UNIVERSITY OF MINNESOTA

Title:	An Open-Label Study to Evaluate DPCP Ointment for the Treatment of Alopecia Areata
Study Drug:	Diphenylcyclopropenone Ointment Provided by: Hapten Pharmaceuticals LLC 151 Merriweather Road Grosse Pointe Farms, MI 48236 313-300-8454 michaelburnsus@aol.com
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List of Abbreviations

AA	Alopecia Areata
AE	Adverse Event
BCC	Basal Cell Carcinoma
CFR	Code of Federal Regulations
CHS	Contact Hypersensitivity
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DNCB	Dinitrochlorobenzene
DPCP	Diphencyprone, Diphenylcyclopropenone
DTH	Delayed Type Hypersensitivity
FDA	Food and Drug Administration
HgB	Hemoglobin
HIPPA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
IDS	Investigational Drug Services Pharmacy
IRB	Institutional Review Board
MCRU	Masonic Clinical Research Unit
MSD	Meso Scale Discovery
PAA	Physician Assessment of Alopecia
PBMCs	Peripheral Blood Mononuclear Cells

PUVA	Psoralen Plus Ultraviolet A
SADBE	Squaric Acid Dibutyl Ester
SALT	Severity of Alopecia Tool
SCC	Squamous Cell Carcinoma
SHLA	Subject Hair Loss Assessment
SST	Serum Separator Tube
TSH	Thyroid Stimulating Hormone
UPIRTSO	Unanticipated Problems Involving Risk to Subjects or Others
USP-NF	The United States Pharmacopeia and The National Formulary
UVB	Ultraviolet B

Study Summary

Protocol Title	An Open Label Study to Evaluate DPCP Ointment for the Treatment of Alopecia Areata
Study Drug	Diphenylcyclopropenone (DPCP) Ointment
Indication	Alopecia Areata
Objectives	Primary Objective: Determine the efficacy of DPCP ointment for the treatment of alopecia areata. Secondary Objectives: • Assess the safety of DPCP ointment in alopecia areata subjects • Determine the best starting dose range for future studies Exploratory Aims: To evaluate the association between cytokine and keratin profiles, Cytomegalovirus UL16 Binding Protein (ULBP) expression and hair regrowth.
Study Design	This is an open labeled study to determine the response and characteristics, safety and efficacy, of the proprietary DPCP ointment composition as a topical immunotherapeutic agent for the treatment of extensive alopecia areata. Up to 10 subjects with 76% to 99% scalp hair loss that, in the opinion of the PI, are eligible for treatment with DPCP will be enrolled. Patients will be recruited from the new and current patient population seen for alopecia areata through the University of Minnesota Medical Center Dermatology Clinic as well as through partnership with the National Alopecia Areata Foundation Clinical Trials Network, and various media outlets. The products to be evaluated are as follows: • 0.05 mL of 0.4% DPCP (sensitization dose) applied topically to the inner aspect of the upper right arm. ("Sensitization and Baseline Sample Collection Visit") If sensitization is not attained after 10 to 14 days, the procedure will be repeated once. • 0.05 mL four dose concentrations of DPCP ointment (0.04, 0.08, 0.12, 0.2%) prepared through dilutions of 0.4% DPCP Ointment with the 0.04% DPCP Ointment (applied to the inner aspect of the left upper thigh) and these will be applied sequentially starting with the lowest concentration and, if no sensitization is attained, sensitization to the next lowest dose will be attempted. This will be repeated until either the participant has a reaction and that concentration will be their dose for the duration of the study or until all concentrations have been unsuccessful in causing a reaction. If this is the case, the participant will be discontinued

	from the study. ("Dose Determination Visit"; 10-14 days after the Sensitization Visit) The subject will return 72 hours after the application of each dose (0.04, 0.08, 0.12, 0.2%) for an assessment by the PI or co-PI. As described above, the lowest dose will be applied first and assessed. If no reaction, the next lowest dose will be applied, etc, until adequate sensitization is attained. The weakest strength concentration that caused a minimal reaction will be used throughout the study. Once adequate sensitization has been obtained, 0.75-1 g of the treatment drug in that dose will be applied in a thin film that covers the entire scalp. The treatment drug will be applied by the PI, a trained study coordinator, or a staff member of the Clinical Research Unit at the University of Minnesota. Eligible subjects may begin receiving the study drug immediately after enrollment and screening. The estimated duration of the study is 22-24 weeks. See Appendix A for the schedule of visits. There will be a total of 42 scheduled visits, beginning with a screening visit followed by a single sensitizing dose of study drug at baseline (Day -16) and an initial dose determination application of 0.04% DPCP at Day -2. Additional visits could include another sensitization attempt as well as dose determination visits for the .04, 08, 0.12, and 0.2% DPCP. Subjects will undergo twice weekly (+/- 2 days) topical applications of an ointment formulation of DPCP during Weeks 1-18. Application will be done twice per week. The drug application will be performed by a board certified dermatologist for the first two treatment visits (Days 0 and 3; Visits 4 and 5), after which a trained member of the Clinical Research Unit staff or a trained study coordinator will apply the drug twice each week (Weeks 2-18; Visits 6-39). The investigator will attend one treatment visit in the Clinical Research Unit each month to evaluate the extent of hair loss and hair growth (Weeks 4, 8, 12, and 16). Scalp biopsy specimen collection will be performed at baseline (Day -16, Visit 2), 72-96 hours after the first treatment (i.e., challenge) application (Day 2, Visit 5), 72-96 hours after the final application (Week 18 +3d, Visit 39) or during treatment when the study subject is determined to have achieved >=50% hair regrowth. Three scalp biopsy samples will be collected by a board certified dermatologist. When possible, each sample will be taken from a balding area near an area with hair preferably in a non-androgen dependent site of the scalp. One of the three samples will be frozen in OCT for histologic examination and immunohistochemical studies. A second biopsy sample from an adjacent area will be placed in 10% buffered

	formalin for histologic examination and assessment of inflammation and hair follicle differentiation. The third sample from each area will immediately be placed in RNAlater (Qiagen, Valencia, CA) for cytokine expression analyses. Peripheral blood collection for the study will include one tube of blood in a serum separator tube (red top SST tube) and one tube of blood in a PAXgene tube for RNA isolation. All blood samples will be transported to the Dermatology laboratory at the University of Minnesota for immediate processing and storage for additional biomarker studies. Research coordinators will be involved in patient recruitment, contact, and scheduling. The PI and research coordinators will all be involved in the collection, analysis, and reporting of collected data.
Concomitant Medications	Medications with no proven effect on the immune system will be acceptable.
Study Population	This study includes subjects ages 18 and above. Patients of the University of Minnesota Dermatology Clinic who meet the inclusion/exclusion criteria will be given the opportunity to participate in this study, as will select participants in the National Alopecia Areata Foundation Clinical Trials Network. Dr. Hordinsky has several Hair Disease clinics and appropriate patients will be offered the opportunity to participate in this study.
Sample Size	Up to 10 subjects
Study Sites	University of Minnesota
Duration of Subject Participation	4-5 month treatment period consisting of a sensitization visit, dose determination visit (at least one, up to 4 total), 36 treatment applications at twice weekly (+/- 2 days) intervals, a post-treatment safety evaluation and biopsy collection procedure 72-96 hours after the last treatment visit, and a visit 7-14 days after the final biopsy collection for suture removal.
Outcome Measures	**Primary Efficacy Endpoints** In this study, the Severity of Alopecia Tool (SALT) will be used. The SALT score is a global AA severity score based on scalp hair loss. To determine the SALT score, 4 main areas are assessed with these representing 18%, 18%, 40%, and 24% of the total scalp surface area. 50% scalp hair regrowth based on SALT score can be expressed as $SALT_{50}$. The primary endpoints ranked below in order of importance are as follows:

	1. The proportion of subjects that has ≥ 75% decrease in their SALT score ($SALT_{75}$), as compared to baseline at the completion of the study or at any time during the study. 2. The proportion of subjects that has ≥ 50% decrease in their SALT score ($SALT_{50}$), as compared to baseline at the completion of the study or at any time during the study. **Exploratory Endpoints** 1. To identify peripheral blood and scalp skin biomarker profiles that characterize a response to DPCP treatment, enabling the future potential for predicting individual responses to treatment and the likelihood of a sustained response. 2. Subject assessment of their hair loss using the subject hair loss assessment scale.
Estimated Start	03/15/2023
Estimated Finish	12/31/2024
Safety	All regulations stated in 21 CFR Parts 50, 56, and 312 and outlined in the ICH Guidelines for Good Clinical Practice will be adhered to throughout this trial. Concomitant medications and adverse events will be monitored and tracked. Adverse events (AEs) may be observed or elicited by the Investigator and/or volunteered by the subjects. AEs will be graded on a four-point scale (mild, moderate, severe, or life-threatening). AEs will be recorded on the Adverse Event Report (Appendix C) and documented in the subjects' files. Serious adverse events (SAEs) will be documented on the Adverse Event Report (Appendix C) and the Serious Adverse Event Report Form (Appendix D). SAEs will be reported to study drug manufacturer, University of Minnesota IRB, and the FDA.
Statistical Considerations	Statistical Considerations: The proportion of subjects with ≥75% and ≥50% decrease in the SALT score (at any time during treatment; endpoints 1 and 2) will be described using the fraction achieving the desired decrease and the associated confidence interval, calculated by the Agresti-Coull exact method. To describe change in SALT score, we will use the average change and associated confidence interval. Statistical power: For a total sample size of 10 we anticipate 6 or 8 participants providing data. For changes in binary outcomes, for 6 participants, the Agresti-Coull 95% confidence intervals for the proportion having an effect range in width from 0.44 (0 to 0.44 if 0 of 6 have an effect or 0.56 to 1 if 6 of 6 have an effect) to 0.62 (i.e., 0.19 to 0.81 if 3 of 6 have an effect). For 8 participants, the Agresti-Coull 95% confidence intervals for the proportion having an effect range in width from 0.37 to 0.56.

	Exploratory Endpoints To identify scalp skin biomarker profiles that characterize a response to DPCP treatment, enabling future potential for predicting individual responses to treatment and the likelihood of a sustained response. The primary outcomes for this analysis are levels of T-cell and DC markers, which will be analyzed using paired t-tests. To estimate power, preliminary data were obtained from a pilot study (Fuentes-Duculan et al. Abstract, ISDS, November, 2014). Assuming the standard deviations are as reported in that abstract, for comparing pre- vs. post-treatment, for 6 participants we have 80% power to detect a 14-fold change (using two-tailed alpha = 0.05); for 8 participants the detectable difference is 8-fold. For the analysis of various biopsy parameters, Dr. Krueger will be supported by a statistician in his group. For the change in subject VAS score, the confidence interval width will be 2.1 standard deviations for 6 participants and 1.7 standard deviations for 8 participants; we lack information needed to supply a standard deviation describing variation between persons in this change.

