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Effect of Corticosteroid on Safety and Efficacy for Actinic Keratosis Therapy with Ingenol Mebutate

Shreya Amin
Yale Physician Associate Program, sba260@gmail.com

EFFECT OF CORTICOSTEROID ON SAFETY AND EFFICACY FOR ACTINIC KERATOSIS THERAPY WITH INGENOL MEBUTATE

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Shreya Amin, PA-SII
Class of 2017
Yale Physician Associate Program

Dr. Suguru Imaeda
Assistant Professor of Dermatology
Director of Dermatology, Yale University Health Services
Director of Dermatology Service, Veteran Affairs Medical Center

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ABSTRACT

Actinic keratoses are common premalignant lesions that occur in fair-skinned individuals with high cumulative ultraviolet exposure. Monotherapy with ingenol mebutate has been known to cause local skin reactions, such as erythema, which reduces safety. Clobetasol propionate has worked well with other actinic keratosis therapies to reduce inflammation, but its role with ingenol mebutate is not well understood. The effect of concurrent application of clobetasol propionate with ingenol mebutate to reduce local skin reactions without impacting efficacy has not yet been investigated. We are proposing a superiority trial for safety as well as a non-inferiority trial for effectiveness. We will conduct an intra-individual, randomized controlled trial at the West Haven Veterans Affairs in patients with multiple actinic keratoses, and analyze local skin reactions at day 4 and efficacy at day 57. If combination therapy has significant advantages compared to monotherapy, it can improve the safety profile and tolerability of ingenol mebutate.

CHAPTER I: INTRODUCTION

1.1 Background

Actinic keratoses (AKs) are common precancerous lesions that form in fair-skinned individuals with high cumulative ultraviolet (UV) exposure.[1-3] In the United States, AKs are among the most common reasons for visits to dermatologists.[2,4] According to the National Ambulatory Medical Care Survey data from 1990 to 1999, AKs were diagnosed in more than 47 million visits over these 10 years accounting for 14% of patient visits to dermatologists.[5] However, this statistic does not reflect the true prevalence of AKs because it represent patients who visit dermatologists, suggesting the actual prevalence in the general population is much greater than 14%.[5,6]

Patients at risk for developing AKs include males with advanced age, fair-skin, high cumulative sun exposure, and prolonged immunosuppression.[7-10] The most common method of AK prevention is sun protection, including avoiding the sun during peak hours from 10am to 3pm, wearing protective clothing, and using sunscreen.[11] Although there has not been a population-based study in the United States to estimate the incidence of AKs, it is thought to be increasing as a result of increasing life expectancy, lifestyle behaviors such as sun tanning, and increasing cumulative sun exposure.[7,8,12]

AKs are a public health concern because if they are not adequately treated they can either persist, progress, or develop into invasive squamous cell carcinomas (SCC).[13,14] Although the actual risk of an individual AK progressing to invasive SCC is unclear, estimations vary from as low as 0.1% to as high as 20%.[13] Lesion directed therapy, such as cryotherapy, is commonly used because of its convenience, cost-effectiveness, and efficacy.[9,15] However, in areas of widespread damage, it is clinically difficult to

distinguish which lesions will convert.[4,16] Therefore, using field therapy to treat both the lesion and the surrounding skin is beneficial because it targets both visible and non-visible subclinical lesions.

First-line field therapies include 5- fluorouracil, diclofenac, imiquimod, and ingenol mebutate.[9,15] Current drawbacks to the self-applied topical field therapies include the long duration of treatment and consequently prolonged local reactions, which leads to decreased adherence.[2] 5- fluorouracil has been preferred over other first line therapies because of its cost effectiveness and widespread availability, but because it requires treatment for four weeks some studies suggest it leads to lower patient satisfaction and adherence.[9,17] The treatment of choice depends on the patient's quality of life, comorbidities that may contribute to adverse effects, and their ability to adhere to their treatment.[9] For example, applying topical treatments for an extended duration can be especially difficult for the elderly and patients who live alone.[15]

Ingenol mebutate (IMB), retrieved from the extract of *Euphorbia peplus* was approved by the FDA in 2012 for the treatment of AK.[18,19] The 0.015% IMB gel is indicated for a three-day treatment course on the face or scalp, while the 0.05% gel is indicated for a two-day treatment course on the trunk or extremities.[4] A major advantage of IMB therapy over other field therapies is the short treatment time required to yield a similar efficacy to 60 days of treatment with diclofenac gel (3.0%) or 16 weeks of treatment with imiquimod (5%).[20] Studies have shown the adherence to IMB is 98%, which can be attributed to the shorter application time.[4] However, barriers to treatment remain because certain side effects are fairly common in patients using this treatment, which can lead to increased cosmetic burden and decreased patient satisfaction.

