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Safety and Efficacy of Lenabasum, a Cannabinoid Receptor Type 2 Agonist, in Dermatomyositis Patients with Refractory Skin Disease

A Randomized Clinical Trial

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ABSTRACT

Background: Treatment options are limited for skin disease in dermatomyositis (DM). Lenabasum is a cannabinoid receptor type 2 agonist that triggers resolution of inflammation.

Objective: Evaluate the safety and efficacy of lenabasum in patients with refractory cutaneous DM.

Design: This study was a single-center, double-blind, randomized, placebo-controlled Phase 2 study conducted from July 2015 to August 2017.

Population: Subjects ≥ 18 years of age with at least moderately active DM skin activity by Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI) activity ≥ 14 and failure or intolerance to hydroxychloroquine.

Intervention: Participants received lenabasum 20 mg daily for 28 days, then 20 mg BID for 56 days, or placebo.

Main outcomes and measures: The primary outcome was change in CDASI activity. Safety and other secondary efficacy assessments were performed to Day 113.

Results: 22 subjects were randomized to lenabasum (n=11) or placebo (n=11). No serious or severe adverse events (AEs) were related to lenabasum, and no participants discontinued the study. The adjusted least squares mean for CDASI activity decreased more for lenabasum, and the difference was significant at Day 113 (least squares mean [SE] difference -6.5 [3.1], p = 0.038). Numerically greater improvements were seen in multiple secondary efficacy outcomes and biomarkers with lenabasum.

Conclusion: Lenabasum treatment was well tolerated and was associated with greater improvement in CDASI activity and multiple efficacy outcomes.

Trial registration: ClinicalTrials.gov Identifier: NCT02466243

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INTRODUCTION

Dermatomyositis (DM) is a rare, systemic autoimmune disease with distinctive cutaneous features that are frequently accompanied by muscle inflammation, interstitial lung disease, and malignancy (Iaccarino et al., 2014). Approximately 80% of DM patients experience recurrent episodes of active skin disease (Chansky et al., 2017, Marie, 2012). Active skin disease can be disfiguring, cause pruritus and photosensitivity, and can impair quality of life (Goreshi et al., 2011). Active skin disease may remain refractory to antimalarials and immunosuppressives, and thus a need exists for more effective and safer therapeutic options than current standard-of-care (Ang and Werth, 2005, Anyanwu et al., 2017, Pelle and Callen, 2002).

The erythema, scale, erosions, and ulcerations that are manifestations of active skin disease in DM are caused by skin inflammation, with increased levels of inflammatory cells including $CD4^+$ T cells, activated macrophages, dendritic cells, and inflammatory cytokines found in skin biopsies (Patel et al., 2021, Wong et al., 2012). Tumor necrosis factor alpha (TNFα), interleukin-1 (IL-1), and interferons (IFNs) appear to contribute to the pathogenesis of DM (Nabatian et al., 2012, Wong et al., 2012). Pruritus, a distinct symptom in patients with DM, correlates with cutaneous activity and could be driven by increased skin IL-31 (Kim et al., 2018).

The endocannabinoid system can modulate inflammatory and fibrotic responses (Rom and Persidsky, 2013). Cannabinoid receptor type 2 (CB2) is mainly expressed on activated immune cells (Rom and Persidsky, 2013) and plays a role in returning an activated immune response to homeostasis through activation of the physiologic process of resolution of inflammation (Serhan et al., 2008). This leads to reduced autoantibody production in animal models of autoimmune

diseases (Servettaz et al., 2010) and reduced levels of Type I IFNs, IL-1, IL-6, TNFα, and IL-17 produced by peripheral blood mononuclear cells (PBMCs) (Kong et al., 2014, Kozela et al., 2013, Parker et al., 2008, Selvi et al., 2008, Zurier et al., 2003).

Lenabasum is a selective CB2 agonist with a greater affinity and functional activity for CB2 than for cannabinoid receptor type 1 (CB1) (Tepper et al., 2014) and limited penetration into the central nervous system, to reduce psychoactive adverse events (AEs) caused by CB1 activation in the brain (Burstein et al., 1992, Loev et al., 1973, Tepper et al., 2014). CB2 is mainly expressed on immune cells and, in DM skin, has highest expression on dendritic cells and B cells (Maddukuri et al., 2021, Motwani et al., 2018). CB2 agonists increase expression of pro-resolving lipoxins and resolvins, while decreasing production of inflammatory prostaglandins and leukotrienes, cytokines, chemokines, adhesion molecules, and polarizing macrophages (Levy et al., 2001, Liu et al., 2003, Motwani et al., 2018, O'sullivan, 2016, Serhan et al., 2008, Zurier et al., 2009). Overall, lenabasum facilitates the resolution of several inflammatory responses.

This Phase 2 study evaluated the safety, tolerability, and efficacy of lenabasum in participants with DM with refractory, moderate-to-severely active skin disease.

RESULTS

Participant disposition

Twenty-nine DM participants were screened, and 22 adult participants were randomized to receive either lenabasum or placebo. All participants in each cohort completed the dosing and were included in safety and efficacy analyses (**Figure 1**).

Baseline demographics and disease characteristics

Demographic (gender, age, body mass index, race), disease characteristic, and baseline immunosuppressive treatments were similar between groups (**Table 1, Table 2**). Overall, 86.4% of participants were taking concomitant immunosuppressant medication. All participants had CDASI activity score ≥ 20 at baseline, with mean CDASI activity scores in the severe range (CDASI activity > 26) (lenabasum arm = 33.3 ± 9.7; placebo arm = 35.8 ± 7.8) (Ahmed et al., 2020), indicating significant skin disease despite the use of immunosuppressant drugs. Baseline scores for the Skindex-29 Symptoms, Emotions and Functioning domains were in the moderate to severe range (Nijsten et al., 2006), indicating significant impairment in the quality of life of enrolled participants (**Table 2**).

