.

The investigator must review the Visit 1 laboratory results for all the required analytes for each subject prior to Visit 2. The subject must not continue in the study and apply study medication if at Visit 2 any of the Visit 1 results are outside normal range for the laboratory AND, in the opinion of the investigator, CS.

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 application or as an AE if found after the first study medication application begins.

7.2.5. Local Tolerability Assessment (LTA)

The LTA is the investigator's assessment of the average overall severity of signs and the subject's assessment of the average overall severity of symptoms associated with irritation on each eyebrow considered separately. The investigator and subject must NOT refer to any other evaluation to assist with these assessments. This is not a comparison with the assessment at any other time point.

At Visits 2-11, the investigator and the subject will evaluate the LTA signs and the LTA symptoms respectively.

LTA Signs (assessed by the investigator for each eyebrow separately):
- Erythema
- Edema
- Scaling/dryness

LTA symptoms (assessed by the subject for each eyebrow separately):
- Stinging
- Burning

The investigator will assess the LTA signs as follows:
- At Visit 2:
  - Prior to the first study medication application
  - 20 (±4) minutes after the application completion time.
- At Visits 3-11

The subject will assess the LTA symptoms as follows:

- At Visit 2:
  - Prior to the first study medication application report the LTA for each symptom over the previous 24 hours
  - 10 (±4) minutes after the application completion time report the average severity since the application completion time
- At Visits 3-11:
  - Report the average LTA for each symptom over the previous 24 hours

The investigator, for the signs, should report the one integer that best describes the average overall severity on each eyebrow separately using the scales below:

Local Tolerability Assessment – Erythema

Grade	Descriptor
0	Clear: No erythema present
1	Mild: Slight erythema
2	Moderate: Definite erythema
3	Severe: Marked, fiery erythema

Local Tolerability Assessment – Edema

Grade	Descriptor
0	Clear: No edema present
1	Mild: Slight edema
2	Moderate: Definite edema
3	Severe: Marked edema

Local Tolerability Assessment – Dryness/Scaling

Grade	Descriptor
0	Clear: No signs of dryness or scaling
1	Mild: Slight roughness (may be more easily felt than seen), barely perceptible scaling
2	Moderate: Moderate roughness, definite scaling
3	Severe: Marked roughness, heavy scaling

The subject, for the symptoms, should report the one integer that best describes the average overall severity on each eyebrow separately using the scales below:

Local Tolerability Assessment – Stinging

Grade	Descriptor
0	Clear: No stinging
1	Mild: Slight tingling, not bothersome
2	Moderate: Definite tingling, slightly bothersome
3	Severe: Intense stinging, bothersome and/or uncomfortable

Local Tolerability Assessment – Burning

Grade	Descriptor
0	Clear: No burning
1	Mild: Slight warmth, not bothersome
2	Moderate: Definite warmth, slightly bothersome
3	Severe: Intense feeling of heat, bothersome and/or uncomfortable

Follow these steps to complete the LTA for each symptom by a subject:

- An investigational center staff member, other than the evaluating investigator, will show the appropriate LTA symptom grading scale to the subject and instruct the subject on the time interval to be considered.
- The staff member should not give any opinion on the meaning of the LTA descriptors.
- The subject should verbally indicate the appropriate grade for each eyebrow for each symptom and the staff member will report the grade in the source document.
- Both the subject and the staff member must sign/initial the source document to indicate the subject performed the LTA for symptoms as instructed.
- The staff member must not influence the subject's assessment.

7.2.6. Urine Pregnancy tests

At Visits 2, and 5-11, a qualified staff member will perform a urine pregnancy test for subjects who are WOCBP. The urine pregnancy test kits used must have a minimum sensitivity of 25-mIU ß-HCG/milliliter (ml) of urine.

Subjects who are WOCBP must have a negative serum pregnancy test result at Visit 1 to continue in the study and a negative urine pregnancy test at Visit 2 to be continue in the study and apply study medication.

If the result of any urine pregnancy test is positive following initial study medication application, the subject will be withdrawn from the study and the subject's pregnancy documented and followed.

7.3. Other Evaluations

7.3.1. Demographics, medical history and alopecia areata history

During Visit 1, the investigator or designee will interview each subject to obtain demographic information including date of birth, sex at birth, Fitzpatrick skin type, race and ethnicity.

The investigator or designee will interview each subject to obtain medical history information related to all medical conditions and disease states that, at Visit 1: are ongoing, require concomitant therapy or are, in the opinion of the investigator, relevant to the subject's study participation. The investigator or designee will also obtain an AA, AU or AT history at Visit 1. 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.

7.3.2. Standardized photography

At Visits 2 and 5-11 a qualified investigational center staff member will take standardized color photographs of each subject's eyebrows.

The photographs are to document the status of each subject's eyebrows. The subject's identity will not be revealed in the study photographs.

At Visit 2, the photographs must be taken prior to the first study medication application.

Equipment, supplies, training and detailed instructions for obtaining and managing the photographs will be provided to the investigational center prior to the initiation of subject enrollment.

8. ADVERSE EVENTS

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

8.1. Definitions

8.1.1. Adverse events (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a study medication 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, or clinically significant worsening of an existing sign, symptom or disease, should be considered an AE.

Worsening of any of the eyebrow assessments should be reported as an AE ONLY if the use of the study medication is interrupted or discontinued or if therapy is required to manage the event.