Barriers to effective AK treatment include concerns associated with cosmetic effects of treatments, side effects including local skin reactions (LSRs), pain, pruritus, cost perceptions, adherence, and long duration time.[12] LSR scores are highest on the face and scalp compared to the trunk and extremities; this can be attributed to thicker epidermis on the scalp and higher absorptive rates compared to other parts of the body.[20] They can be inconvenient for patients and affect their quality of life if LSRs are located in areas such as the face or hands.[12]

1.2 Statement of the Problem

Several studies have shown the efficacy and high adherence rates of IMB, but LSRs, one of the most common side effects of IMB, remain a barrier to effective treatment of AKs. LSR scores peak at the 4th day of IMB treatment and usually completely heal in 2 weeks.[20,21] There are several studies that have shown the extent to which LSR reactions can lead to reduced quality of life.[12] Multiple studies have also shown that when compared to another field therapy, IMB has worse LSR, pain, and pruritus scores, giving preference to other treatments.[22] This can lead to reduced patient satisfaction and decreased usage of IMB as a treatment for AKs, especially in the elderly and frail population.

Corticosteroids have been used in many aspects of dermatology, including eczema and psoriasis, to reduce inflammation. Glucocorticoids, such as clobetasol propionate have immunosuppressive, anti-inflammatory, and vasoconstrictive properties. Although LSRs are common side effects of field therapies, there is limited research investigating the effectiveness of corticosteroids to reduce LSRs.

Combination therapy with corticosteroids has shown reduction in inflammation caused by cryotherapy and photodynamic therapy. Research into the effectiveness of corticosteroids as combination therapies with IMB to reduce LSR is especially limited. Erlendsson et al. is the only randomized controlled trial that has looked at the effect of clobetasol propionate after IMB treatment on inflammation and efficacy.[23] In their study, clobetasol propionate was applied on day 4, when LSR was most severe; no difference was found in LSR reduction or in AK clearance.

Inflammation in IMB is caused by a neutrophil-mediated response, which is required to prevent relapse against residual tumor cells.[24] The current problem lies in reducing this inflammation, without impacting the efficacy of IMB. Research has shown that neutrophil invasion, which is prevented by corticosteroids, is most pronounced in the early phase of IMB-induced inflammation, therefore, starting clobetasol propionate on day 4 may be too late to impact LSR. Further research is needed to investigate the effect of earlier clobetasol propionate treatment on reducing IMB-induced LSR for AK treatment. This study will also add to our understanding about the effect of earlier corticosteroid combination therapy on the efficacy of IMB treatment.

1.3 Goals and Objectives

Our goal is to create a randomized controlled trial that will examine the safety and efficacy of earlier corticosteroid application in reducing the severity of LSRs associated with IMB therapy. Our primary objectives are to evaluate the mean reduction of LSR scores on day 4 and AK clearance on day 57 in combination therapy with IMB plus corticosteroids as compared to IMB alone. We will evaluate the severity of LSR by using

the standardized LSR grading scale created by Rosen et al.[25] We will evaluate AK clearance clinically and with the use of dermoscopy. Our secondary objectives are to compare the composite pain and pruritus scores of the two groups before and after treatment. We will also assess long-term efficacy by evaluating AK clearance at 12 months.

1.4 Hypothesis

Primary Hypotheses:

We hypothesize that there will be a statistically significant difference of 2.0 in the mean composite LSR score at day 4 in AKs that receive combination therapy with ingenol mebutate and clobetasol propionate compared to monotherapy with ingenol mebutate alone.

We also hypothesize that there will be no difference in the visible or dermoscopy clearance of AKs treated with combination therapy with ingenol mebutate and clobetasol propionate compared to monotherapy with ingenol mebutate alone at day 57.