Efficacy outcomes

There was a trend for the change from baseline CDASI to be greater in lenabasum versus placebo starting at Day 43, 2 weeks after the dose of lenabasum was increased from 20 mg daily to 20 mg twice daily. The LS mean differences (95% confidence interval) and P-values were: Day 43 [-4.5 (-11.5, 0.8), P = 0.0857]; Day 57 [-6.0 (-12.1, 0.1), P = 0.0536]; Day 85 [-3.4 (-9.5, 2.7), P = 0.2761]; and Day 113 [-6.5 (-12.6, -0.4), P = 0.0382] (**Figure 2**). At the end of the study in the lenabasum group, 2 (18.2%) participants had CDASI activity scores < 14, 3 (27.3%) had scores between 14 and 19, and 6 (54.5%) had scores ≥ 20, whereas all participants in the placebo group ended the study with CDASI activity score ≥ 20, P = 0.0351, 2-sided exact test. At the same time, improvements (reduction) in CDASI of at least -5 points were observed in 7 lenabasum-treated participants versus 5 placebo-treated participant (not significant), and

improvement of at least 40% of baseline were seen in 5 lenabasum-treated participants versus no placebo-treated participants (p = 0.0235, exact test, post-hoc analysis).

Evaluation of secondary efficacy outcomes showed numerical improvements that were greater in the lenabasum group than placebo group at multiple visits starting at Day 57 and shown for the end of study Day 113, although the majority of these outcomes did not reach statistical significance (**Table 2)**. All participants had skin-predominant disease and had normal, maximal resistance on muscle testing upon trial entry and throughout the study.

Safety

All but 1 participant in the placebo group had at least 1 AE. No serious or severe AEs and no study drug discontinuations or study discontinuations for AEs or any reason occurred in either treatment group. Four participants in each group (36.4%) had AEs with maximum severity of moderate, all other participants had AEs with maximum severity of mild.

The most common AEs in the study and numbers of particpants in the lenabasum versus placebo groups with those AEs, respectively, were mild dizziness (5 versus 2), mild or moderate fatigue (3 versus 3), mild dry mouth (4 versus 2), and mild diarrhea (3 versus 1). The dizziness AEs all recovered or resolved, and all but 1 recovered or resolved in no more than 5 days. Mild psychiatric AEs were observed in 3 lenabasum-treated participants (1 participant each with daydreaming, abnormal dreams, and 1 subject with agitation, depressed mood, and irritability) and 2 placebo-treated participants (1 participants each with anxiety, insomnia). No participant reported euphoria. Overall these side effects were short-lived and spontaneously resolved

without medical intervention. There were no interruptions to intake of lenabasum/placebo during the trial. Changes from baseline in vital signs, non-skin-related findings on physical examination, and laboratory tests were limited and similar to those in placebo group.

Biomarker outcomes

Treatment with lenabasum resulted in statistically significant reductions ($p \leq 0.05$) from baseline in IFN-β and IFN-γ levels quantified from signal intensity in immunostained skin sections at Day 85 (**Table 3**). Additional biomarker results were not significant (**Table 3**). Changes in CD4+ T cells correlated with change in CDASI activity score in both lenabasum-treated subjects ($r = 0.80$, $p = 0.03$) and placebo-treated subjects ($r = 0.64$, $p = 0.25$). No significant changes from baseline were observed in IL-13, IL-33, or PPAR-γ protein levels or CD8+, CD11c+ or mast cell numbers in either treatment group. When patient biopsies were grouped into disease responders (those who had an improvement of CDASI greater than 5 (Anyanwu et al., 2015) and non-responders, responders had a significant reduction in IL-31 % area. Further grouping the patients into itch responders (those who had improvement in Skindex-29 Symptoms itch score) and non-responders, there was a significant reduction in mean intensity of IL-31 among the responders (**Figure 3**).

Skin biopsies obtained at baseline (Day 1) showed overall no significant difference in gene expression of cytokines IFN-β, IFN-γ, IL-31, or IL-4 between the 2 treatment groups (**Table 3**). At Day 85, the decrease from baseline in gene expression of these cytokines was numerically greater in the lenabasum group, compared to placebo, and statistically significant for IFN-β ($p = 0.0303$) and IFN-γ gene expression levels ($p = 0.0480$). There was no decrease in PBMC mRNA

expression for IFN-β, IFN-γ, IL-31, or IL-4 in the lenabasum group, compared to the placebo group (data not shown).

DISCUSSION

This phase 2 trial is tested the potential beneficial effects of activating the endocannabinoid system in patients with DM, using lenabasum, a preferential CB2 agonist.

The endocannabinoid system has 2 principal receptors: CB1, mainly expressed on cells in the nervous systems, and CB2, mainly expressed on activated immune cells (Munro et al., 1993). Cell surface expression of CB2 is up-regulated on multiple immune cell types during immune activation and returns to baseline low levels when the immune response resolves (Carayon et al., 1998). Activation of CB2, class A serpentine receptors that couple primarily to $G_{i/o}$ proteins, modulates multiple signaling pathways leading to regulation of multiple cell functions (Dhopeshwarkar and Mackie, 2014, 2016). In response to challenges that activate innate immune responses, CB2 knockout mice have more severe inflammation and fibrosis mediated through increases in nuclear factor kappa beta activation, production of inflammatory adhesion molecules, chemokines, cytokines, and superoxide generating enzymes, recruitment of immune cells, and reduced apoptosis of lymphocytes (Deveaux et al., 2009, Engel et al., 2010, Mukhopadhyay et al., 2010, Servettaz et al., 2010, Trebicka et al., 2011). In humans, genetic polymorphisms in the CB2 gene that lead to dysfunction of CB2 are associated with more severe inflammation (Rossi et al., 2012) and increased risk for autoimmunity (Bellini et al., 2015, Sipe et al., 2005). Importantly, in a model of acute inflammation in the skin of healthy human volunteers, lenabasum 20 mg BID had a magnitude of effect similar to prednisolone 15 mg daily (Motwani et al., 2018). As further rationale for testing effects of lenabasum in DM, lenabasum

suppressed production of IFNα, IFNγ, TNFα, IL-1β, and IL-31 from *in vitro* lipopolysaccharide-stimulated or CpG-stimulated PBMC from patients with DM (Robinson et al., 2017). In DM skin, CB2R is upregulated on dendritic cells, B cells, T cells, and macrophages, with much less CB2R present on PBMCS (Maddukuri et al., 2022).