The investigator must, for any AE in a study medication treatment area, question the subject in detail to determine if there are any confounding factors (*e.g.*, irritation by cosmetics) for any such AE.

Every new episode or clinically significant worsening of a chronic condition (*e.g.*, headaches, seasonal allergies, depression, and 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 application should be reported as medical history, not as an AE.

8.1.2. 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 was 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.

8.1.3. Adverse event reporting period

The investigator must start reporting non-serious AEs and SAEs starting with the subject's first study medication application continuing until the end of the subject's last study visit.

8.1.4. Severity

The investigator must 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.

8.1.5. 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 possibility that there is a causal relationship between the study medication and the AE.

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

The term "reasonable causal relationship" means there are facts or arguments to suggest a causal relationship (International Conference on Harmonization (ICH) E2A).

8.2. Reporting Procedures

8.2.1. Procedures for reporting adverse events

At each post randomization visit (Visit 2), the investigator or designee 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?"

At Visit 2, the investigator or designee will monitor the subject for at least 20 minutes after the first study medication application at Visit 2 to elicit AEs in a similar manner.

If appropriate, based on the subject's response to non-directed questioning regarding AEs, the investigator or designee 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.

8.2.2. 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 medication, the investigator must:

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

8.2.3. 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

8.2.4. Study Medication Interruption and Discontinuation

8.2.4.1 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 1.

Table 1: Study Medication Interruption Criteria

Laboratory Test	Interrupt 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 1, 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 1.

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. Application of study medication may be resumed upon resolution of the abnormal laboratory values to the levels specified in Table 1 above.

8.2.4.2 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.

9. PREGNANCY

9.1. 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 and monthly throughout the study.

9.2. 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 as described in Section 8.2.2.

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.

If the outcome of the pregnancy meets the criteria for an SAE (i.e., ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly [in a live born, a terminated fetus, an intrauterine fetal demise, or a neonatal death]), the investigator should follow the procedures for reporting SAEs.

10. STATISTICAL ANALYSES

10.1. Statistical Methods

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

Mean change from baseline in eyebrow growth will be calculated as the mean change in the Clinician's Eyebrow Assessment (CEA) from Visit 2 (Baseline) compared to end of treatment (Visit 10). 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 Visit 2 (Baseline) to end of treatment (Visit 10).

Mean change from baseline in the Subject's Eyebrow Satisfaction (SES) will be calculated from Visit 2 (Baseline) to end of treatment (Visit 10).

All efficacy summaries will be based on the per-protocol population including all subjects who have Baseline (Visit 2) and Visit 10 efficacy data and no major protocol violations.

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 Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class. Vital signs and clinically significant abnormal laboratory results will also be

tabulated and presented. Change from baseline in the Local Tolerability Assessments (LTA) to end of treatment (Visit 10) will be summarized.

Data from all enrolled and treated subjects will be presented and summarized. Safety summaries 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.

10.2. Sample Size

This is the first US-based study with topical ATI-50002 Topical Solution for the treatment of eyebrow loss due to AA, AU/AT. No formal power calculations will be performed.

11. TRAINING, MONITORING, DATA MANAGEMENT AND QUALITY ASSURANCE

11.1. 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.

11.2. 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. representatives designee and/or and 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.

11.3. 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.

11.4. 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.

12. ETHICS AND GENERAL STUDY CONDUCT CONSIDERATIONS

12.1. 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.

12.2. Ethical Conduct of the Study

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

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

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

12.3. 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 sites, with HIPAA. The ICF will contain all the required elements in compliance with the current ICH E6 GCP guideline, 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.

12.4. 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 Aclaris Therapeutics, Inc. and prior review and documented approval from the IRB/EC.

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

A protocol violation is defined as any divergence from the protocol-specific study procedures or schedules that may 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.

12.5. 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.

12.6. 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.

12.7. Data Collection and Archiving

12.7.1. 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.

12.7.2. 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.

12.7.3. 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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14. APPENDIX A: PARTICIPANT INSTRUCTION SHEET

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 8 to 12 hours apart. Once you apply study medication, do not wash your face and eyebrow area or participate in strenuous exercise that would cause profuse sweating for at least 6 hours.
7. Remember to bring your study medication bottles, both used and unused, to each study visit.
8. Avoid exposing your face to excessive natural or artificial ultraviolet radiation (e.g., sunlight, tanning beds) and use sunscreen on the face including the eyebrows, if excessive sun exposure cannot be avoided.
9. Remove any products applied to the eyebrow area at least 1 hour before study visits. Do not apply study medication less than 6 hours before a study visit. If your visit is in the morning you should wait until after the visit to apply your study medication.
10. Each bottle of study medication should be used for 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 your clean, dry washcloth over one eye. Swipe the applicator across your affected eyebrow ridge above the covered eye, applying a thin layer of study medication over the entire affected eyebrow area. Your eyebrow area should be wet, but not dripping wet. Dispose of the applicator. **Do not dip the same applicator in the study medication bottle more than once.**
4. If you need additional study medication to cover your entire affected eyebrow, use a new applicator and repeat the application process as described in #2 and #3.
5. If you are instructed by the study doctor to treat both eyebrows, apply study medication to your other eyebrow following instructions in #2, #3, and #4.

After Study Medication Application

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

Missed Doses

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

Storage

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