Definitions

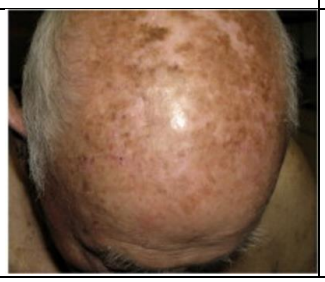
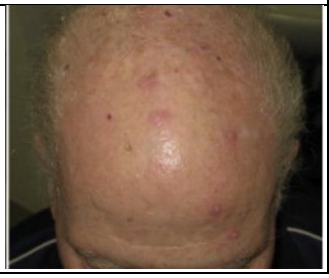
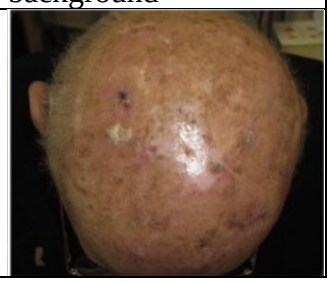
Safety: mean composite LSR score, pain, and pruritus

Efficacy: dichotomous outcome that will be evaluated as being greater than 75% visible and dermoscopy AK clearance or less than 75% visible and dermoscopy AK clearance at day 57.

Split-face studies: intra-individual studies that compare the application of the control on one side of the face to the treatment on the other side of the face.

Intra-individual studies: similar to split-face, except they do not divide patients based on sides of the face. Instead one discrete area is chosen as the control and another as the treatment and both areas can be on the same side of the face.

Clinical, Dermoscopy, and Visible Characteristics of Actinic Keratosis Grades[13]

	Grade I	Grade II	Grade III
Clinical	Slightly palpable AK, which are better felt than seen	Moderately thick AK	Very thick, hyperkeratotic
Dermo-scopy	Red pseudo-network pattern and discrete white scales	Background erythema intermingled by keratotic follicular openings	White-yellow areas with no structure or large follicular openings filled with keratotic plugs over a scaly and white-yellow-appearing background
Visible			

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CHAPTER II: REVIEW OF THE LITERATURE

2.1 Introduction

We conducted a comprehensive medical literature review between November 2016 and May 2017 to develop this proposed randomized controlled trial. We searched the databases PubMed, Ovid MEDLINE, and Cochrane using the following keywords in a variety of combinations: *ingenol mebutate, PEP005, 5- fluorouracil, photodynamic therapy, cryotherapy, adherence, corticosteroid, clobetasol propionate, actinic keratosis, local skin reactions, skin disease, safety, efficacy, and field therapy.* All articles written between January 1976 and May 2017 in English were reviewed for significance and analyzed. Studies looking at immunocompromised patients or pediatric populations were excluded.

2.2 Review of relevant studies

This section will summarize the existing evidence relevant to the use of IMB in the treatment of AKs and focus on measures of safety, which include LSRs, pain, and pruritus. We will also focus on current literature analyzing the long-term efficacy of IMB and how this differs from other first-line field therapies. Data regarding combination therapy of IMB and corticosteroids are limited, thus studies analyzing the effect of combination therapies with other field treatments for AKs will be reviewed. We will focus on previous research that successfully used corticosteroids to reduce inflammation caused by cryotherapy, 5- fluorouracil, and photodynamic therapy without compromising efficacy. Limitations of these studies will also be discussed.

2.2.1 Mechanism of Action of Ingenol Mebutate and Corticosteroids

Several studies have attempted to analyze how IMB works to clear AKs. Rosen et al. described a dual mechanism of action by IMB, which includes both rapid lesion necrosis and neutrophil-mediated, antibody-dependent cellular cytotoxicity.[1-3] Mice studies showed that rapid lesion necrosis begins 1 to 2 hours after application and is followed by a robust inflammatory response.[2] Morphologic manifestation of necrotic cell death, which was marked by swelling of the mitochondria via an electron microscope, was evident as early as 3.5 hours after the addition of IMB in vitro.[2] This process likely begins with the release of pro-inflammatory cytokines from keratinocytes undergoing necrosis, which mediates the neutrophil recruitment.[4] The activation and upregulation of vascular endothelial adhesion molecules is necessary to allow neutrophils to attach to the microvascular endothelium and transmigrate through the vessel wall to reach the treatment site.[1]

Challacombe et al. showed infiltration of neutrophils 6 hours after IMB application in mice.[5] This infiltrative process was clinically apparent on the skin 24 hours later.[5] This neutrophilic reaction is a key component required to prevent relapse against residual tumor cells.[1,5,6] Neutrophilic activity also results in the inflammation that may cause severe LSRs in patients treated with IMB.[1,2] More research needs to be conducted on combination therapies that can reduce this neutrophil-mediated inflammation without compromising the efficacy of IMB.