This Phase 2 study was a randomized, placebo-controlled clinical trial of lenabasum in participants with DM with moderate to severe skin involvement as well as moderate to severe symptoms by several patient-reported outcomes despite standard treatments. All subjects were on a stable dose of background DM and non-DM therapy during screening and throughout the trial. Participants had skin-predominant disease and had normal, maximal resistance on muscle testing throughout the study.

In this study, there was a trend for participants treated with lenabasum to have improved CDASI scores compared to placebo beginning on Day 43, two weeks after the lenabasum dose was increased from 20 mg daily to 20 mg BID. The differences reached statistical significance four weeks after the patients discontinued lenabasum (on Day 113) suggesting that the modulation of the inflammatory response by lenabasum continued beyond its last dose. The lenabasum-treated group also had greater improvement in physician-reported and patient-reported VAS scores of overall disease activity and in patient-reported VAS scores for global skin disease and pain. In addition, participants treated with lenabasum reported greater improvement in the Skindex-29 symptoms score, concern about hair loss, and PROMIS-29 physical function and pain interference domains. Several other secondary efficacy outcomes suggested improvement at Day 113 compared to baseline, although these did not reach statistical significance.

Itch is a highly prevalent symptom in DM (Hundley et al., 2006). About half of DM patients have moderate to severe itch, which contributes to a significant impairment of their quality of life (Kim et al., 2018). IL-31, a protein that is implicated in pruritic skin conditions, has been shown previously to be elevated in lesional DM skin and to correlate with cutaneous activity (Kim et al., 2018). Lenabasum improved the VAS itch score of patients at Day 113, although the difference was not statistically significant. Immunohistochemistry of lesional skin biopsies showed that improvement in Skindex Symptoms scores, a more objective measure of itch, was associated with a significant reduction in IL-31 intensity in lesional skin, whereas improvement in cutaneous disease activity was associated with a significant reduction in IL-31 area (**Figure 3**). Since a majority of the responders were on lenabasum, this decrease in IL-31 protein expression could explain the trend towards improvement of pruritus in DM subjects on active treatment. On the other hand, cannabinoids have also been recently found to activate other receptors such as the ligand-gated, non-selective ion channel transient receptor potential cation channel sub-family V member 1 or TRPV-1 that are found on central and peripheral nerve cells as well as immune cells (Muller et al., 2019). Interestingly, the activation of TRPV on these cells have been shown to decrease the release of certain neuropeptides implicated in itch (Bíró et al., 2007).

This study showed that lenabasum was generally safe and well-tolerated, with no severe or serious AEs related to lenabasum and no study drug or study discontinuation due to lenabasum. Adverse events consistent with cannabinoid class effects that were seen more frequently in the lenabasum-treated group than the placebo-treated group included mild dizziness and mild dry mouth (Pertwee, 2009). These AEs were also reported with lenabasum in a trial in diffuse cutaneous systemic sclerosis (Spiera et al., 2020). There were no major differences in psychiatric

AEs in the lenabasum-treated and placebo-treated groups to suggest psychoactivity of lenabasum in this study.

Comparisons of skin biopsies before and after treatment showed biologic activity of lenabasum, with significant reduction in certain cytokines relevant to DM pathogenesis in participants treated with lenabasum relative to placebo. Participants on lenabasum had a downward trend in the CD4+ T cell population, which correlated with the decrease in the CDASI activity score. Lenabasum also had a significant effect on IFN-β gene expression in the skin. This result is consistent with a recent report which demonstrated that lenabasum reduced type I interferon levels produced *in vitro* by PBMCs from DM patients (Robinson et al., 2017). Lenabasum reduced IFN-γ protein and mRNA expression as well. The decrease in IL-31 protein expression, a cytokine associated with itch of the skin, with lenabasum treatment may have contributed to improvements in Skindex-29 symptom and VAS itch scores.

CB2 expression in DM skin is upregulated on plasmacytoid dendritic cells (pDCs), myeloid dendritic cells (mDCs) and B cells in DM skin compared to skin of healthy controls (Maddukuri et al., 2020). Plasmacytoid DCs and mDCs play an important role in DM inflammation, with mDCs much more prevalent in skin and having a more significant role in IFN-β production in skin, which could further explain the CB2-induced immunomodulation by lenabasum in DM (Chen et al., 2021, Patel et al., 2021).

Strengths of this study include use of a randomized and double-blind trial design and similar baseline characteristics of participants in both treatment groups, focus on skin-predominant DM

and treatment of active skin disease as a window to show potential efficacy in a small short study (Cordeiro and Isenberg, 2006), and exclusion of patients with active muscle disease requiring high dose steroids that could confound treatment efficacy. Another strength of this study was selection of CDASI, a validated and sensitive measure for skin activity in DM, as the primary efficacy outcome (Aggarwal et al., 2020, Anyanwu et al., 2015, Klein et al., 2008, Paik et al., 2020, Tiao et al., 2017, Yassaee et al., 2010). The CDASI activity score correlates well with serum IFNβ levels in DM patients (Huard et al., 2017) and quality-of-life measures (Robinson et al., 2015). Finally, an important strength of this study was the inclusion of biomarker studies in the target organ, to support findings suggesting clinical benefit.

Limitations of this study include a small sample size, short study duration, use of a single center, lack of correction for multiple comparisons, and lack of generalizability to other systemic aspects of DM.