1. INTRODUCTION

Alopecia Areata (AA): AA is an autoimmune disease characterized by immune targeting of anagen hair follicles (growing stage of the hair cycle) and subsequent disruption of the hair cycle.[1] The first National Health and Nutrition Examination Survey conducted from 1971 through 1974 found the prevalence of AA in the United States to be 158 per 100,000 persons, which is roughly 0.1 to 0.2% of the population.[2] AA presents as patchy AA or extensive, involving all of the scalp (alopecia totalis) or scalp and body hair (alopecia universalis). It is estimated that AA accounts for approximately 4% of visits to dermatologists. AA has a considerable impact on quality of life and can be a devastating disease.[3–7] Support for a multifactorial inheritance pattern comes from many sources.[8–11] Transplantation studies in severe-combined immunodeficient mice show T-cell immunity is directly involved in the disease.[12] A hallmark of active AA is the presence of peribulbar lymphocytes around anagen follicles and the presence of killer CD8 T cells attracted by NKG2DL.[13] Immune privilege may be lost in AA.[14,15] A genome-wide association study showed the involvement of both innate and acquired immunity.[11] More recently investigators have also demonstrated in scalp biopsies from AA patients with mild to extensive disease that lesional AA samples had an "inflammatory" AA subset characterized by higher expression of T-cell (CD4, CD2, CD3D, CD3G, and CD28) and dendritic cell markers (CD11c, CE1b, CD83 and CD86). More extensive scalp involvement was also found to be associated with higher genomic dysregulation and multiple lines of evidence suggest that there are shared genetic risk factors between AA and other autoimmune diseases.[11]

Treatments: Many therapies are available off-label and current treatment choices are frequently based on disease extent, duration, and age of the patient.[16–19] In June 2022, the FDA approved an oral janus kinase inhibitor for the treatment of AA, however, this medication is associated with immunosuppression and potential risk. (Ref 20-21) Assessing efficacy of treatments for AA is complicated because there are few published randomized controlled trials. However, there are several uncontrolled trials and reports with non-ideal criteria to evaluate treatment progression and long-term follow-up is not included in most of the published works. In 1999 and 2004 the AA Investigational Assessment Guidelines were published and have been subsequently applied in up to 30% of new published investigations, providing some standardization to the description of disease extent and percent improvement in response to therapy.[20,21]

Sensitizers

Three different topical sensitizers have been used to treat alopecia areata with some evidence of efficacy. These three include dinitrochlorobenzene (DNCB), diphencyprone (DPCP), and squaric acid dibutyl ester (SADBE). The theory of immunomodulation was proposed by Happle et al in 1986 when they showed that skin treated with topical sensitizers showed changes in the peribulbar CD4/CD8 lymphocyte ratio.[22] In 2006 Herbst et al reinforced and complemented the theory providing evidence that long term treatment with a contact sensitizer allows for the recovery of the hair follicle by driving autoreactive T cells into activation-induced cell death.[23]

DNCB was first used for alopecia areata in 1976,[24] but due to concerns over the mutagenic properties of the drug in ***Salmonella enteritides*** serotype Typhimurium and its absorption through the skin with ultimate excretion in urine, its use has in large part been discontinued.[25] Because SADBE is stated to be relatively unstable in acetone solution , DPCP is

the most commonly used sensitizer in clinical practice.[26,27] Happle et al enthusiastically introduced the use of DPCP for the treatment of alopecia areata when they reported that 67% of treated patients had a satisfactory response to this treatment.[26]

However, in a larger study of 139 patients completed by the same investigator, a response rate of 50.4% was subsequently reported.[28] Some years later a review of 17 reported cases series concluded that though 50-60% of patients may achieve a response to DPCP, the range of reported responses was very wide, between 9 to 87%.[29] In a follow-up study, Gordon et al included a total of 32 patients and demonstrated that only 9 patients maintained regrowth without further DPCP treatment for an average of 19.8 months, while 9 had poor regrowth with continued use of the drug and 5 discontinued the treatment.[30]

A review of AA treatments in 2003 presented the evaluation of the 17 most significant controlled trials from 1983 to 2001, using DPCP and SABDE studies to test the efficacy of treating AA.[31] The response rate of treatments represented by cosmetically acceptable hair regrowth varied from 29% to 78%. The authors suggested that these differences could be explained by the different extent and durations of alopecia areata present in the patient populations prior to the treatment in each study and by the different treatment methodologies.[31] In yet another retrospective chart review of patients from 1996 to 1999, 33% were found to attain cosmetically acceptable or total regrowth with topically applied SADBE.[32]

Additional studies have been performed more recently around the globe, and have continued to support the efficacy of contact sensitizers for some patients with AA. An Indian study in 2006 involved 70 patients treated with SADBE for 4 months. The overall success rate was 43%. For subjects with < 50% of scalp involvement the rate was 68%, however for those with > 50% involvement the success rate was 29%. Additionally 81% of the responders (21 in 26) had relapse occurrence.[33] In a study completed in Greece 64 patients with alopecia areata were sensitized with 2% DPCP and treated with graduated concentrations adjusted to maintain erythema and pruritus on the scalp for 48 hours. The follow up was 2 years. A total of 83.3% of the subjects responded to therapy, although a significant difference in responses was noted and varied between regrowth of vellus hair, sparse pigmented terminal hairs, terminal hairs in patches of alopecia as well as regrowth of terminal hair on the entire scalp. Relapses were noted to occur in approximately 70% of the patients.[34] A Chinese study published in 2012 investigated the efficacy of DPCP in treating 31 steroid non-responders with extensive AA (defined as >30 percent hair loss) after 6 months of weekly treatment. Similar to the Greek study, participants were sensitized with 2% DPCP and treated with graduated concentrations adjusted until an erythematic reaction was maintained for 48 hours. The authors reported 37.9% of participants exhibited complete (n = 4) or partial (n = 7) remission of AA, with a 69.2% relapse rate in the subsequent 18 months (n = 9/13).[35]

The side effects of topical immunotherapy include the desired mild dermatitis inherent to the treatment and in 2-5% of patients vesicular and bullous reactions at the initiation of treatment before appropriate individual concentrations are determined, dissemination of allergic contact dermatitis, urticaria or erythema multiforme-like reactions and pigmentary disturbances such as postinflamatory hyperpigmentation. Notably, no long term side effects have been reported after 18 years use of DCPC and 21 years of SADBE treatments worldwide.[36]

Cytokine Profiling

Studies designed at investigating the role of cytokine expression during active disease have resulted in an increased comprehension of the molecular basis of disease, as well as, the

potential of more targeted therapies. A study by Czarnowicki et al. explored the extent of T-cell activation in atopic dermatitis (AD) and psoriasis and found that patients with AD had higher and more persistent activation of central and effector memory T cells which may explain the systemic and frequent disease in these patients. This type of study allows for identification of cytokine profiles that characterize a disease.[37]

A previous clinical study that investigated the role of cytokines, specifically IL-17A, found that this cytokine plays a role in the pathogenesis of psoriasis. Krueger et al. found that Th17 cell cytokine interleukin-17 (IL-17) is likely causing inflammation and epidermal hyperplasia in psoriasis. This study combined an experimental therapeutic drug trial with cytokine profiling. Skin biopsies were obtained from participants undergoing subcutaneous injections of ixekizumab. A combination of histology, RT-PCR, and a gene array were used to analyze cytokine concentrations in order to better understand their role and contribution to disease. IL-17 was identified as the major "driver" cytokine for the disease phenotype in psoriasis. This study provides support for the investigation of the role of cytokines as an indicator and biomarker of disease in psoriasis and other related diseases including alopecia areata.[38]

Recent studies have found differences in the transcriptomes between lesional and non-lesional areas of disease. Specifically, a greater immune and keratin dysregulation corresponding to greater scalp involvement (>25%) was found. Activation of cytokines, T_H2, T_H1, IL-23, and IL-9/T_H9 were identified to be a signature for alopecia areata.[39] A study also performed at the Krueger Laboratory identified dysregulation of T cell expansion in a subtype of psoriasis specific to the Asian population. IL-17A and IL-17- were found to be highly expressed specifically in Asian small plaque psoriasis, compared to Western large plaque psoriasis. Similar differences worldwide in alopecia areata remain to be reported. In summary, cytokine profiling studies allows for a deeper understanding of the main mechanism of disease development.[40]

DPCP Non-clinical Studies

Although it has not received FDA or EMA approval to date, several investigator sponsored trials and research programs have generated data to support the safe use of DPCP for the treatment of several indications, including warts, alopecia areata and cutaneous metastases of melanoma.

Toxicity and Safety Studies

Genetic Toxicity

Two standard *in vitro* genetic toxicity studies, a bacterial mutagenicity assay (Ames test) and a chromosomal aberration assay, were conducted with DPCP obtained from the API supplier, Carbosynth. Both tests were negative under the conditions of the assays, attesting to the absence of a risk for genetic toxicity. In addition, an *in vivo* micronucleus assay was performed to evaluate DPCP for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte cells (mnPCEs) in mouse bone marrow. DPCP administered by intraperitoneal injection did not induce a significant increase in the incidence of mnPCEs in bone marrow at any dose tested. DPCP was concluded to be negative in an *in vivo* micronucleus test designed to test for *in vivo* clastogenic activity.

DPCP Ointment has been evaluated in studies carried out by Hapten Pharmaceuticals under BLA 10,223 and IND 77,043. DPCP Ointment is also being evaluated in the treatment of

cutaneous metastases of melanoma in Investigator Sponsored individual patient INDs. The status and results to date of these studies are summarized below.

Diagnostic Use as a Measure of Immune Competence

A clinical study was conducted by Pharmaderm Associates, LLC (now Hapten Pharmaceuticals) to determine if DPCP can be used as a diagnostic for assessing immunocompetency in HIV-positive subjects.[41] Forty HIV-positive subjects were enrolled and divided into four groups to be treated with one of the following DPCP concentrations (0.4, 0.2, 0.1, or 0.05%). The DPCP was formulated in 50/50 polysorbate 80/isopropyl myristate and applied on a one square inch latex-free adhesive pad to the medial surface of the right arm and left in place for 48 hours. Skin reactivity was assessed weekly on a scale of 1 to 4 with ≥ 2 considered positive for immunocompetence. The skin reactivity scores correlated with CD4 T cell counts, suggesting DPCP could be used as a simple test for immunocompetency. This study also clearly demonstrated that 0.4% DPCP can successfully elicit a DTH response in most patients with CD4 T cell counts in the normal range. In these subjects there were no skin reactivity scores of greater than 2 (erythema and induration) even at the highest dose of DPCP of 0.4%.

Healthy Volunteer Biomarker Study

In a study by Gulati et al., 11 healthy volunteers were sensitized with sensitizing DPCP ointment (0.4%) followed by challenge applications of treatment DPCP ointment (0.04%) as well as topical placebo applications. The treated sites were biopsied at different time points for comprehensive cellular and molecular profiling. All volunteers tolerated treatment well, with the only adverse events being the expected inflammatory skin reactions to DPCP ointment. No serious adverse events, unexpected adverse events, or other types of adverse events occurred.[42]

The analysis of biopsy samples included gene array approaches, and ~7,500 mRNA transcripts were modulated during the peak inflammatory responses induced by DPCP. These responses were characterized by high expression of cytokines that define all major effector T cell subsets. The study suggests the presence of active regulatory mechanisms in the resolution of inflammation induced by DPCP, proving the utility of using DPCP to study mechanisms of immune regulation in human skin**.**

Cutaneous Metastasis of Melanoma

There have been two investigator initiated individual patient INDs in which DPCP ointment was evaluated for treatment of cutaneous metastases of melanoma. Gulati et al. (2016a) demonstrated dramatic reduction of cutaneous metastases in a patient who received three months of combined pembrolizumab (an immune checkpoint inhibitor) and DPCP therapy (alternating 0.4% and 0.04% concentrations applied twice weekly). Treatment using either agent alone produced mixed results. The patient also developed vitiligo on the contralateral leg not treated with DPCP, which suggests a synergistic immune response against melanocyte lineage cells.[43]

Gulati (2016b) characterized the immune response to DPCP in a cohort of six patients with cutaneous melanoma metastases. Immune reactions induced by DPCP were characterized using both immunohistochemical (IHC) and gene expression approaches. The authors reported that five patients achieved partial or complete regression of their cutaneous metastases after minimum 14 weeks (range: 14-28 weeks) of 0.4% DPCP treatment, applied twice weekly, as indicated by reduced IHC staining of the melanocyte marker MLANA. Further evidence of

melanoma regression was demonstrated through gene expression microarray analysis. This analysis showed downregulation of hallmark melanoma and melanocyte gene expression post-DPCP treatment compared to pre-DPCP treatment. The genes most significantly downregulated included five "melanoma signature" genes *PRAME*, *TYR*, *OCA2*, *DCT*, and *MLANA*, which were downregulated 12- to 22-fold. Notably, one patient left the trial before sensitization could be induced, and was not included in these results.[44]

Common Warts

A study was carried out to evaluate DPCP ointment for the treatment of common warts (verruca vulgaris). This Phase 2a trial, sponsored by PharmaDerm Associates, LLC (later Hapten Pharmaceuticals), was a randomized, double blind, placebo controlled study to determine the safety and efficacy of DPCP ointment as a topical immunotherapy for the treatment of common warts. The study enrolled adults with at least one 5-20 mm non-genital wart. The treatment consisted of a sensitization dose applied to the inner arm and one target wart with Sensitizing DPCP ointment (0.4%) or placebo (ointment with no DPCP) on Day 0, followed by seven (7) weekly administrations of treatment DPCP ointment (0.04%) or placebo applied to each target wart (up to 4 per subject) beginning on Day 15. A final follow-up examination was carried out two weeks after the last treatment dose. The response to sensitization was graded at the Day 15 visit on a scale of 0-4. Adverse reactions, immunotherapeutic response and efficacy using IGAS and Lesion Surface Area scores were assessed during each visit throughout the treatment and follow-up period.