There are limited studies investigating the usage of corticosteroids to decrease inflammation caused by AK field therapies. Studies looking at the impact of corticosteroids on neutrophils have been mixed. Systemic and topical corticosteroids are

the mainstay of treatments for diseases such Sweets syndrome, which is a neutrophil-mediated infiltration in the upper dermis.[7] However, some studies have shown that corticosteroids prolong the life of neutrophils by preventing apoptosis, which would theoretically increase inflammation.[8]

Corticosteroids are known to have anti-inflammatory, anti-proliferative, immunosuppressive, and vasoconstrictive effects.[9] Schaefer et al. suggests that the mechanism of anti-inflammation is multifactorial; one of these mechanisms is the inhibition of the formation of inflammatory proteins released by keratinocytes, fibroblasts, and infiltrating leukocytes.[10] The anti-inflammatory effects of corticosteroids work by inhibiting dermal edema, capillary dilatation, and vascular permeability, which prevents the transmission of leukocytes through the vessel wall, and hinders their recruitment to the inflammation site.[9,11] Glucocorticoids also inhibit cytokine gene transcription, T-cell proliferation, and T-cell dependent immunity.[11] Generally, topical glucocorticoids have both immediate effects that cause membrane stabilization, as well as delayed effects that are due to glucocorticoid alteration of DNA transcription.[12]

In contrast to Challacombe, Liles et al. suggested that glucocorticoids prolong neutrophil survival in vitro by inhibiting apoptosis, which increases the survival of circulating neutrophils.[8] However, Parrillo et al. hypothesized that circulating neutrophils may also increase due to enhanced release from bone marrow cells, or because the neutrophils are unable to transmigrate to the site of inflammation.[13]

Cronstein et al. showed that pretreatment of endothelial cells with corticosteroids prevents them from becoming more adhesive to neutrophils by diminishing stimulated expression of ICAM-1 and ELAM-1, which are molecules critical for neutrophil

adhesion.[14] Earlier use of corticosteroids can prevent the expression of molecules that allow neutrophils to adhere to the microvascular endothelium, which may cause a decrease in the inflammatory response caused by IMB.[14]

Another variant in determining the effectiveness of corticosteroids is their potency, which is determined by their anti-inflammatory activity, vasoconstriction abilities, and their effect on carbohydrate metabolism.[15-17] Clobetasol propionate is a Class I, super-potent corticosteroid; in several studies it has been successfully used to reduce inflammation caused by AK treatments.[18-20] Since the LSRs induced by IMB occurs rapidly,[15,21-23] we are using a more potent corticosteroid, such as clobetasol propionate, that will cause greater vasoconstriction and reduce inflammation quicker than a milder corticosteroid.[15] Local side effects have been minimal when clobetasol propionate has been used for short periods of time (<3 weeks), with transient burning and pruritus being the most prominent adverse effects.[24]

In conclusion, the effectiveness of clobetasol propionate relates to its anti-proliferative, immunosuppressive, vasoconstrictive, and anti-inflammatory effects. There is conflicting evidence on how the mechanism of corticosteroids will affect the neutrophil-induced inflammatory response of IMB. However, most recent research has shown that circulating neutrophils may increase after the usage of corticosteroids because they cannot transmigrate to the inflammatory loci. We do not currently understand to what extent this will decrease the neutrophil-mediated response, and how this affects the efficacy of IMB. As a result, more studies, such as this one, are needed to investigate the role of corticosteroids to reduce IMB-induced LSR without compromising efficacy.

2.2.2 Role of Ingenol Mebutate in Actinic Keratosis

Numerous studies have investigated the safety and efficacy of IMB on the treatment of Grades I and II AKs.[23,25-27] In a multi-centered, randomized, double-blinded study Lebwohl et.al, analyzed 547 patients with face or scalp AKs (277 received IMB and 270 placebo) and 458 patients with trunk or extremities AKs (226 received IMB and 232 placebo) that were treated with IMB 0.015% and 0.05% gel respectively.