In conclusion, in aggregate, improvement in physician-assessed, patient-reported, and biomarker outcomes suggest clinical benefit of lenabasum in DM patients with refractory skin disease occurred in this study. Lenabasum demonstrated a favorable safety and tolerability with no severe or serious AEs or study discontinuations. The degree and consistency of clinical benefit, combined with a favorable safety prolife, warrant further evaluation of lenabasum in DM.

MATERIALS AND METHODS

Protocol

This was a randomized, double-bind, placebo-controlled, single-center, Phase 2 trial conducted at a single tertiary referral center for autoimmune connective tissue diseases (Department of Dermatology, University of Pennsylvania, Philadelphia, PA). The first visit was in July 2015 and the last visit was in August 2017.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The University of Pennsylvania Institutional Review Board approved the study protocol and amendments and informed consent form. Participants gave written, informed consent prior to any study procedures.

Participants

Eligible participants ≥ 18 and ≤ 70 years of age had a diagnosis of DM either by the Bohan and Peter criteria for classic DM (Bohan and Peter, Bohan and Peter, 1975) or the Sontheimer diagnostic criteria for amyopathic DM (Sontheimer, 2002). Classification of DM activity was based on a CDASI activity score ≥ 14, corresponding to moderate-to-severely active skin disease (Anyanwu et al., 2015). Participants had to have documented efficacy failure or intolerance to hydroxychloroquine and minimal current muscle involvement. Additional inclusion and exclusion criteria are described in the supplementary text.

Randomization

Treatment assignment was determined through a central web-based randomization system. Participants were randomized in a 1:1 ratio to receive either lenabasum 20 mg daily on Days 1-

28, followed by lenabasum 20 mg twice per day (BID) on Days 29-84, or matching placebo administered in a similar manner (**Figure 1**).

Procedures

Each subject was maintained on baseline medications for DM during the entire study period. However, modifications to treatment were allowed at the discretion of the investigator/physician in case of significant disease flares in order to provide the best possible medical care.

Safety and efficacy outcomes were assessed from Day 1 through end of study at Day 113, with a total of 7 study visits. The efficacy of the drug was evaluated in terms of DM skin activity by measuring the changes in the CDASI scores from baseline. Secondary efficacy objectives were to evaluate effects of lenabasum on measures of quality of life and disease activity, using the Physician Global Assessment, Patient Global Assessment, Skindex-29+3, the Patient-reported Outcomes Measurement Information System (PROMIS)-29 short form, and the CDASI damage score. The CDASI, Physician Global Assessment, Patient Global Assessment, and Skindex-29+3, were assessed at each visit, whereas the PROMIS-29 short form was assessed on Days 1, 29, 57, 85, and 113. Safety and tolerability were evaluated by AE monitoring, laboratory (complete blood count, urinalysis, serum chemistries, creatine phosphokinase, aldolase, C-reactive protein), electrocardiogram and vital signs assessment, and physical exams. The subjects consented to photography and publication of the images.

Optional punch biopsies of lesional and non-lesional skin were taken on Day 1 and repeated in lesional skin at nearby anatomical sites on Days 29 and 85, for assessment of skin biomarkers

and histology. Peripheral blood mononuclear cells were obtained at the same three time points, and plasma was used for determination of cytokine levels. Optional skin photography was also done at Days 1, 29, and 85. Finally, for female participants of childbearing potential, urine pregnancy tests were performed at each study visit.

Immunostaining

Paraffin-embedded skin tissue samples were cut into 5 μm sections and stained for CD4, CD8, CD11c, mast cells, as well as IL-13, IL-33, PPAR-γ protein, IFN-γ, and anti-IFN-β. Sections were analyzed using the Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan). Staining quantification was performed using NIS Elements Software (Nikon). The percent of area stained, defined as the percentage of the dermis with positive staining, and the mean cell intensity, defined as the average cell intensity in the epidermis and dermis, were calculated.

RNA and cDNA isolation and quantitative real-time reverse transcription polymerase chain reaction (QRT-PCR)

RNA was extracted from formalin-fixed, paraffin-embedded blocks containing the tissue samples using the RNeasy FFPE Kit (Qiagen, Valencia, CA). RNA samples were converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) for subsequent use in PCR. A similar approach was used to isolate mRNA from PBMCs. Gene expression was measured by performing QRT-PCR on cDNA samples using the TaqMan custom designed array card assay (Applied Biosystems, Foster City, CA) per manufacturer's protocol (Applied Biosystems; IFNG, Hs00989291_m1; IL1RL1 (ST2), Hs00249384_m1; IL31, Hs01098710_m1; IL33, Hs04931857_m1; IL4, Hs00174122_m1;

STAT6, Hs00598625_m1). The assay was performed on the VIIA 7 Real-Time PCR (Applied Biosystems, Foster City, CA). Gene transcript levels were normalized to human GAPDH (Applied Biosystems, Hs99999905_m1) and relative gene expression was calculated using the comparative CT method.

Statistical Analyses

Categorical variables were summarized by frequencies and percentages and continuous variables by means and standard deviations. The CDASI scores and changes from baseline were summarized by visit and treatment group. The least squares (LS) mean change and standard error (SE) from baseline for CDASI at all follow-up visits was compared between lenabasum and placebo, using a mixed model with repeated measures (MMRM) where change from baseline was the dependent variable, treatment, visit, and treatment-visit interaction were factors, prednisone use was a time-varying factor, baseline was a covariance, and subject was a repeated random effect using a compound symmetry covariance matrix. The LS mean (SE) difference and 95% confidence intervals (CI) were calculated for change in CDASI activity score. A similar MMRM analysis was used for Physician Global Assessment, Patient Global Assessment, Skindex-29+3, and PROMIS-29 Short Form. Efficacy analyses used the modified intent-to-treat (mITT) population (all randomized participants who received at least 1 dose of study drug). The statistical test for the primary efficacy outcome was two-sided at a significance level of 0.05. One-sided analyses with a significance level of 0.1 were used for secondary efficacy outcomes in this small, first-in-DM study. No formal statistical testing was performed to compare the safety in different cohorts.