The percent clearance by wart was calculated for the 'Per Protocol' population (completing all treatments and assessment visits). At least 50% clearance was reported in 47.6% of the DPCP ointment treated wart lesions compared to 6.7% of the wart lesions treated with placebo ointment. This result is statistically significant ($p = 0.011$) indicating a clinical benefit in the clearance of warts with DPCP ointment.

Drug-related adverse events reported in the study were local reactions due to the sensitization and challenge responses in the skin. The most commonly reported adverse events (AEs) were rash, pruritus, and erythema. The highest rate of reported AEs occurred after the sensitization step (reported at the Day 15 visit).

2. OBJECTIVES

Primary Objective: To determine the efficacy of DPCP ointment for the treatment of alopecia areata.

Secondary Objectives: 1) To assess the safety of DPCP ointment in alopecia areata subjects.
2) To determine the best starting dose range for future studies

Exploratory Aims: 1) To evaluate the association between cytokine and keratin profiles, Cytomegalovirus UL16 Binding Protein (ULBP) expression and hair regrowth.

3. STUDY DESIGN

- The products to be evaluated are 0.4% DPCP and four strengths of DPCP ointment (0.04, 0.08, 0.12, 0.2%) (Add preparation) Four dose concentrations of DPCP ointment (0.04, 0.08, 0.12, 0.2%) will be prepared through dilutions of 0.4% DPCP Ointment (applied to

the inner aspect of the left upper thigh) and these will be applied sequentially starting with the lowest concentration and, if no sensitization is attained, sensitization to the next lowest dose will be attempted. This will be repeated until either the participant has a reaction and that concentration will be their dose for the duration of the study or until all concentrations have been unsuccessful in causing a reaction. If this is the case, the participant will be discontinued from the study. ("Dose Determination Visit"; 10-14 days after the Sensitization Visit)

For sensitization, 0.05 mL of the 0.4% concentration will be applied topically to the right upper inner arm. If sensitization is not attained after 10 to 14 days, the procedure will be repeated once. After sensitization is established, the lowest treatment dose will be applied to the inner aspect of the upper left thigh. If sufficient sensitization is not attained, the next lowest treatment dose will be applied, etc, until sensitization is attained. If no sensitization is attained to any of the 4 concentrations, the participant will be discontinued from the study. The treatment dose will be selected based on lowest concentration of DPCP that causes minimal reaction. This treatment dose will be used throughout the study. The treatment phase of the study will consist of twice-weekly applications of 0.75-1 g DPCP ointment to the entirety of the scalp. Every four weeks during treatment, the investigator will assess hair loss as described below:

1) Efficacy will be assessed as follows:
    a. Severity of Alopecia Tool (SALT) Score: Percentage of hair loss on the scalp will be determined using the SALT score, a global alopecia areata severity score based on scalp hair loss.[20,21] The SALT score is calculated by visually examining *four* discrete areas of the scalp, measuring the percentage of terminal hair loss in each area, and then totaling the results (See Appendix B).
    b. Scalp Photography.
    c. Hair Pull Tests (positive or negative).

2) Safety will be assessed by monitoring side effects at each visit and recording adverse events for each subject on the Adverse Event Report (Appendix C). Adverse events will be tracked for each subject from the time of the first sensitization does application of the drug until the subject completes the study. Adverse events will be assessed for severity by the investigator and graded on a four-point scale (mild, moderate, severe, or life-threatening). Serious adverse events will also be recorded on the Severe Adverse Event Report (Appendix D), and the study drug manufacturer and the U of MN IRB, and the FDA will be notified. Skin reaction scores will be recorded during the sensitization assessment (Appendix I) to monitor reactions, and may constitute an adverse event based on severity of reaction.

3) Molecular and immunological characterization of the Alopecia Areata disease process and how it changes throughout the course of treatment with DPCP will be achieved through mRNA expression profiling in blood and scalp biopsy specimens, and histopathology analyses.

4) Molecular predictors of positive and negative response to DPCP treatment will be identified through expression profiling targeted to mRNA transcripts for cytokines, T cell

subsets, and hair keratins in scalp biopsy specimens. Peripheral blood mononuclear cells will be frozen and also serum frozen for future studies looking downstream for possible changes with DPCP in T-cell activation markers or circulating cytokines such as gamma-interferon, IL-13, IL-17, and/or an increase in Tregs (CD25+/CD127- population or Foxp3+ population). This will not need to happen in blood if all the changes however are found to be happening in skin/scalp.

3.1 Study Design

This is an open labeled study to determine the response and characteristics, safety and efficacy, of the proprietary DPCP ointment composition as a topical immunotherapeutic agent for the treatment of extensive Alopecia Areata. The products to be evaluated are 0.4% DPCP and four strengths of DPCP ointment (0.04, 0.08, 0.12, 0.2%), with each dose determined for every participant individually.

Up to 10 (minimum of 6) subjects with diagnosed extensive alopecia areata (76%-99% scalp hair loss as determined by the SALT score; see Appendix B, Part I) that are eligible for DPCP treatments will be consented and enrolled in the study. Patients with alopecia areata will be recruited from the new and current patient population seen in the University of Minnesota Medical Center Dermatology Clinics as well as through partnership with the National Alopecia Areata Foundation Clinical Trials Network. Potential subjects will also be recruited using flyers posted at the University of Minnesota and in surrounding communities. The study may be advertised using various media outlets, including newsletters, campus wide mailings, campus email announcements, the Dermatology department website, and listservs in the Twin Cities area (including the listserv for the Minnesota Alopecia Areata Support Group). All materials used in recruitment will be approved by the Institutional Review Board (IRB) prior to implementation.

There will be a total of 42 scheduled visits, beginning with a screening visit. Subjects will undergo 38 scheduled topical applications of a an ointment formulation of DPCP, including a sensitizing dose of study drug at Day -16, an initial dose determining application at Day -2, followed by a challenge at Day 0, and twice weekly (+/- 2 days) treatment doses from Weeks 1-18. Additional applications of DPCP could include another sensitization visit or additional dose determination visits for the higher concentrations, if necessary. There will be a scalp biopsy collection at baseline (Visit 2, Day -16), and visits for scalp biopsy collection 72-96 hours after the first treatment, and 72-96 hours after $\geq$50% hair regrowth *or* 72-96 hours after application of the final treatment dose. Suture removal will take place during treatment visits or scheduled 7-14 days after each biopsy visit. Several questionnaires and forms will be completed throughout the duration of the study.

Research coordinators will be involved in patient recruitment, contact, and scheduling. The PI and research coordinators will all be involved in the collection, analysis, and reporting of collected data.

All visits will take place either in the M Health Clinical Research Unit (CRU), part of the Clinical and Translational Science Award at the University of Minnesota, or in the LCRU. There are no additional collaborating sites for this study.

Schedule of Visits

	Visit							
Procedure	**1[a]**	**2[b]**	**3[c]**	**4[d]**	**5**	**6-40[e]**	**41**	**42[f]**
Informed consent	X							
Screening for eligibility	X							
Blood tests	X					X (1 time at week 12)	X	
Pregnancy test	X					X (1 time a month)	X	
Vital signs	X	X			X	X (1 time a mo.)	X	
Adverse events		X	X	X	X	X		X
Assessment of hair loss	X		X			X (1 time a month)	X	
Photographs		X	X			X (1 time a month)	X	
Scalp biopsy procedure		X			X	X (if 50% regrowth)	X	
Suture removal				X		X (as needed)		X
Blood draw		X			X	X (if 50% regrowth)	X	
DPCP ointment - sensitizing		X						
DPCP ointment – dose determination for each concentration: 0.04, 0.08, 0.12, 0.20			X					
DPCP ointment - challenge				X				
DPCP ointment - treatment				X	X	X (twice per week For 17 weeks, visit 40 will occur during week 18)		
Study completion								X

a. Screening visit
b. Sensitization and baseline sample collection – visit can be repeated if participant not a responder to DPCP the first time. Blood draw and scalp biopsy procedure will only take place if sensitization has occurred and the participant is a responder to DPCP.
c. Dose determination visit – see following schedule of events for more details.
d. Challenge visit
e. Participants will return twice per week for application of the previously selected dose of DPCP. Every four weeks during treatment, the investigator will attend the treatment visit to perform assessments of hair loss.
f. Suture removal and study completion

Dose Determination visit schedule:

	Visit			
Procedure	**3[1]**	**3[2]**	**3[3]**	**3[4]**
Adverse events	X	X	X	X
Assessment of hair loss	X			
Photographs	X	X	X	X
DPCP ointment – 0.04%	X			
DPCP ointment – 0.08%		X		
DPCP ointment – 0.12%			X	
DPCP ointment – 0.2%				X

1: DPCP ointment 0.04% concentration is the starting concentration for all participants

2: DPCP ointment 0.08% will only be applied if participant does not have a strong enough response [defined as skin reaction score 1+] to the 0.04% concentration of DPCP. Thus, this visit will begin with an assessment of the sensitization reaction to the 0.04% dose, If there is not a strong enough response, 0.08% concentration DPCP will be applied to a different area of the inner upper left thigh in the same manner as the 0.005% dose. If the participant is a responder to the 0.04% DPCP, patient will proceed to the challenge visit (visit 4) as described above.

3: DPCP ointment 0.12% will only be applied if participant does not have a strong enough response [defined as skin reaction score 1+] to the 0.08% concentration of DPCP. Thus, this visit will begin with an assessment of the sensitization reaction to the 0.08% dose, If there is not a strong enough response, 0.12% concentration DPCP will be applied to a different area of the inner upper left thigh in the same manner as the 0.08% dose. If the participant is a responder to the 0.08% DPCP, patient will proceed to the challenge visit (visit 4) as described above.

4: DPCP ointment 0.2% will only be applied if participant does not have a strong enough response [defined as skin reaction score 1+] to the 0.12% concentration of DPCP. Thus, this visit will begin with an assessment of the sensitization reaction to the 0.12% dose, If there is not a strong enough response, 0.2% concentration DPCP will be applied to a different area of the inner upper left thigh in the same manner as the 0.12% dose. If the participant is a responder to the 0.2% DPCP, patient will proceed to the challenge visit (visit 4) as described above.

Screening Visit

At the screening visit the PI will obtain signed, informed consent and HIPAA approval. Demographic information, weight and vital signs, medical history including concomitant medications will be recorded on the Demographics and Baseline Assessment form (Appendix D). Research personnel will review eligibility with the subject. Lifetime treatment history will also be recorded (Appendix E). A dermatologic exam will be performed to confirm that the extent of alopecia areata is 76-99% hair loss according to the SALT score (Appendix B, Part I). Laboratory tests will be drawn to measure TSH, Hemoglobin (HgB), and Vitamin D level, and thus to reduce the likelihood a common underlying medical problem that impacts hair growth exists prior to study participation. Additional laboratory tests will be draw as part of safety monitoring as described in section 9.7. Pregnancy test will be administered to all women of childbearing potential, and must be negative to participate. Safety laboratory results will be documented in the subjects' medical record.

An appointment for the sensitization visit will be scheduled with research personnel. If a washout period is required prior to treatment, the subject will return for the sensitization visit at the appropriate time. Otherwise, the research subject may immediately proceed to the sensitization part of the study or may be scheduled at a determined date in the future for the sensitization and baseline sample collection visit described below.