Rate of complete clearance at day 57 was higher in patients treated with IMB on the face and scalp as compared to placebo (42.2% vs 3.7%; $p<0.001$). Partial clearance was also higher in the treatment group (63.9% vs 7.4%; $p<0.001$). Efficacy analyses by anatomic location of IMB versus placebo demonstrated greater rates of complete clearance on the face (47.3% vs 4.1%; $p < .001$) compared to the scalp (22.8% vs 2.0%; $p = .001$). Results showed a mean reduction of 83% in the number of AKs treated with IMB compared to baseline. The rate of complete clearance at day 57 was also higher with IMB than with placebo for the treatment of trunk and extremities (34.1% vs. 4.7%, $p<0.001$). Partial clearance was also higher in the treatment group (49.1% vs 6.9%, $p<0.001$), while the mean percentage in reduction in number of AKs from baseline was 75%.

Although this study was pivotal in showing the effectiveness of IMB in treatment of AKs, there are several limitations of this study. Due to formation of LSRs, participants in this study could not be effectively blinded. Additionally, treatment areas were limited to 25 cm^2, so the effect of IMB on larger areas was not studied. The protocol of this study prevented adjunctive treatments, so the effect of combination therapy was not assessed. Lastly, because patients diagnosed with AKs have a recurrence rate of 15-53%

in the next year,[26] it is important to do a follow-up and assess efficacy after day 57, which was not performed in this study.

Realizing this limitation, Lebwohl et al. used their previously published data and analyzed the 3, 6, 9, and 12-month recurrence rate and safety in patients with IMB-cleared AKs from their previous study.[25] The percentage reduction in AKs at 12 months from the number of lesions at baseline was 87.2% for the face or scalp and 86.8% for the trunk or extremities. In 53.9% of patients (95% confidence interval [CI]; 44.6 to 63.7) one or more lesions developed or recurred in the treatment field. The sustained clearance rate after 12 months of follow-up was 46.1% for patients treated on the face or scalp and 44.0% for patients treated on the trunk or extremities.

To compare this to the efficacy of other first-line field therapies for AKs, Krawtchenko et al. studied application of fluorouracil twice daily for 4 weeks (n=24), and 1 or 2 courses of topical imiquimod 5% administered 3 times per week for 4 weeks each (n=26) in AKs.[28] Results showed that sustained clearance of treatment at 12 months after initial evaluation was 33% for 5-fluorouracil and 73% for imiquimod ($p < 0.01$). Although this study is limited by its small sample size, it showed that long term IMB efficacy is greater than that of 5-fluorouracil.

In order to study improvements in efficacy after reapplication of treatment, Garbe et al conduced a randomized, double-blinded study.[26] In this study, 450 patients with 4–8 clinically visible AKs on the face or scalp were treated with 3 days of 0.015% IMB. After initial treatment, 61.6% patients showed complete clearance at 8 weeks. Remaining patients were randomized to IMB (n = 134) or placebo (n = 69). For patients who received a second treatment cycle, IMB showed significantly higher clearance rate

than placebo after 8 weeks (46.7% vs. 18.4%; $p < 0.01$). AKs that emerged at week 26 were also randomized to IMB (n=14) and placebo (n=8) and showed the same success in clearance rate (59.5% vs. 25%; $p = 0.01$). Overall the complete clearance of AKs increased from 61.6% after the initial 3-day treatment to a total of 79.5% by combining patients who were completely cleared initially and those who received follow-up treatment at 8 weeks. This study was helpful in proving the long-term efficacy of IMB and showed the benefit in repeating treatment for lesions not initially cleared. Limitations of the study included the difficulty in blinding patients owing to the lack of LSRs in the intervention group during the repeat treatment.

Lastly, Siller et al. studied the effect of different IMB concentrations on efficacy at day 85 and LSRs on day of treatment.[3] In a randomized, double-blinded, multi-centered trial, five preselected lesions were treated with IMB gel 0.0025% (n=15), 0.01% (n=16), 0.05% (n=15), or vehicle gel (n=12), on days 1 and 2 (Arm A) or days 1 and 8 (Arm B). Results showed that there was a statistically significant difference in complete clinical clearance of 71% of treated lesions in patients that used 0.05% IMB gel compared to vehicle gel ($P < 0.0001$). The study also showed 80% or greater complete clinical lesion clearance in 0.05% IMB gel compared to vehicle ($P = 0.0185$). There was not a dose response seen histologically, however, this could be due to the small sample size. Limitations of the study include its inter-patient variability, which were avoided in other studies that used an intra-individual design and larger sample size. Although the baseline characteristics in the control and treatment groups were matched, the vehicle group had more females compared to the treatment group (25% vs 7%) and it is unclear if this had any effect on the results.