Each biomarker and mRNA expression of cytokines were log-transformed and change from baseline to Day 85 was compared between treatment groups using a student t-test. The statistical test for biomarkers was two-sided at a significance level of 0.05. Statistical analyses of biomarker data were performed using R version 3.4.

DATA SHARING STATEMENT

Lenabasum is an investigational drug under development. Individual deidentified participant data and data dictionaries will be available by request directed to the primary investigator, Dr. Victoria Werth, and to the Sponsor, Corbus Pharamaceuticals. Safety results are posted to clinicaltrials.gov, NCT02466243. The rest of the study documents such as the study protocol, statistical analysis plan and informed consent form will be made available only to academic investigators, and requests should be made as above.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS STATEMENT

Conceptualization: VPW, EH, SMP, JH, BW; Data Curation: VPW, EH, SMP, JH, BW; Formal Analysis: VPW, RF, BW; Investigation: VPW, EH, SMP, JH, JSSC, JO; Methodology: VPW, EH, SMP, JH; Project Administration: VPW, JO; Resources: VPW; Supervision: VPW, ND, SC, BW; Visualization: VPW, RF, SC, ND, BW; Writing - Original Draft Preparation: VPW, EH, SMP, JH, MZ, NR, JO, MB, KG, ASJ, JSSC; Writing - Review and Editing: VPW, JSSC, BW

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Table 1. Baseline Subject Demographics, Disease Characteristics, and Background Immunomodulating Treatments

Baseline	Lenabasum n/11 (%) or mean ± SD	Placebo n/11 (%) or mean ± SD
Demographics		
Female	10 (90.9)	11 (100.0)
Age, years	53.1 ± 9.31	52.5 ± 10.44
Body mass index, kg/m^2	26.4 ± 5.78	27.3 ± 7.40
Race		
White (non-Hispanic/non-Latino)	8 (72.7)	10 (90.9)
Black or African American	0	1 (9.1)
Hispanic or Latino	3 (27.3)	0
Background Systemic Immunomodulating Treatments		
Any systemic immunomodulator use	9 (81.8)	10 (90.9)
Glucocorticoids	3 (27.3)	2 (18.2)
Hydroxychloroquine	4 (36.4)	6 (54.5)
Mycophenolate mofetil	3 (27.3)	4 (36.4)
Methotrexate	3 (27.3)	2 (18.2)
Other (Quinacrine, tacrolimus, azathioprine)	2 (18.2)	2 (18.2)
≥ 2 systemic immunomodulator treatments	5 (45.5)	6 (54.5)
≥ 1 systemic immunomodulator treatment other than hydroxychloroquine	9 (81.8)	10 (90.0)
Topical corticosteroids	4 (36.4)	5 (45.5)

Table 2. Efficacy outcomes at baseline and end of study, Day 113

Endpoint	Lenabasum		Placebo		LS mean difference (CI)	P
	Baseline Mean (SD)	Change from baseline Mean (SD)	Baseline Mean (SD)	Change from baseline Mean (SD)		
Primary Endpoint						
CDASI activity score	33.3 (9.74)	-9.3 (10.99)	35.8 (7.77)	-3.7 (6.83)	-6.5 (-12.6, -0.4)	**0.0382**
Secondary Endpoints						
CDASI damage score	2.4 (2.16)	1.1 (1.76)	3.8 (3.92)	1.5 (2.54)	-0.5 (-1.5, 0.6)	0.2869
Patient VAS						
Overall disease	4.6 (2.18)	-0.8 (3.44)	6.4 (2.59)	-0.1 (1.18)	-1.3 (-2.5, 0.0)	**0.0937**
Skin global	4.9 (2.20)	-1.1 (3.38)	6.7 (2.51)	0.0 (1.14)	-1.6 (-2.8, -0.5)	**0.0374**
Pain, 24 h	2.7 (2.53)	-1.0 (2.18)	3.7 (3.79)	0.0 (2.47)	-1.2 (-2.5, 0.0)	0.1056
Itch, 24 h	6.1 (2.67)	-1.2 (2.47)	5.1 (3.53)	-0.3 (1.91)	-0.7 (-2.0, 0.5)	0.2265
Physician VAS						
Overall disease	4.7 (1.27)	-0.8 (3.44)	5.4 (1.42)	-0.1 (1.18)	-1.3 (-2.5, 0.0)	**0.0937**
Skin disease	5.5 (1.31)	-0.6 (1.84)	6.5 (1.60)	-0.6 (1.61)	-0.5 (-1.2, 0.3)	0.2202
Skin activity	5.8 (1.47)	-0.8 (1.71)	6.6 (1.44)	-0.7 (1.51)	-0.4 (-1.1, 0.3)	0.2429
Skin damage	0.4 (0.51)	0.2 (0.25)	1.9 (2.77)	0.3 (0.64)	-0.1 (-0.4, 0.2)	0.3759
Muscle disease	0 (0.06)	0.0 (0.08)	0 (0.0)	0 (0.00)	0.0 (0.0. 0.1)	0.9381
Extramuscular disease	4.5 (1.92)	-0.5 (2.98)	5.8 (1.60)	-1.0 (1.17)	-0.5 (-1.4, 0.5)	0.2589
Skindex-29+3						
Overall	42.2 (17.7)	-10.1 (12.80)	43.7 (26.3)	-6.1 (9.25)	-4.1 (-10.3, 2.0)	0.1929
Emotions	45.0 (24.21)	-10.5 (14.57)	56.6 (32.12)	-8.6 (12.67)	-2.7 (-10.4, 5.0)	0.3254
Symptoms	61.0 (20.23)	-16.9 (14.91)	52.3 (24.29)	-7.8 (10.08)	-8.1 (-15.6, -0.5)	**0.0861**
Functioning	27.8 (15.74)	-5.1 (12.79)	27.3 (26.65)	-3.3 (10.42)	-1.8 (-8.0, 4.5)	0.3586
Photosensitivity	55.7 (31.31)	-6.8 (23.29)	39.8 (35.72)	2.3 (16.60)	-6.7 (-17.1, 3.7)	0.2021
Hair loss	63.6 (39.31)	-18.2 (16.17)	68.2 (40.45)	-2.3 (13.48)	-16.2 (-24.8, -7.7)	**0.0081**
PROMIS-29						
Physical function	50.56 (7.445)	2.61 (5.173)	53.96 (6.688)	-2.03 (5.579)	5.12 (2.07, 8.18)	**0.0336**
Social role	54.20 (6.437)	0.86 (3.282)	55.59 (8.851)	0.21 (5.924)	0.48 (-1.96, 2.92)	0.7996
Anxiety	49.40 (8.168)	-2.67 (4.314)	49.86 (10.109)	-2.50 (8.006)	-0.29 (-3.33, 2.74)	0.4502
Depression	50.39 (6.777)	-4.65 (6.438)	48.70 (7.368)	-0.95 (12.189)	-2.77 (-7.01, 1.47)	0.2004
Fatigue	50.30 (9.897)	-1.85 (11.079)	51.33 (9.857)	-1.47 (9.431)	-0.80 (-5.90, 4.29)	0.4194
Sleep disturbance	54.04 (7.840)	-1.45 (9.53)	55.59(7.176)	-1.25 (6.372)	-0.60 (-4.75, 3.55)	0.4260
Pain interference	51.29 (7.846)	-5.16 (7.362)	47.59(8.976)	2.06 (6.240)	-5.99 (-9.50, -2.48)	**0.0154**
Pain intensity	2.91 (2.508)	-1.18 (1.888)	3.55 (3.236)	-0.82 (1.250)	-0.49 (-1.44, 0.46)	0.2542