Sensitization and Baseline Sample Collection Visit, Day -16
Inclusion and exclusion criteria will be reviewed again, especially if there was a washout period between the screening and sensitization visits. Interim medical history, concomitant medications, and vital signs will be recorded on a Case Report Form (CRF). Assessments of hair loss, including the SALT score, the Physician Assessment of Alopecia (PAA; Appendix F), and the Subject Hair Loss Assessment (SHLA; Appendix G) will be completed by the physician or subject. Pregnancy test will be administered to all women of childbearing potential, and must be negative to participate. AEs (Appendix C) will be monitored and recorded.

Three 4-mm scalp biopsy samples will be collected from perilesional scalp by a board certified dermatologist knowing that in some patients this site may be by just a few tufts of fibers. One of the three samples will be frozen in OCT for histologic examination and immunohistochemical studies. Those samples will be stored in a -80 degree freezer and batch shipped to Dr. Krueger and the Laboratory of Investigative Dermatology at Rockefeller University in New York, NY on dry ice every one to two months. A second biopsy sample from each area will be bisected and placed in 10% buffered formalin and Zamboni's fixative for histologic examination and assessment of inflammation and hair follicle differentiation in the Dermatology Laboratory at the University of Minnesota. The third sample from each area will immediately be placed in RNAlater (Qiagen, Valencia, CA) and shipped to the Krueger Laboratory in the Laboratory of Investigative Dermatology at Rockefeller University in New York, NY for mRNA-level cytokine analyses.

Peripheral blood samples will be collected by a trained staff member and will include 1 serum separator tube (SST) and 1 PAXgene tube for RNA isolation.

A sensitizing dose of 0.4% DPCP ointment will be applied directly to the inner aspect of the upper right arm. The site where the dose is applied will be marked and photographed as will the extent of scalp disease.

Dose Determination Visit, Day -2 (10 to 14 days post-sensitization)
Assessments of hair loss, including the SALT score and PAA will be completed. AEs (Appendix C) will be monitored and recorded. The sensitization reaction will be recorded by the physician on the Sensitization Assessment form using accepted methods (Appendix I, Part I).[42] Subjects are considered DTH Responders with skin reaction scores ≥ 2+ at the inner arm sensitization site. Subjects having skin reaction scores of 0 or 1+ are considered non-responders. In the event the subject has a response that is 4+, this will be considered an AE and recorded as such. Reactions scoring less than 4+ can still be considered an AE at the investigator's discretion.

Non-responders (i.e., subjects who do not have a sensitization response) will receive another sensitization cycle in the manner previously described. If a subject is found to be a non-responder through two cycles of sensitization, they will be discontinued from the study.

A concentration of DPCP 0.04 will be applied by the PI or co-PI in the amount of 50 ul per concentration to the inner aspect of the upper left thigh. The site where the dose is applied with be marked and photographed. The site will be covered with a clear, plastic, waterproof dressing such as Tegaderm. The sensitizing test site is to be discontinued 6-8 hours after application by

the subject removing the Tegaderm and cleansing the test site. The subject will report to the study site in 72 hours. If the patient has a sufficient reaction (DTH skin reaction score of 1+), this will be their treatment dose for the duration of the study and the participant will proceed directly to the challenge visit listed below.

- If no reaction to the DPCP 0.04% preparation, an additional test sensitizing to an alternate site in the inner aspect of the upper left thigh with DPCP 0.08% prepared in the same dilutions as previous dosage. The site where the dose is applied with be marked and photographed. The sensitizing test is to be discontinued 6-8 hours after application by the subject removing the Tegadem and cleansing the test site. The subject will report to the study site in 72 hours. If the patient has a sufficient reaction [defined as skin reaction score 1+], this will be their treatment dose for the duration of the study and the participant will proceed directly to the challenge visit listed below.
- If no reaction to the DPCP 0.08% preparation, an additional test sensitizing to an alternate site of the inner aspect of the upper left thigh with DPCP 0.12% prepared in the same dilutions as previous dosage. The site where the dose is applied with be marked and photographed. The sensitizing test is to be discontinued 6-8 hours after application by the subject removing the Tegaderm and cleansing the test site. The subject will report to the study site in 72 hours. If the patient has a sufficient reaction [defined as skin reaction score 1+], this will be their treatment dose for the duration of the study and the participant will proceed directly to the challenge visit listed below.
- If no reaction to the DPCP 0.12% preparation, an additional test sensitizing to an alternate site in the inner aspect of the upper left thigh with DPCP 0.2% prepared in the same dilutions as previous dosage. The site where the dose is applied with be marked and photographed. The sensitizing test is to be discontinued 6-8 hours after application by the subject removing the Tegaderm and cleansing the test site. The subjects will return to the study site in 72 hours after test applications. If the patient has a sufficient reaction [defined as skin reaction score 1+], this will be their treatment dose for the duration of the study and the participant will proceed directly to the challenge visit listed below.
- If the subject does not have a reaction to the DPCP 0.2%, they will be discontinued from the study. If a subject is discontinued from DPCP treatment or study participation, they should complete an Early Termination Visit one week after the last drug application.

Challenge Visit, Day 0
AEs (Appendix C) will be monitored and recorded.

This first treatment of the drug of the determined dose will be applied to the entire scalp (0.75-1 g of treatment drug) by the PI or co-PI, both board certified dermatologists. Photographs will be taken prior to the application of the treatment drug to document extent of hair loss.

Post-First Treatment Sample Collection Visit and Second Treatment, Day 3
A visit will occur a minimum of 72-96 hours after the first application of drug in which scalp biopsy and blood specimens will be collected. Vital signs and AEs (Appendix C) will be monitored and recorded. Handling of biopsy and blood specimens will follow the same protocol

as was used at the Sensitization and Baseline Sample Collection Visit, Day -16.

The biopsies taken 3-4 days after application of the first challenge application of DPCP of the determined dose will be approximately 2 inches from the first set of biopsies. These biopsies are being done to define the initial DTH response to DPCP in the scalp. The expectation is that the initial response will differ from a chronic response that becomes more Th1 centered with chronic treatment.

The second treatment with study drug will again be applied by the PI or co-PI. Photographs will be taken to document extent of hair loss.

Treatment Visits, Twice per Week for Weeks 1-18
Subjects will return to Dermatology Clinical Research twice per week for application of the previously selected dose of DPCP to the whole scalp. Week 18 will only have one treatment application visit. Treatment visits must be at least 24 hours apart. The drug will be applied by trained a member of the MCRU and/or Dermatology clinical research staff. AEs will be recorded each visit (Appendix C) and will be monitored.

Investigator Assessment Visits (Weeks 4, 8, 12, and 16)
Every four weeks during treatment, the investigator will attend one of the treatment visits at the CRU or Dermatology Clinical Research to perform assessments of hair loss, including the SALT score and PAA, in addition to the regular treatments and data collection performed at every treatment visit. Photography will also be completed. Vital signs will be recorded and monitored at these visits. Pregnancy test will be administered to all women of childbearing potential once monthly at these visits. Laboratory tests related to safety monitoring will be drawn at week 12 of treatment.

Regrowth Sample Collection Visit
A visit will occur a minimum of 72-96 hours after a treatment visit in which the physician determines the subject has achieved a $\geq$50% decrease in hair loss. Scalp biopsy and blood specimens will be collected. Vital signs and AEs (Appendix C) will be monitored and recorded. Handling of biopsy and blood specimens will follow the same protocol as was used at the Sensitization and Baseline Sample Collection Visit, Week 0. These samples will be obtained from an area of hair regrowth and ideally, in the same scalp region where the initial biopsy samples were obtained.

Follow-up Visit, Week 18
The follow-up visit will occur a minimum of 72-96 hours after the final application of the drug. The post-treatment scalp biopsy and blood specimens will be collected at this time. Vital signs and AEs (Appendix C) will be monitored and recorded. Laboratory test related to safety monitoring will be drawn at this time. Pregnancy test will be administered to all women of childbearing potential. No treatment will be provided. Handling of biopsy and blood specimens will follow the same protocol as was used at Week 0. Photography will be completed and assessments of hair loss, including the SALT score, the Physician Assessment of Alopecia (PAA; Appendix G), and the Subject Hair Loss Assessment (SHLA; Appendix H) will be done by the physician or subject.

Follow-up Visit, Week 20
Subjects will return to the CRU or Dermatology Clinical Research 10-14 days after the final biopsy collection procedure for suture removal. AEs (Appendix C) will be monitored and recorded. A Study Completion Form (Appendix J) will be completed by the investigator or study coordinator.

Early Termination Visit
If a participant withdraws from the study at any time between the Baseline/Sensitization Visit and the final week of treatment, the early termination visit should take place at the time of subject withdrawal. AEs (Appendix C) will be monitored and recorded. No treatment will be provided. The investigator will perform assessments of hair loss, including the SALT score and PAA. The subject will complete the final SHLA (Appendix H). Photography will also be completed. A Study Completion Form (Appendix J) will be completed by the investigator or study coordinator.

4. RATIONALE FOR STUDY DESIGN

The active ingredient used in this study is Diphenylcyclopropenone (DPCP), also known as Diphencyprone. The synthesis of DPCP is well established and is readily available from commercial sources. DPCP is well accepted in the scientific community as a Delayed type Hypersensitivity (DTH) or Contact Hypersensitivity (CHS) Hapten. DPCP has been used for decades throughout the world for the treatment of Alopecia Areata (AA), as described in the Background section.

Diphenylcyclopropenone is a potent contact sensitizer that has distinct advantages over other alternatives. DPCP is not mutagenic in the Ames test and is shelf stable at room temperature. DPCP however can break down in the presence of Ultraviolet light and therefore should be stored in amber bottles or some other light-blocking container while not in use.

DPCP is not mutagenic in the Ames test, and teratogenicity and organ toxicity could not be detected in the hen's egg test or in the mouse teratogenicity assay. Moreover, DPCP is presumed to be not absorbed after topical administration because it is not detected in serum or urine.[45]

Despite the positive results for treating AA, DPCP has not been FDA approved for the treatment because the drug had previously been made in individual pharmacies or clinics and no standardized product was available for study. Furthermore, because there was no standardized product, many hospitals and clinics are unable to offer this treatment to patients. Hapten Pharmaceuticals recently created a standardized formulation of the drug (DPCP Ointment) with an IND and studies are needed to demonstrate the effectiveness of this drug in treating AA.

This study includes subjects who have been diagnosed with severe alopecia areata (76-99% hair loss) and, in the opinion of the Principal Investigator, are eligible for treatment with DPCP Ointment. Subjects with extensive disease are particularly suited to this study as to demonstrate the effects of treatment with DPCP Ointment.

4.1 Trial Treatment Descriptions

Diphencyprone, Diphenylcyclopropenone

Brand Name:	Diphenylcyclopropenone Ointment
Manufacturer:	**Ferndale Laboratories, Inc.** 780 West 8-Mile Road Ferndale, MI 48220 for Hapten Pharmaceuticals, LLC 151 Merriweather Road Grosse Pointe, MI 48236 (313) 300-8454
Dosage Form	(1) 0.4% and 0.04% DPCP Ointment formulations provided by Hapten Pharmaceuticals (2) 3 additional strengths of DPCP 0.08%, 0.12%, 0.2% will be compounded at the U of MN Investigational Drug Services Pharmacy using the 0.4% and 0.04% DPCP Ointments (provided by Hapten Pharmaceuticals); treatment dosage will be selected based on weakest strength concentration that caused minimal reaction
Color	Clear
Route of Administration	Topical
Pregnancy Category	B
Preservative	Methyl Paraben Propyl Paraben
Package size	5 gram tubes of 0.4% DPCP and 0.04% ointments (provided for dilution purposes)
Storage	Store at room temp Avoid excessive heat or exposure to UV light
Drug Interaction	None listed

Reported Adverse Events	Contact dermatitis Mild eczema Lymph node swelling Hyperpigmentation Local blistering and swelling Pompholyxlike eruptions Passive transfer of the sensitizer causing eczema distant from the application site and regional lymphadenopathy. Erythema multiformelike reactions Fever Palpitations Flu-like symptoms Headache
Contraindications	Allergy or reaction to any component of the vehicle formulation.
Warnings/Precautions	Diphenylcyclopropenone (DPCP) is a potent contact sensitizer. Sensitization of pharmacy, medical and nursing staff involved in its preparation and use is a potential hazard. Gloves should be worn when handling DPCP and all used containers, applicators, and gloves should be disposed of in a safe manner to avoid unintended sensitization to DPCP. Potential for hyper- or hypopigmentation, especially in patients with dark skin tones.