2.2.3 Safety of Ingenol Mebutate treatment

Several studies have analyzed the safety profile of IMB as measured via LSR, pain, and pruritus. Recent post-marketing reports by the U.S. Food and Drug Administration have reported severe allergic reactions, herpes zoster, and eye injuries after IMB treatment.[29] Results of safety in IMB have been mixed, proving the irregularity in predicting which patients are more prone to experiencing IMB-induced side effects.

A meta-analysis conducted by Tzogani et al. looked at 13 IMB clinical trials that included 1165 patients.[30] The analysis found that most LSRs reported were transient, peaked early in the treatment, and resolved within 2 weeks. However, severe LSRs occurred with an incidence of 29% on the face and scalp and 17% on the trunk and extremities.

In addition to measuring efficacy, Lebwohl et al. also looked at safety via LSR scores.[23] The study noted a peak in the mean maximum composite LSR scores on the face and scalp of 9.1±4.1 as compared to 1.8±1.6 in the placebo group. LSRs generally peaked on day 4 and declined afterwards. In the face or scalp studies, 24% of patients experienced severe erythema, 9% experienced severe flaking/scaling, and 6% experienced severe crusting. In the trunk and extremities LSRs were less common (6.8±3.5 vs 1.6±1.5) and peaked on days 3, 8, and 15. The study found the most common adverse effect reported was pain (13.9%) and pruritus (8%).

Goldenberg et al. conducted a randomized, double-blinded, vehicle-controlled trial to evaluate the safety, tolerability, and efficacy of 0.015% IMB applied 3 weeks after cryotherapy to AKs on the face and scalp.[31] The mean (95% CI) composite LSR score at day 3 in patients treated on the face (n=120) was higher 9.3 (95% CI; 8.5–10.1) compared

to the scalp (n=36) 5.8 (95% CI; 4.3–7.4). This may be due to thinner skin and fewer hair follicles on the face compared to the scalp.[31] Scalp AKs can be thicker, and therefore absorb IMB less efficiently than the face.[31] This study showed that face LSR severity in patients receiving combination therapy with cryotherapy and IMB was similar to LSR scores seen with IMB alone in Lebwohl et al.[23]

Bettencourt et al. conducted a retrospective chart review from a community dermatology practice to study 78 males treated with 0.05% IMB on the scalp.[32] Usually, for the scalp, 0.015% IMB is recommended. However, the author noted that in his own practice, 41% of patients who received 0.015% formulation had persistent AKs, so he used 0.05% instead. Additionally, 83% of the patients reviewed had received cryosurgery 2 weeks before IMB treatment for the scalp AKs. The study noted all patients experienced erythema (n=78, 100%), and a majority had flaking/scaling (n=76, 97%) and crusting (n=48, 66%). Most patients experienced mild to moderate reactions that resolved in 2 weeks, except one patient whose reaction did not resolve until day 20. This study shows that IMB-induced LSR affects a majority of patients

A limitation of this study is that AK clearance in patients was not compared to those treated with cryosurgery alone or with 0.05% IMB gel alone. Since most patients had undergone cryotherapy 2 weeks prior to IMB, it is difficult to determine how much LSR was caused by IMB versus residual inflammation from cryotherapy. Therefore, the author cannot know to what extent the 0.05% IMB formulation contributed to AK clearance or LSRs. Also this was a retrospective study conducted from a single dermatology clinic, which provides a very limited sample size. This study only looked at males, so we also do not understand the effects this would have on a female population.

Both IMB and methyl-aminolevulinate photodynamic therapy (MAL-PDT) are preferred field therapies because of their short duration times. MAL-PDT needs only one therapeutic session, with a second session required only if there is partial removal. However, both field therapies have significant side effects including erythema and pain.