P-value ≤ 0.05 based on 2-sided test for CDASI activity score and P ≤ 0.10, 1-sided test for other analyses. 95% confidence intervals are provided for change in CDASI activity score and 80% confidence intervals for change in other efficacy outcomes.

Table 3. Change from Baseline for mRNA Gene Expression of Cytokines from Skin Biopsy

	Pre-treatment Baseline			Change (Post – Pre)		
	Placebo (n = 5)	Lenabasum (n = 7)	P value*	Placebo change (post – pre)	Lenabasum	P value*
IFN-β	5.68 (4.33-18.26)	25.61 (21.24-31.49)	0.0732	-0.73 (-2.00 - 0.10)	-14.59 (-20.93- -5.52)	**0.0303**
IFN-γ	1.45 (1.20-1.50)	1.33 (0.79-2.19)	0.9999	0.34 (0.27-1.96)	-0.67 (-1.66- - 0.37)	**0.0480**
IL-31	5.34 (0.63-15.26)	5.53 (2.92-27.13)	0.7746	1.28 (-2.80-7.12)	0.6 (-0.84-9.78)	0.8763
CD4+ T cell	60.4 (49.8-63.8)	96 (74.5-143.1)	0.1490	-7.75 (-16.8 - 9.0)	-43.2 (-46.9- - 10.8)	0.2677
IL-4	4.60 (1.02-5.98)	1.75 (1.16-10.46)	0.9999	-0.48 (-2.65-4.49)	0.42 (-0.21-0.58)	0.8763
IL1-β	1.76 (0.69-2.05)	1.36 (0.59-3.23)	0.9999	-0.27 (-0.75 - 0.47)	-0.08 (-0.66-1.25)	0.9999
PPAR-γ	3.06 (1.19-4.26)	2.68 (1.99-4.63)	0.7551	0.80 (-1.48-3.81)	0.18 (-1.37-2.36)	0.8763
MX1	5.49 (4.71-16.18)	23.98 (8.84-35.49)	0.3434	0.3 (-0.46-8.45)	1.2 (-7.00-2.75)	0.8763
Stat6	0.81 (0.55-1.57)	1.54 (1.13-2.79)	0.3434	0.05 (-0.05-0.17)	0.65 (-0.86-1.07)	0.4318
TNF-α	2.01 (1.62-2.97)	5.57 (2.00-7.89)	0.6389	4.73 (-0.36 – 4.73)	1.97 (-1.10 - 5.97)	0.5273
St2	0.46 (0.46-0.59)	0.67 (0.44-1.11)	0.5152	0.43 (0.08-0.67)	0.00 (-0.15 - 0.75)	0.3434

Values are mean ± standard error; *p-value ≤ 0.05 for change from baseline (Day 85)

Figure legends

Figure 1. Enrollment, Randomization, and Treatment Assignment. There were 11 participants in each cohort (lenabasum and placebo) who completed the dosing and were included in the safety and efficacy analyses.

Figure 2. Change from baseline in DM skin activity. (a) Mean (SD) change from baseline in CDASI activity score *Lenabasum 20 mg daily on Days 1-28, followed by lenabasum 20 mg BID on Days 29-84. *P-value = 2-sided, MMRM, lenabasum vs. placebo. **(b)** Subject in the lenabasum arm showing change in skin activity from baseline**.** Subject 5 (lenabasum arm) had recalcitrant skin disease despite being on plaquenil 400 mg/day and mycophenolate mofetil 3,000 mg/day. Top row: baseline with dark red erythema of the upper lateral arms (left) versus last visit with pink erythema and decreased surface area from baseline (right). Bottom row: baseline with dark red erythema of chest (left) versus last visit with pink erythema and poikiloderma of chest (right). The subject consented to the publication of these images.