All medications will be maintained by the Investigational Drug Services (IDS) pharmacy and administered within the Clinical Research Unit or Clinical Research Division of the Department of Dermatology. Administration will be verified in the numerical identification study file for each patient.

Patients will receive all study-related treatments free of charge during the course of the study.

5. SELECTION OF STUDY POPULATION

This study includes subjects ages 18 and above. Patients of the University of Minnesota Dermatology Clinic or affiliated faculty and patients recruited through partnership with the National Alopecia Areata Foundation Clinical Trials Network who meet the inclusion/exclusion criteria will be given the opportunity to participate in this study.

5.1 Inclusion Criteria

Subjects may be included in the study if they meet **all** of the following criteria:

1. Subject has clinical diagnosis of extensive alopecia areata (76%-99% involvement as determined by SALT score, Appendix B, Part I).

2. Written informed consent and HIPAA authorization have been obtained.

3. Subject is ≥ 18 to years of age.

4. Female subjects of childbearing potential have a negative pregnancy test and agree to use an acceptable, highly effective method of birth control (i.e., failure rate of less than 1% per year) to prevent pregnancy.

5. Subject agrees to comply with protocol requirements and attend all required study visits and is considered to be a good study subject.

6. Subject meets concomitant medication washout requirements.

5.2 Exclusion Criteria

Subjects will be excluded from the study if they meet **any** of the following criteria:

1. Subject has <76 or greater than 99% hair loss.

2. Subject is pregnant or lactating.

3. Subject has current controlled or uncontrolled bacterial, viral (with the exception of herpes simplex), fungal, atypical, or opportunistic infection(s).

4. Subject has a history of substance abuse within the past five years.

5. Immunosuppression (history of transplantation, chemotherapy, splenectomy, HIV).

6. Administration of systemic treatment (e.g., Imuran, biologics) that have an immunomodulatory mechanism of action in the preceding 3 months.

7. Previous treatment with DPCP.

8. Application of topical immunomodulating agent in the preceding 6 weeks.

9. Application of topical or intralesional corticosteroids on the scalp within the past 6 weeks.

10. Systemic (oral, inhaled, or intravenous) administration of corticosteroid or other systemic treatment (i.e., prednisone) with an immunosuppressive mechanism of action within the past 3 months.

11. Use of light treatments (e.g., PUVA, narrow band UVB) in the preceding 6 weeks.

12. Use of Anthralin in preceding 6 weeks.

13. Use of minoxidil, topical or oral, in the preceding 4 weeks.

14. Subject is currently or has undergone systemic therapy for malignancy within the past five years except for adequately treated Squamous Cell Carcinoma (SCC) or Basal Cell Carcinoma (BCC) of the skin.

15. Clinical evidence of secondary skin infection (i.e., folliculitis).

16. Participation in other therapeutic investigational clinical trials within 4 weeks of enrollment.

17. Evidence of anemia, thyroid disease, sarcoidosis or other medical condition that could be adversely affected by participating in the study.

18. Subject has any medical condition that, in the judgment of the Investigator, would jeopardize the subject's safety following exposure to the administered medications.

Vulnerable Populations:

Population / Group	Identify whether any of the following populations will be focus of the research (targeted), included, but not necessarily the focus or excluded from participation in the study.
Children	Excluded
Pregnant women	Excluded
Fetuses	Excluded
Neonates	Excluded
Prisoners	Excluded.
Adults lacking capacity to consent and/or adults with diminished or fluctuating capacity to consent	Excluded

Non-English speakers	Excluded
Those unable to read (illiterate)	included but not the focus
Employees of the researcher	Excluded
Students of the researcher	Excluded
Undervalued or disenfranchised social group	included but not the focus.
Active members of the military (service members), DoD personnel (including civilian employees)	included but not the focus.
Individual or group that is approached for participation in research during a stressful situation such as emergency room setting, childbirth (labor), etc.	Excluded
Individual or group that is disadvantaged in the distribution of social goods and services such as income, housing, or healthcare.	included but not the focus
Individual or group with a serious health condition for which there are no satisfactory standard treatments.	Excluded
Individual or group with a fear of negative consequences for not participating in the research (e.g. institutionalization, deportation, disclosure of stigmatizing behavior).	included but not the focus
Any other circumstance/dynamic that could increase vulnerability to coercion or exploitation that might influence consent to research or decision to continue in research.	included but not the focus.

6. SUBJECT DISPOSITION AND QUALITY ASSURANCE CRITERIA

6.1 Subject Withdrawal Criteria

Any subject may discontinue participation in the study for any reason at any time. Subjects who withdraw from the study prematurely will complete an Early Termination Visit at the time of withdrawal. The procedures to be performed at the Early Termination Visit are specified under "Early Termination Visit." Follow-up evaluations two weeks after the last dose will be obtained, if possible. The reason(s) for a subject's discontinuation from the study will be clearly documented in the subject's medical records, the Case Report Form (See 11.2 Reporting and Recording of Data), and on the Study Completion Form (Appendix I).

Patients who withdraw will not be eligible to continue DPCP therapy with the current formulation because the drug is only available at this institution on a research basis.

6.2 Quality Assurance

To ensure accurate, complete, and reliable data, we will keep records of laboratory tests, clinical notes, and the subject's medical records in the subject's files as original source documents for the study.

The study may be audited by Human Subjects Research Compliance Program of the University of Minnesota its representatives and/or regulatory agencies at any time.

7. TREATMENT OF SUBJECTS

7.1 Enrollment

Patients will be recruited by advertising this study to faculty affiliated with the University of Minnesota Department of Dermatology and from the investigators' patients as well as through partnership with the National Alopecia Areata Foundation Clinical Trials Network.

Once screening is completed, subject eligibility is confirmed, and written consent obtained, any further data, test results, or assessments will be maintained within the patient's medical file and be available to the PI and co-investigators for use in patient care and data collection.

The site contact is:
Maria Hordinsky, MD
612-625-1493
Hordi001@umn.edu

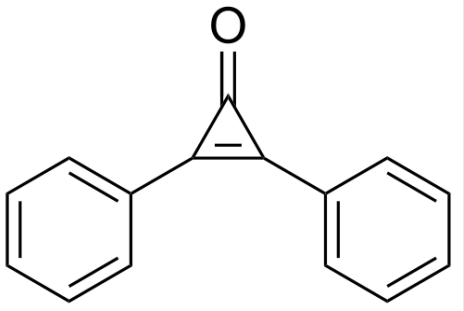
7.2 Formulation

The drug substance to be used for this study is:

Diphenylcyclopropenone (Diphencyprone, DPCP) Chemical name: 2,3-diphenylcycloprop2-en-1-one
Molecular formula: $C_{15}H_{10}O$
Molecular weight: 206.25

Chemical Abstracts (CAS) registry: #886-38-4
Purity: ≥99% pure crystalline powder. Contains ≤ 0.05% dibromodibenzylketone.
Commercial Source: Chiracon, Biotechnology Park, 14943 Luckenwalde, Germany
Contracted by: Carbosynth Ltd, 10 Langley Business Park, Beedon, Berkshire, RG20 8RY, UK

Figure 3.1 Structure of 2,3-diphenylcycloprop-2-en-1-one



Historically, DPCP has been formulated at various strength solutions in acetone. Typically, an initial sensitizing dose at a strength of 2-4% in acetone is administered at a distal site (inner arm or upper thigh) followed at two weeks by a series of weekly or bi-weekly challenge doses at strengths ranging from 0.001 – 0.4% in acetone at the site to be treated. Despite its efficacy, this volatile DPCP formulation has faced challenges, mostly due to lack of standardization of strength and to side effects caused by over-sensitization. The volatility results in lack of control of the actual DPCP concentration on the skin or scalp which most likely contributes to the risk of adverse reactions.

The DPCP Ointment formulation (Samcyprone) being used for the present study is manufactured by Ferndale Laboratories, Inc., Michigan under contract to Hapten Pharmaceuticals, LLC, Michigan. The drug is manufactured in compliance with FDA's current Good Manufacturing Practices (cGMP) requirements and has been granted Investigational New Drug approval by the FDA for indications including warts, melanoma, cutaneous metastasis and neurofibromatosis.

Sensitizing DPCP Ointment (0.4%) and Treatment DPCP Ointment (0.04%) are comprised of the excipients shown in the table below.

Ingredient	Sensitizing DPCP Ointment (0.4%), %w/w	Treatment DPCP Ointment (0.04%), %w/w
DPCP	0.4	0.04
Butylated hydroxytoluene (BHT) NF	0.1	0.1
Methylparaben NF	0.1	0.1
Propylparaben, NF	0.05	0.05
Cetyl esters wax, NF	10.0	10.0
White wax, NF	10.0	10.0
Polysorbate 80, NF	39.675	39.855
Isopropyl myristate, NF	39.675	39.855

7.3 Dosage, Administration and Storage
All subjects will be administered a sensitization dose of 0.05 mL 0.4% DPCP ointment formulation topically in the inner aspect of the upper right arm at Day -16, and 0.05 mL of four concentrations (0.04, 0.08, 0.12, 0.2%), prepared through dilutions in the ointment vehicle, topically on the inner aspect of the left thigh sequentially beginning with the lowest dose (0.04%). If no sensitization is obtained with the 0.04% concentration of DPCP, then the subject will return and the process will be repeated with the 0.08% concentration and, subsequently, the 0.12% and the 0.2% if necessary, depending on the reaction of the subject. The weakest strength that may cause a minimal reaction (DTH skin reaction score of 1+) after 72 hours will be chosen, and 0.75-1 g of that concentration will be applied to the scalp starting at Week 1 and administered subsequently twice a week for 18 weeks.

If the research participant has not been sensitized with the first application of 0.4% DPCP a second try will be made on the left inner arm. If, again, the patient has not been sensitized, no further attempts will be made to sensitize the patient and the patient will be disqualified from the study.

Missed Doses
Patients missing > 2 consecutive treatment visits or 20% of treatment visits in total will not be included in subsequent analysis and will not be eligible to continue receiving DPCP therapy through our center.

Storage

The test articles will be stored at Room Temperature (15-25 ^{o}C / 59-77 ^{o}F) and should not be placed in the freezer.

7.4 Treatment Schedule

(See Appendix A)

Pretreatment Period

The Demographics and Baseline Assessment Form (Appendix E) and Treatment History Form (Appendix F) will be completed at the Screening Visit (and updated at Baseline/Sensitization if necessary) to document the preceding 6 weeks before therapy.

Treatment Periods

Patients will undergo a single sensitization dose of 0.4% DPCP ointment, a dose 0.05 mL each of four concentrations (0.2, 0.12, 0.08, 0.04%) DPCP ointment to determine the treatment dose starting with the lowest concentration (0.045%) and the sequentially applying the others by increasing concentration until sensitization has been achieved, followed by a 18-week period that will involve twice weekly (+/- 2 days) treatment of the scalp with the chosen treatment concentration of DPCP ointment.

Follow-up Period

Patients will follow-up 72-96 hours after their final DPCP treatment application with a final blood and scalp biopsy specimen collection, analysis of safety issues, and hair loss examination. The participant must return 10 to 14 days after the biopsy collection for suture removal.

7.5 Concurrent Medications / Therapies

All current medications used by the subject throughout the study and all prior medications used to treat alopecia areata within 6 weeks prior to the Baseline/Sensitization Visit (Day -16) will be recorded in the subject's medical records, on the Demographics and Baseline Assessment Form (Appendix E) and the Treatment History Form (Appendix F).

7.6 Prohibited Concomitant Medication and Treatment

- Any other therapeutic investigational drugs
- Systemic treatment (e.g., Imuran, or biologics) with an immunomodulatory mechanism
- Topical immunomodulating agent
- Oral, intravenous, intralesional (on the scalp), inhaled, or topical (on the scalp) corticosteroids
- Systemic immunosuppressants
- Topical Immunotherapy
- PUVA
- Narrow band UVB
- Anthralin
- Minoxidil, topical or oral
- Systemic therapy for malignancy within the past five years (except for adequately treated SCC or BCC of the skin).