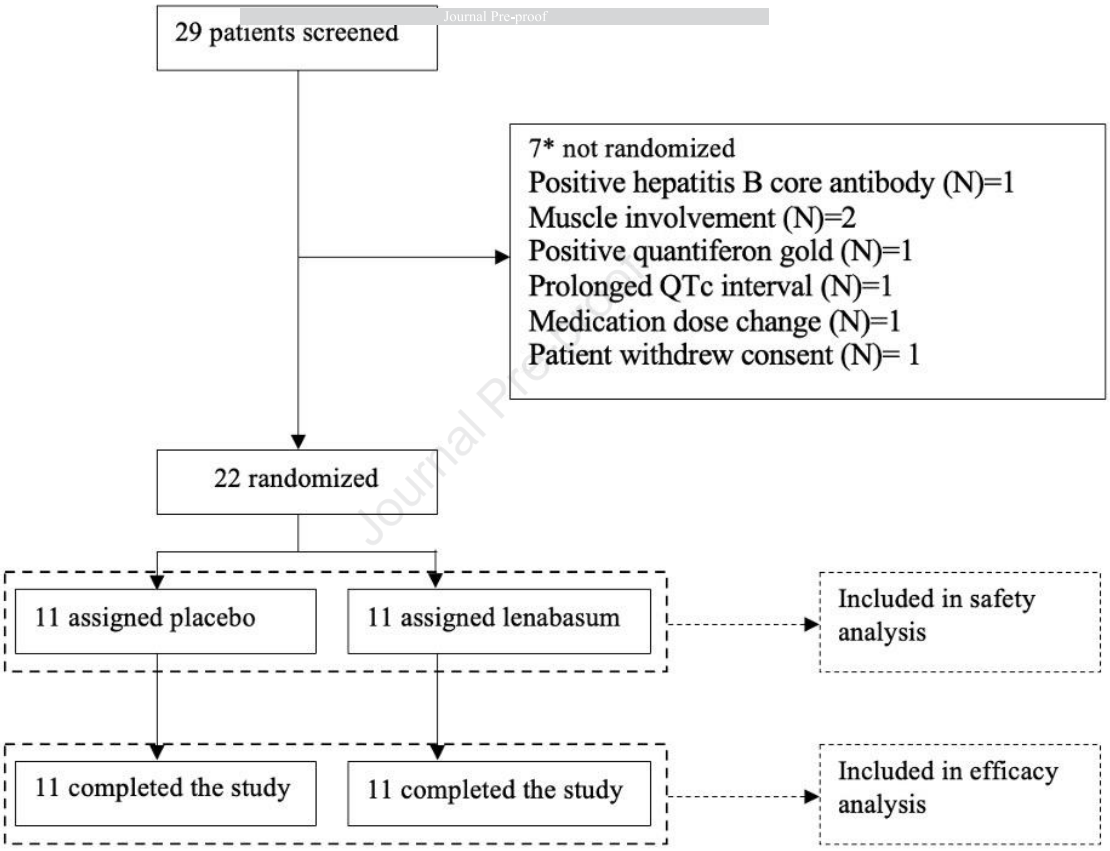
Figure 3. IL-31 immunohistochemistry. (a) IL-31 % area decreases in disease responders vs. non-responders. The responder group, defined as subjects who had a greater than a 5-point change in the CDASI activity score, had a significant reduction in IL-31 % area in lesional skin, compared to the non-responders. **(b)** IL-31 intensity decreases in itch responders vs. non-responders. The responder group, defined as subjects who had a notable decrease in their Skindex Symptoms score, had a significant reduction in IL-31 intensity in lesional skin, compared to the non-responders. Scale bars: 200 μm

SUPPLEMENT

Study design and participants

Additional Inclusion and Exclusion Criteria

Participants should have minimal muscle disease activity with no difficulty with lifting or walking, CPK or aldolase not more than 1.5x the upper limit of normal, and no requirement for corticosteroid treatment for muscle disease. They had to be on a stable regimen of background DM medications for ≥ 28 days. Glucocorticoid dose was limited to ≤ 10 mg/day prednisone or equivalent within 28 days before Day 1. Other immunosuppressive drugs were allowed, provided doses had not increased within 8 weeks before Day 1. Other immunosuppressive drugs were allowed, provided doses had not increased within 8 weeks before Day 1. Participants who were exposed to an anti-TNF medication or had prior treatment with B cell-depleting monoclonal antibodies within 6 months of visit 1 were excluded. Participants with significant conditions other than DM such as recurrent severe infections, positive serology for acute/chronic hepatitis B, C, or human immunodeficiency virus infection, malignancy within 5 years except for successfully treated skin basal cell or squamous cell carcinoma or cervical carcinoma in-situ, or uncontrolled cardiac conditions or baseline prolongation of QT/QTc were also excluded from entry. Furthermore, a positive pregnancy test, QuantiFERON test, and severe alterations in the hematologic profile and serum chemistries were exclusions to enrollment.



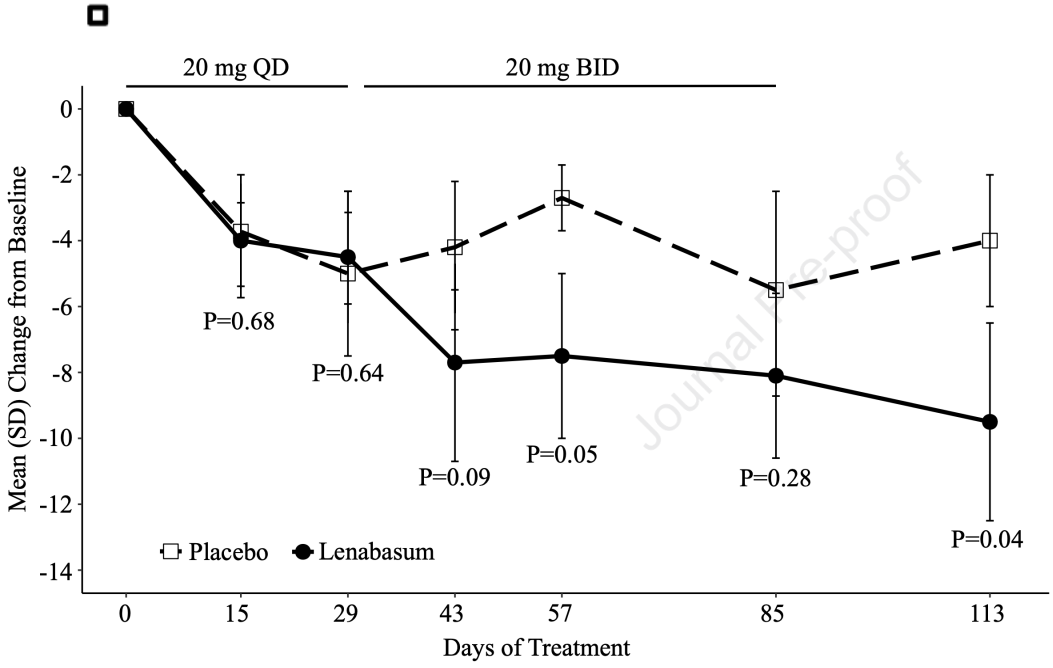


Figure 2a

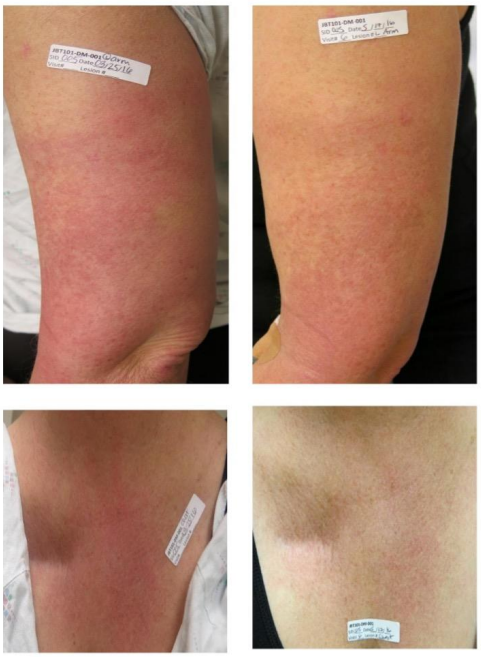


Figure 2b

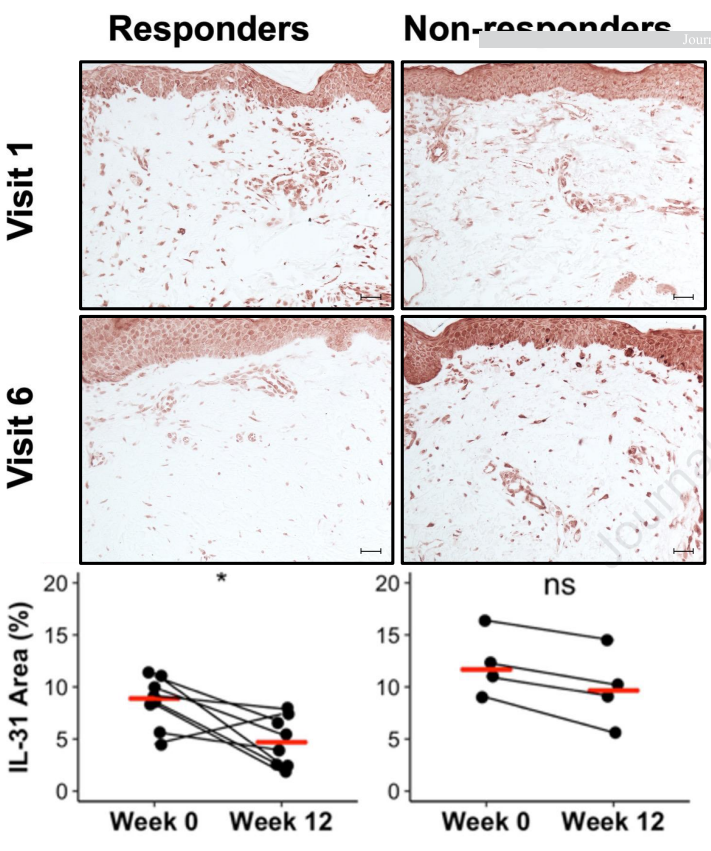


Figure 3a

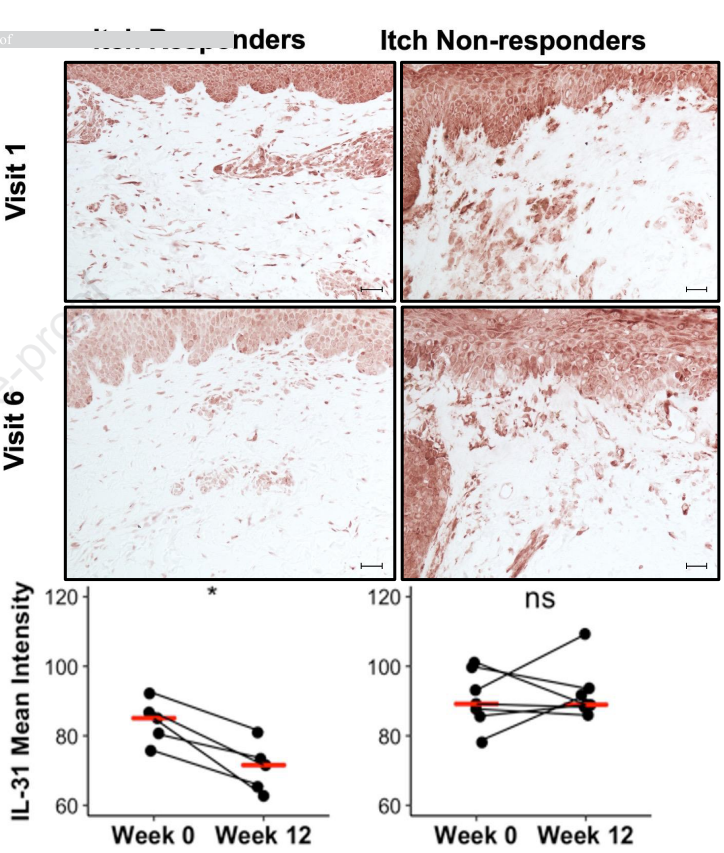


Figure 3b