A subject who receives a prohibited concomitant therapy will be asked to withdraw from the study.

8. EFFICACY ANALYSIS

8.1 Primary Efficacy Endpoints

Our primary endpoint of efficacy will be assessed by measuring percentage hair loss at each visit. Percentage hair loss will be measured using the Severity of Alopecia Tool (SALT).[19-20] The percentage of hair loss is determined through a visual examination of four regions of the scalp; the percent of terminal hair loss visible in each area is recorded and the percentages are aggregated, using SALT scores.

The primary endpoints are as follows, ranked in order of importance:

1. The proportion of subjects who have $\geq$75% decrease in their SALT score ($SALT_{75}$), as compared to baseline at the completion of the study or at any time during the study.

2. The proportion of subjects who have $\geq$50% decrease in their SALT score ($SALT_{50}$), as compared to baseline at the completion of the study or at any time during the study.

8.2 Exploratory Endpoints

To identify scalp skin biomarker profiles that characterize a response to DPCP treatment, enabling the future potential for predicting individual responses to treatment and the likelihood of a sustained response with the opportunity to study blood samples in the future if needed.

Subject assessment of hair loss/growth with the subject hair loss assessment and investigator assessment of the total SALT score.

Gene expression and histologic analysis (immunohistochemistry) on skin biopsy specimens will be done. mRNA will be prepared from skin samples stored frozen in RNA Later solution. Expression of cytokine genes that define different polar sets of T-cells will be measured: interferon-gamma, IL-13, IL-17A, IL-22, IL-9, IL-32; other inflammation associated genes such as IL-12, IL-23, IL-1B, and IL-15 will also be measured. mRNAs for a series of negative immune regulatory genes, e.g., Foxp3, CTLA4, PD-1, etc. will be measured by RT-PCR. Re-initiation of hair growth will be assessed by measuring expression of mRNAs encoding hair-associated keratins and keratin-associated proteins. A subset of patients showing good responses of hair re-growth will be selected for whole transcriptome analysis. Pre and post-treatment lesional biopsies will have global mRNA profiles established using Affymetrix U133 2.0Plus arrays. From frozen samples in OCT blocks, frozen sections will be prepared that will be stained initially with antibodies to T-cells (CD3, CD8), dermal dendritic cells (CD11c), and the regulatory marker Foxp3. Once the gene expression analysis is completed, expression of negative immune regulatory proteins will be examined, as guided by the genes with highest up-regulation in post-treatment samples in patients with good hair regrowth.

9. ASSESSMENT OF SAFETY

Subject safety will be monitored by evaluating all AEs that occur following the first application of topical DPCP Ointment and at subsequent treatment visits.

If at any time, evidence of clinical and/or laboratory toxicity is noticed, the Investigator will take appropriate and prompt measures to elucidate and treat, if necessary, the etiology of the condition. The subjects will be followed until the condition resolves or becomes chronic or stable.

9.1 Adverse Events (AE)

An AE is defined as any untoward medical occurrence (e.g., sign, symptom, disease, syndrome, concurrent illness, abnormal laboratory finding) that emerges or worsens relative to pretreatment baseline during the study, regardless of the suspected cause. Untoward medical events that occur from the time the patient signs the informed consent form to the time the administration of the drug starts are not considered adverse events, and will be recorded under medical history.

Adverse events will be recorded as events that are newly appeared, increased in frequency, or worsened in severity following application of topical DPCP Ointment. AEs will be recorded from the first sensitization does application of the drug until the subject completes the study.

The incidence, severity and type of AEs for all treatment-related AEs (defined as definitely, probably, or possibly related to the study drug) will be summarized by body system and preferred term.

The occurrence of an AE will be determined based on observed or volunteered signs and symptoms, as well as changes in the subject's physical examination and laboratory results.

Patients will be asked at each visit to report any concurrent medications, therapies, illnesses, and adverse effects that take place during the study.

All AEs will be evaluated by the Investigator for the seriousness, severity, relationship to topical DPCP Ointment, and outcome. Participants will not be compensated monetarily for research-related injuries. Medical care related to any research-related injuries will be billed to a participant's insurance company.

A non-serious AE is defined as a change from baseline (pre-treatment) in a subject's medical health that is not life-threatening, does not require hospitalization, does not prolong a current hospitalization, and is not disabling. All non-serious AEs must be reported in the AE form (Appendix C) regardless of whether or not they are considered to be related to the study drug.

An AE occurring will be classified as serious if:

- It resulted in death (i.e., caused or led to death).
- It was life-threatening (i.e., placed subject at immediate risk of death; not, however, if it could hypothetically have caused death if it were more serious).
- It required or prolonged inpatient hospitalization (i.e., required at least a 24-hr inpatient hospitalization or prolonged a hospitalization beyond expected length of stay; hospitalization for elective medical/surgical procedures, scheduled treatments, or routine check-ups are not AEs by this criteria).

- It was disabling (i.e., resulted in substantial disruption of the subject's ability to carry out normal life functions).
- It resulted in a congenital anomaly/birth defect (i.e., adverse outcome in a child or fetus of a subject exposed to the molecule or study drug prior to conception or during pregnancy).
- It does not meet any of the above criteria for a serious AE but may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above.

Serious adverse events will be reported to the University of Minnesota IRB, to Hapten Pharmaceuticals within 24 hours, and to the FDA.

9.2 Baseline Medical/Preexisting Conditions

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

9.3 Procedures for Eliciting, Recording, and Reporting Adverse Events

AEs may be directly observed or elicited by the Investigator, and/or volunteered by the subjects.

In recording AEs, these guidelines will be observed:

- Whenever possible, medical terms will not be abbreviated
- If known, record the diagnosis (disease or syndrome) rather than component signs and symptoms unless they are unrelated to an encountered syndrome or disease.
- Adverse events occurring secondary to other events will be identified by primary cause.

9.4 Special Reporting Situations

Fatal or life-threatening events thought to be caused by topical treatment with the investigational drug should be immediately reported by telephone.

Death: Event resulting in death should be recorded and reported on the Adverse Event Report (See Appendix C) and the Serious Adverse Event Report (Appendix D).

Hospitalization: The illness leading to the surgical or diagnostic procedure will be recorded as the AE, not the procedure itself.

9.5 Post Treatment Follow-Up

All subjects will be followed for 2-3 weeks after the final application of DPCP Ointment. All protocol-defined AEs occurring in treatment periods should be fully evaluated by the Investigator and recorded on the appropriate Adverse Event Report (Appendix C) documented in the subjects' files.

Subjects who prematurely discontinue from the study will be encouraged to follow up 6 weeks following their last dose.

9.6 Post Adverse Event Follow-Up

All subjects with AEs will be followed until the event resolves or until participation in the study ends. Resolution of those deemed probably related to the administration of the DPCP Ointment will be followed until the events resolve or until the Investigator judges the event to be "chronic" or "stable."

9.7 Laboratory Tests

Blood specimens for hematology and chemistry will be collected at the Screening Visit, week 12, and at the Follow-up Visit (Week 18 +3d) for all subjects. The following tests will be performed:

Laboratory Tests	
Hematology	**Chemistry**
Hemoglobin Hematocrit Platelets WBC	Sodium Potassium Chloride Bicarbonate BUN/Urea Creatinine Total protein Albumin Alkaline phosphatase AST (SGOT) ALT (SGPT) Calcium CRP

Sample collection, handling, labeling, and shipping should be conducted following the instructions provided by the relevant certified laboratory and the applicable local regulations.

The investigator will review the subjects' laboratory reports in a timely manner. Screening laboratory abnormalities viewed as clinically significant by the investigator that do not exclude the subject from study participation will be documented in the subject's medical record. The investigator will complete an appropriate AE form for any new or worsening abnormal test results that are identified as clinically significant after baseline. If a subject experiences a cardiovascular or hematologic event (including laboratory abnormalities), Grade 2 or higher, the study drug will be discontinued.

AEs that may be associated with venipuncture include:

- Pain
- Bruising
- Bleeding at the puncture site
- Fainting
- Inflammation of the vein

10. STATISTICAL METHODS

10.1 Data to be Analyzed

Data will be analyzed for efficacy. For primary endpoint analysis, subject data will be analyzed up to the point in which compliance is maintained.

Subjects missing > 2 consecutive treatment sessions or 20% of treatment visits in total will not be included in subsequent analysis and will not be eligible to continue receiving DPCP therapy through our center.

10.2 Determination of Sample Size

Statistical considerations

The primary endpoints ranked below in order of importance are as follows:

1. The proportion of ***subjects*** who have ≥ 75% or ≥ 50% decrease in their SALT score ($SALT_{75}$ and $SALT_{50}$, respectively), as compared to baseline at the completion of the study or at any time during the study.
2. Subject assessment of total scalp hair regrowth or loss using a visual analog scale and Investigator assessment of the total SALT score.

The proportion of subjects with ≥75% or ≥50% decrease in the SALT score will be described using the fraction achieving the desired decrease and the associated confidence interval, computed using the exact Agresti-Coull method. To describe change in SALT score, we will use the average change and associated confidence interval.

For a total sample size of 10 participants we anticipate 6 or 8 providing the primary outcomes. For changes in the binary outcomes, i.e., proportions having ≥ 75% or ≥ 50% decrease in their SALT score, the 95% confidence interval for this proportion is as follows for each possible outcome:

- 6 participants providing data: 0 of 6 meeting the criterion gives an interval of 0 to 0.44; 1/6, 0 to 0.58; 2/6, 0.09 to 0.70; 3/6, 0.19 to 0.81; 4/6, 0.30 to 0.91; 5/6 0.42 to 1; and 6/6, 0.56 to 1.
- 8 participants providing data: 0/8, 0 to 0.37; 1/8, 0 to 0.49; 2/8, 0.06 to 0.60; 3/8, 0.13 to 0.70; 4/8, 0.22 to 0.78; 5/8 0.30 to 0.87; 6/8, 0.40 to 0.94; 7/8, 0.51 to 1; and 8/8, 0.63 to 1.

Exploratory Endpoints

The primary outcomes for the analysis are levels of T-cell and DC markers. We will compute confidence intervals and do paired t-tests for changes in these measures. These analyses will most likely use the logarithms of these measures, as is commonly done; the decision to use or not use logarithms will be based on preliminary analyses.

For change in a participant's rating of hair loss using a visual analog scale, the confidence interval width is 2 x [97.5th percentile of the t distribution] x the standard deviation of a single measurement / square-root (sample size). For 6 and 8 participants, the 97.5th percentiles are

2.57 and 2.36. Thus, the respective confidence interval widths are 2.1 standard deviations and 1.7 standard deviations. We currently lack information needed to supply a standard deviation describing variation between persons in the change in this VAS rating; one of the goals of this pilot study is to collect this information.

To estimate power, preliminary data were obtained from a pilot study (Fuentes-Duculan et al. Abstract, ISDS, November, 2014). Confidence interval widths are computed as above. Assuming the standard deviations are as reported in that abstract, and that the analyses are done on the common logarithm scale as in the abstract and analyses are done on the common-log scale, the confidence interval widths re-expressed on the original (i.e., non-logarithmically transformed) scale are 46-fold and 20-fold differences for 6 and 8 subjects respectively. For tests, again assuming the standard deviations are as reported in that aforementioned abstract, for 6 and 8 participants with data we have 80% power to detect a 14-fold change (using two-tailed alpha = 0.05).

For the analysis of various biopsy parameters, Dr. Krueger will be supported a statistician in his group.

10.3 Blinding

No blinding will occur or be necessary. All patients will receive the same treatment plan.

10.4 Subject Disposition

The disposition of subjects will describe the number of subjects enrolled, the number of subjects treated, and the number of subjects withdrawn prematurely from the study (including the reasons).

10.5 Handling of Dropouts and Missing Data

The last available SALT scores will be used to determine percent hair regrowth for dropouts and subjects who were lost to follow-up.

11. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

11.1 Overview

To decrease variability in this study any manual measurements such as SALT scoring should be done as consistently as possible, and be performed by the same individual performing the original assessments. Photographs will be taken to verify measurements, if needed.

11.2 Reporting and Recording of Data

Case Report Forms (CRF) will be developed and supplied by the University of Minnesota Department of Dermatology and will involve laboratory values and other assessments.

A CRF will be completed for each subject enrolled under this protocol. Information collected must be verifiable in the source documents. Source documents for this trial will include hospital records, clinic records, and any study-specific worksheets. Subject medical records will contain reference to the study title and assigned subject identification number. The signed consent form

must be filed with the subject's medical record.

CRF completion will be kept current to reflect subject status during the course of the trial and will be completed in a neat and legible manner to ensure accurate interpretation of data.

Study subjects will be identified by subject ID number, initials, and date of birth.

Results from the study will be available on clinicaltrials.gov. If a participant contacts us wanting additional information regarding results, we will provide them with this information.

12. ETHICAL CONSIDERATIONS

12.1 Informed Consent

The informed consent document will be used to explain in simple terms, before the subject is entered into the study, the risks and benefits to the subject. The informed consent document will contain a statement that consent is freely given, that the subject is aware of the risks and benefits of entering the study, and that the subject is free to withdraw from the study at any time.

The final IRB-approved documents must be provided to the University of Minnesota and Sponsors for regulatory purposes.

The Investigator is responsible for seeing that informed consent is obtained from each subject or legal representative and for obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the application of topical DPCP ointment formulation, or collection of serum samples and scalp biopsy specimens.

As used in this protocol, the term "informed consent" includes all consent given by subjects or their legal representatives.

12.2 Institutional Review

The University of Minnesota Institutional Review Board will approve the protocol and informed consent document and, if appropriate, agree to monitor the conduct of the study and agree to review it periodically. The Investigator will provide the IRB with documentation demonstrating that the IRB has approved the study before the study begins.

In addition, the Investigator will provide the following documentations:

- The IRB's annual re-approval of the protocol
- The IRB's approvals of any revisions to the informed consent document or amendments to the protocol

12.3 Ethical Considerations

The study will be conducted in accordance with the ethical principles stated in the most recent version of the Declaration of Helsinki or the applicable guidelines on Good Clinical Practice; whichever affords the greater protection to the individual.

12.4 Data Integrity Monitoring

A CTSI monitor will be used to review the study binders every 6 months while we are actively recruiting and enrolling participants. The objective of the monitor will be to ensure completion of forms including adverse event forms, reporting, and to assess for other protocol deviations. The monitor will be expected to write a report of major and minor protocol deviations and provide to the study staff.

15. FINANCING AND INSURANCE

Considerations will be provided by the University of Minnesota Department of Dermatology fund for Graduate Research and the National Alopecia Areata Foundation.

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Add reference

17. Appendices

An Open Label Study to Evaluate DPCP Ointment for the Treatment of Alopecia Areata

Appendices:

A. Flowchart of Study Procedures
B. Instructions for Severity of Alopecia Tool Assessments
C. Adverse Events Report Log
D. Serious Adverse Event Report Form
E. Demographics and Baseline Assessment
F. Treatment History Form
G. Physician Assessment of Alopecia
H. Subject Hair Loss Assessment
I. Sensitization Assessment
J. Study Completion Form

APPENDIX A: <u>Flowchart of Study Procedures</u>

Week(s)	**>= -2**		**-1**	**0**		**1**	**2**	**3**	**4**		**5**	**6**	**7**	**8**		**9**	**10**	**11**	**12**		**13**	**14**	**15**	**16**		**17**	**18**		**20**
Visit(s)	**1[a]**	**2**	**3[n]**	**4**	**5**	**6-7[b]**	**8-9**	**10-11**	**12**	**13**	**14-15**	**16-17**	**18-19**	**20**	**21**	**22-23**	**24-25**	**26-27**	**28**	**29**	**30-31**	**32-33**	**34-35**	**36**	**37**	**38-39**	**40**	**41**	**42**
Study Event	Screening	Baseline, Sensitization	Dose	Challenge	Treatment					Assessment	Treatment				Assessment	Treatment				Assessment	Treatment				Assessment	Treatment		Assessment	Suture Removal
Informed Consent	X																											
Review of Inclusion and Exclusion Criteria	X	X																										
Document Interim Medical Hx/Adverse Experiences		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X			X					X					X					X					X		X	
SALT Score	X	X	X							X					X					X					X		X	
Physician Assessment of Alopecia		X	X							X					X					X					X		X	
Subject Hair Loss Assessment[s]		X																									X	
Skin Biopsies[c]		X			X																						X[d]	
Phlebotomy[e]		X			X																						X[d]	
Suture Removal[f]				X		X[g]																						X[h]
Photography		X	X		X					X					X					X					X		X	
Drug Application		X[i]	X[j]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Assessment of Sensitization			X	X																								
Demographic & Baseline Assessment	X																											
Laboratory Tests[k,l]	X[k,l]																			X[l]							X[l]	
Pregnancy Test[m]	X	X								X					X					X					X		X	

[a] Screening visit can but does not have to occur concurrently with sensitization/baseline.
[b] Subjects treatment visits twice per week for 18 weeks after the challenge visit.

[c] Scalp skin biopsies will be performed at baseline, 3 days after the first treatment administration, at the time of hair regrowth (if >=50%) **OR** after the final treatment administration
[d] 3rd biopsy and phlebotomy collection will occur at Visit 41 **OR** a prior date at the time of regrowth (if >=50%)
[e] Whole blood, serum collection
[f] Sutures will be removed 10-14 days after the biopsy procedure
[g] Of Visits 6-7, suture removal will only take place on Visit 6
[h] Suture removal will occur at Visit 41 **OR** at a prior visit 10-14 days after the time of regrowth (if >=50%)
[i] Drug sensitization procedure
[j] Dose determination procedure
[k] Pre-treatment labs include TSH, HgB, and Vitamin D
[l] Safety monitoring labs include hemoglobin, hematocrit, platelets, WBC, sodium, potassium, chloride, bicarbonate, BUN/urea, total protein, albumin, alkaline phosphate, AST (SGOT), ALT (SGPT), calcium, CRP
[m] Women of child bearing potential only
[n] See following schedule of events for dose determination visit(s):

	Visit			
Procedure	**3[1]**	**3[2]**	**3[3]**	**3[4]**
Adverse events	X	X	X	X
Assessment of hair loss	X			
Photographs	X	X	X	X
DPCP ointment – 0.04%	X			
DPCP ointment – 0.08%		X		
DPCP ointment – 0.12%			X	
DPCP ointment – 0.2%				X

1: DPCP ointment 0.04% concentration is the starting concentration for all participants

2: DPCP ointment 0.08% will only be applied if participant does not have a strong enough response to the 0.005% concentration of DPCP. Thus, this visit will begin with an assessment of the sensitization reaction to the 0.005% dose, If there is not a strong enough response, 0.01% concentration DPCP will be applied to a different area of the inner upper left thigh in the same manner as the 0.005% dose. If the participant is a responder to the 0.005% DPCP, patient will proceed to the challenge visit (visit 4) as described above.

3: DPCP ointment 0.12% will only be applied if participant does not have a strong enough response to the 0.01% concentration of DPCP. Thus, this visit will begin with an assessment of the sensitization reaction to the 0.01% dose, If there is not a strong enough response, 0.05% concentration DPCP will be applied to a different area of the inner upper left thigh in the same manner as the 0.01% dose. If the participant is a responder to the 0.01% DPCP, patient will proceed to the challenge visit (visit 4) as described above.

4: DPCP ointment 0.2% will only be applied if participant does not have a strong enough response to the 0.05% concentration of DPCP. Thus, this visit will begin with an assessment of the sensitization reaction to the 0.05% dose, If there is not a strong enough response, 0.1% concentration DPCP will be applied to a different area of the inner upper left thigh in the same manner as the 0.05% dose. If the participant is a responder to the 0.05% DPCP, patient will proceed to the challenge visit (visit 4) as described above.

APPENDIX B: Instructions for Severity OF Alopecia Tool Assessments

Part I: The Severity of Alopecia Tool (SALT)

Overview

The SALT score is a global alopecia areata severity score based on scalp hair loss, as it provides a measure of the percentage of total scalp alopecia. The SALT score is calculated by visually examining four discrete areas of the scalp, measuring the percent of terminal hair loss in each area and then totaling the results. (See below)

The SALT score will be measured to the nearest 1%, following the instructions below, at screening to determine study eligibility and at all following appointments as one measure of the extent of hair loss.

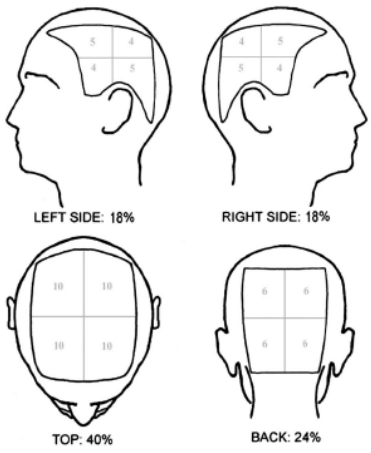
Evaluator Requirements

Dr. Maria Hordinsky and Dr. Ronda Farah will be the primary evaluators and will be performing all percentage hair loss assessments for all individual subjects throughout the trial.

Determining SALT score

To determine the SALT score, four main scalp areas are assessed: the left side, the right side, the top and the back. These areas correspond, respectively, to 18%, 18%, 40%, and 24% of the total scalp surface area (see Figure A, "Visual Aid for Determining SALT Score").

Figure A: Visual Aid for Determining SALT Score



Reference: Figure 1. "Alopecia Areata Investigational Assessment Guidelines –Part II" (Olsen, et. al. 2004).

Determining SALT Score (cont)

The subject's SALT score will be determined as follows:

1. For each of the four main scalp surface areas, the percentage of missing hair will be documented in Row 1.
2. The percentages recorded in Row 1 will be multiplied by the percentages in Row 2 of the same column (Areas as % of Total Scalp Area). The results will be entered in the appropriate columns of Row 3.
3. The four results in Row 3 will be summed to get the percentage hair loss (Row 4).

Sample Worksheet for Determining SALT Score

Row	Parameter	Left	Right	Top	Back
1	Enter % Hair loss for Each Scalp Surface Area				
2	Area as % of Total Scalp Surface	X 0.18	X 0.18	X0.4	X 0.24
3	Multiply Row 1 x Row 2				
4	**Total % Hair Loss** (Sum the Products from Row 3)				

50% scalp hair regrowth based on SALT score can be expressed as $SALT_{50}$
75% scalp hair regrowth based on SALT score can be expressed as $SALT_{25}$

APPENDIX C: Adverse Event Report Log

ADVERSE EVENTS

Has the Subject experienced any Adverse Events since receiving the study treatment? ❒ **Yes** ❒ **No**

Description of Event	**Serious Y/N***	**DLT Y/N**	**Related to Treatment Y/N**	**Severity****	**Onset Date** (dd/mmm/yyyy)	**Resolution Date or Ongoing** (dd/mmm/yyyy)	**Relationship To Study Drug‡**	**Action Taken Regarding Study Drug#**	**Action Taken to Treat Adverse Event ^ (if 3,4 or 5, specify)**	**Outcome^^**

***If yes, complete an SAE CRF.**

****Severity**	**‡Relationship**	**#Action Taken Regarding Study Drug**	**^Action Taken to Treat Adverse Event**	**^^Outcome**
1 Mild 2 Moderate 3 Severe 4 Life-Threatening	1 Definite 2 Probable 3 Possible 4 Not Related	1 None – N/A 2 Dose Discontinued	1 None 2 Concomitant Medication 3 Blood Product 4 Medical or Surgical Procedure 5 Other Intervention	1 Continuing 2 Resolved 3 Lost to Follow-up 4 Unknown 5 Resolved with sequelae 6 Fatal

Investigator's Signature:_____ **Date** ☐/☐/**20**☐ (dd/mmm/yyyy)

APPENDIX D: Serious Adverse Event Report

(Fax completed form to 888-929-9821)

Protocol No.	Site No.	Principal Investigator
Date Form Completed	☐ Initial Report ☐ Follow-Up Report #_____ ☐ Final Report	
Reporter Name	Title	Telephone Number

Serious Adverse Event	**Onset Date**	**Resolution Date** ☐ or Ongoing

Subject ID	Gender	Race	Age	Height	Weight

Study Design

☐ Open-Label ☐ Double-Blind ☐ Other (specify): Single Blind

Investigational Drug DPCP (Samcyprone™)	Dose Date	Dose	Route of Administration Topical
Prescription #			

Criteria for Seriousness (check all that apply)	**Action Taken For Event**
☐ Death	☐ None
☐ Life Threatening	☐ Investigational Drug Dose Discontinued
☐ Inpatient Hospitalization *	☐ Required Therapy (specify on page 2)
☐ Prolongation of Existing Hospitalization *	
☐ Persistent or Significant Disability / Incapacity	
☐ Congenital Anomaly / Birth Defect	
☐ Required Intervention to Prevent Permanent Damage	
☐ Other (specify) _____	

*** Dates of Hospitalization**	**Admission Date**	**Discharge Date**

Event Status at Time of This Report	Grade of Event (CTCAE)	Investigator Assessment of Relationship to Investigational Drug	Was Investigational Drug Resumed?
☐ Recovered/Resolved ☐ Died	☐ 1 ☐ 4	☐ Related	☐ Yes
☐ Not Recovered/Resolved ☐ Unknown	☐ 2 ☐ 5	☐ Not Related	☐ No
☐ Recovered/Resolved with Sequelae	☐ 3		☐ N/A

Protocol No.	Site No.	Subject ID

Known Medications at the Time of the Event

1) **Exclude those used to treat the event**
2) **A copy of the Concomitant Medication CRF may be attached in place of completing this section**

Drug (brand name if known)	Total Daily Dose	Units	Route	Indication	Start Date	Stop Date

Medications Used to Treat the Event

Drug (brand name if known)	Total Daily Dose	Units	Route	Indication	Start Date	Stop Date

Relevant Tests / Laboratory Data

1) **A copy of the lab reports may be attached in place of completing this section**

Clinical Laboratory Test	Normal Range	Value / Date	Value / Date	Value / Date

Relevant Background Information

1) **Include medical history and demographic data, including pre-existing medical conditions**

Protocol No.	Site No.	Subject ID

Narrative

1) **Please include any other important information that is not included on the form.**
2) **Supplemental information or supporting documents may be attached.**

Investigator's Signature	Date

Please Email copy of this completed form to Hapten Pharmaceuticals: michaelburnsus@aol.com

APPENDIX E: Demographics & Baseline Assessment

(Visit 1, Date of visit: ____/____/____)

1. Subject Initials: ___ ___ ___ 2. Subject study number (ID #): ___ ___ ___

3. Date of Birth: ___/ ___/___ 4. Gender: ☐ Male ☐ Female

5. Height? __ ft. __ in. / __m. __ cm. 6. Weight? _____lb. / _____ kg.

7. Blood Pressure: _____ mmHg 8. Pulse _____ bpm

5. If female, urine pregnancy test: ☐ Positive ☐ Negative

6. Ethnicity**:** ☐ Hispanic/Latino ☐ Not Hispanic/Latino

7. Race: ☐ Caucasian ☐Black/African American ☐ American Indian
☐ Asian ☐ Pacific Islander ☐ Multi-racial ☐
Other: _____ ☐ Refused

8. Fitzpatrick Skin Type:
☐ I – Always burns, never tans. ☐ IV – Rarely burns, tans well
☐ II – Always burns, rarely tans ☐ V – Rarely burns, always tans
☐ III – Sometimes burns, gradually tans ☐ VI – Never burns, always tans

9. Significant or Recent Medical History:

Condition/Illness/Surgery/Hospitalizations	**Condition Status (check if condition is ongoing or resolved)**	
	Ongoing	**Resolved**
☐ Subjects denies any significant medical history, hospitalizations or surgeries		

10. Medications Used in Past 6 weeks (Including Current):

Medication	**Dosage**	**Frequency**	**Indication**	**Date Started**
☐ Subject denies any medication or OTC regimen				

APPENDIX F: Treatment History Form

(Visit 1, Date of visit: ____/____/____)
Of these commonly used therapies for alopecia areata, check all that you use now and have used in the past in the following table:

Therapies:	***Current Treatment* And Dose**	***Past Therapies used***	***Check if Hair Re-growth achieved***	***Any Side effects of therapy?***
None	❑_____	❑	❑	
Systemic Steroids "cortisone" Oral (prednisone, medrol) Intramuscular	 ❑_____ ❑_____	 ❑ ❑	 ❑ ❑	 ❑No ❑Yes _____ ❑No ❑Yes _____
Intralesional steroids	❑	❑	❑	❑No ❑Yes
Topical steroids Gel or mousse Lotion, Cream or ointment	 ❑_____ ❑_____	 ❑ ❑	 ❑ ❑	 ❑No ❑Yes _____ ❑No ❑Yes _____
Topical Immunotherapy FK506 (protopic, tacrolimus) Cyclosporine Aldara Elidel Other	 ❑_____ ❑_____ ❑_____ ❑_____ ❑_____	 ❑ ❑ ❑ ❑ ❑	 ❑ ❑ ❑ ❑ ❑	 ❑No ❑Yes _____ ❑No ❑Yes _____ ❑No ❑Yes _____ ❑No ❑Yes _____ ❑No ❑Yes _____
Oral Immunotherapy FK506 (tacrolimus) Cyclosporin Other	 ❑_____ ❑_____ ❑_____	 ❑ ❑ ❑	 ❑ ❑ ❑	 ❑No ❑Yes _____ ❑No ❑Yes _____ ❑No ❑Yes _____
Phototherapy NB UVB PUVA – systemic PUVA – topical Excimer laser LLLT	 ❑_____ ❑_____ ❑_____ ❑_____	 ❑ ❑ ❑ ❑	 ❑ ❑ ❑ ❑	 ❑No ❑Yes _____ ❑No ❑Yes _____ ❑No ❑Yes _____ ❑No ❑Yes _____
Contact Sensitizer DNCB Dinitrochlorobenzene Diphencyprone Squaric acid dibutylester	 ❑_____ ❑_____ ❑_____	 ❑ ❑ ❑	 ❑ ❑ ❑	 ❑No ❑Yes _____ ❑No ❑Yes _____ ❑No❑ Yes _____
Retinoids (Vitamin A) Topical _____ Oral _____	 ❑_____ ❑_____	 ❑ ❑	 ❑ ❑	 ❑No ❑Yes _____ ❑No ❑Yes _____
Topical Rogaine/Minoxidil 2% 5% Topical Anthraline/Dithrocream	 ❑_____ ❑_____ ❑_____	 ❑ ❑ ❑	 ❑ ❑ ❑	 ❑No ❑Yes _____ ❑No ❑Yes _____ ❑No ❑Yes _____
Other_____ Other_____ Other_____	❑_____ ❑_____ ❑_____	❑ ❑ ❑	❑ ❑ ❑	❑No ❑Yes _____ ❑No ❑Yes _____ ❑No ❑Yes _____

APPENDIX G: <u>Physician Assessment of Alopecia</u>

The Physician Assessment of Alopecia includes body hair assessment, nail involvement assessment, duration of current episode of scalp hair loss pattern of scalp hair loss, and SALT scoring assessment.

The proportion of scalp involvement is determined by dividing the scalp into 4 quadrants and estimating the scalp surface that all the alopecic areas would occupy if placed together. The following groups will be used:

- ❑ S0 (no scalp hair loss)
- ❑ S1 (<=25% hair loss)
- ❑ S2 (26-50% hair loss)
- ❑ S3 (51-75% hair loss)
- ❑ S4 (76-99% hair loss)
- ❑ S5 (100% hair loss)

Other areas of alopecia or involvement by alopecia areata may be noted:

B. Extent of Body Hair Loss on the exam today:

- ❑ B0 (no body hair loss)
- ❑ B2 (100% body hair loss)
- ❑ B1 (some body hair loss):
  - ❑ Eyelashes
  - ❑ Eyebrows
  - ❑ Beard
  - ❑ Axilla
  - ❑ Arms
  - ❑ Trunk
  - ❑ Pubic area
  - ❑ Legs

N. Degree of Nail Involvement:

- ❑ N_0 (no nail involvement)
- ❑ N_1 (some nail involvement)
- ❑ N_2 (all nails) 20 nail dystrophy

Area: ❑ Fingernails ❑ Toenails ❑ Both

Type: ❑ Pitting ❑ Onycholysis ❑ Onychomycosis ❑ Dystrophy

Global assessment: overall improvement. This takes into account extent and density of regrowth by the SALT scoring system. First note regrowth, then categorize by:

- ❑ A0 = no change or further loss
- ❑ A1 = 1-24% regrowth
- ❑ A2 = 25-49% regrowth
- ❑ A3 = 50-74% regrowth
- ❑ A4 = 75-99% regrowth
- ❑ A5 = 100% regrowth

APPENDIX H: Subject Hair Loss Assessment

Visit Date: ___/___/___ Week: _____

Instructions: To be completed by the subject. Grade your perception of the extent of your hair loss for your scalp and the rest of your body. Check only one box per row.

Region	Severe	Moderate to Severe	Moderate	Mild to Moderate	Mild	Trace	None
Scalp							
Non-Scalp							

Subject Signature: _____ Date: _____

APPENDIX I: Sensitization Assessment

Part I: Sensitizing Dose

Immunotherapeutic Response Skin Reaction Scale

Score	Observation
0	No skin reaction
1+	Erythema only
2+	Erythema and cutaneous induration
3+	Erythema, papules and small vesicles
4+	Large vesicles, bullae, and severe local reaction besides erythema

***First Attempt*: (Visit ___, Date: ___/___/___)**

1. Did skin sensitization occur? ☐ Yes ☐ No

SKIN REACTION SCORE	0	1+	2+	3+	4+

If **no**, was another sensitization dose applied? ☐ Yes ☐ No

2. Did the subject experience any adverse reactions to the sensitizing dose?
   ☐ Yes ☐ No
   * If **yes**, note reaction and file AE form: _____

_____

_____

***Second Attempt*: (Visit ___, Date: ___/___/___)**

1. Did skin sensitization occur? ☐ Yes ☐ No

SKIN REACTION SCORE	0	1+	2+	3+	4+

2. Did the subject experience any adverse reactions to the sensitizing dose?
   ☐ Yes ☐ No
   * If **yes**, note reaction and file AE form: _____

_____

_____

Part II: Dose Determination

(Visit ___, Date: ___/___/___)

1. Complete the grid below with the DTH Reaction score for each region of the dose application matrix.

Concentration	SKIN REACTION SCORE
.2%	
.12%	
.08%	
.04%	

Immunotherapeutic Response Skin Reaction Scale

Score	Observation
0	No skin reaction
1+	Erythema only
2+	Erythema and cutaneous induration
3+	Erythema, papules and small vesicles
4+	Large vesicles, bullae, and severe local reaction besides erythema

Determined Dose: _____%

2. Did the subject experience any adverse reactions to the dose determination drug application?

☐ Yes ☐ No

* If **yes**, note reaction and file AE form: _____

_____

_____

3. Was first treatment dose applied? ☐ Yes ☐ No

APPENDIX J: Study Completion Form

Comments:

Please use this space to summarize any issues not covered in the previous forms or to explain procedural deviations:

Did subject complete the study? ❑ Yes ❑ No If No, Please complete the following: Subject Dropped/Withdrawn at Visit #: _____ Date subject dropped/withdrawn : ___/___/___
Check reason for subject withdrawal or termination: ❑ Sensitization failure: _____ ❑ Adverse Event: _____ ❑ Subject non-compliance: _____ ❑ Subject withdrawal of Consent: _____ ❑ Subject lost to follow-up: _____ ❑ Investigator or Sponsor request to withdraw subject: _____ ❑ Other: _____
Investigator Signature _____ Date: ____/_____/____