Genovese et al. conducted an intra-individual study that compared the effectiveness, tolerability, and patient preference of daylight-photodynamic therapy with methyl-aminolevulinate (D-PDT-MAL) vs IMB in patients with grade I and II AKs on the face and scalp.[33] This study analyzed 27 patients with a total of 323 AKs, of which 215 were grade I and 108 were grade II. The mean number of AKs in a treatment group were similar (D-PDT-MAL 6.2 ± 63.4 vs IMB 5.7 ± 63.6; p=0.4). Mean AK clearance rate at 3 months was found to be similar between the two groups (D-PDT-MAL 72.4% vs IMB 73.6%; p=0.74). D-PDT-MAL was associated with lower LSR scores at week 1 and month 1 as compared to IMB. At week 1 average LSR score was 2± 1.1 (range 0–4), for D-PDT-MAL areas and 8.9± 3.8 (range 2–15) for IMB areas (ΔLSR= 6.9; p<0.0001). At 1 month, mean LSR score was 0.5± 0.7 (range 0–3) for D-PDT-MAL areas and 3.2± 1.9 (range 0–6) for IMB areas (ΔLSR= 2.7; p<0.0001). Average patient Visual Analog Scale (VAS) satisfaction score was greater for D-PDT-MAL compared to IMB (8 ± 2.1 vs 7.5 ±2.1; p=0.15). Although the results were not statistically significant, 14 (56%) of patients preferred D-PDT-MAL compared to 3 (12%) that preferred IMB. This study also noted that clearance rate of grade I and grade II AKs treated with IMB was similar at 3 months (76.7% vs. 72.8%; p=0.28).

In a similar randomized, split-faced study, Moggio et al. evaluated treatment outcomes, such as erythema, pain, and clearance for D-PDT-MAL vs IMB.[34] In this

study, a total of 22 patients with 311 AKs were enrolled at the University of Brescia, Northern Italy. The mean pain VAS score was higher in IMB compared to D-PDT-MAL (3.55 ± 1.82 vs 2.05 ± 0.72; $p < 0.01$). The mean LSR score was also higher in IMB (9.91 ± 4.24 vs 4.59 ± 4.03; $p < 0.01$). However, AK clearance at 3 months was found to be similar between IMB and D-PDT-MAL (75.8% vs 77.9%; 95% CI from −0.21 to 0.11).

Similarly, Zane et al. conducted a single-center, prospective, open-label, split-face, clinical trial to compare efficacy, LSR, and patient preference of MAL-PDT versus 0.015% IMB on the treatment of face and scalp AKs. Using 35 patients, complete clearance of AKs was similar between IMB and MAL-PDT at 3 months (62.9% vs 67.1%; p= non-significant). Pain score was determined using the VAS, which showed that IMB was less painful than MAL-PDT (3.74 ± 2.28 vs 5.46 ± 3.05; $p < 0.01$), which differs from Moggio et al. However, LSR was still found to be more severe with IMB compared to MAL-PDT (11.17 ± 5.29 vs 6.69 ± 2.88; $p < 0.01$). In a survey eliciting patient preference, it showed patients preferred MAL-PDT (60% vs 40%). Both these studies demonstrated that the efficacy between MAL-PDT and IMB was not statistically different, but patients preferred MAL-PDT over IMB, which could be due to better cosmetic outcome. This can have a strong impact on the adherence of patients, especially if they are elderly or frail, and can influence the effectiveness of the treatment in real life.[35]

Reviewing studies of IMB-induced LSR shows a wide variability in patients. This is parallel to what Longo et al., reported in a case series (n=4) of patients treated with IMB with maximum LSR scores on the face and scalp ranging from 11 to 23.[36] Although studies state that patients experience mostly mild to moderate LSRs during AK treatment

with IMB, there is an unpredictability in knowing who will experience severe reactions. We have also seen that higher concentrations of IMB are more efficacious, but are generally less tolerated as they induce more LSRs.

2.2.4 Relationship Between Inflammation and Efficacy of IMB

Jim et al. conducted a regression analysis to determine if the extent of AK clearance was determined by the intensity of the inflammatory LSRs. They collected data from 2 double-blinded, randomized studies (n=218) that evaluated IMB 0.015% for treating AKs of the face and scalp. The analysis looked at week 8 AK count, compared to baseline, along with day 4 LSR to create a 90% prediction for percent reduction in AK count. Results showed the mean reduction from baseline in AK count was 78% (95% CI; 73%-81%) and the mean day 4 composite LSR score was 9.2 (95% CI; 8.7- 9.8). The regression predicted that a composite LSR score of 15 will create an expected percentage reduction in AKs of 88.1%, LSR score of 10 an expected reduction of 80.7%, and LSR score 5 an expected reduction of 68.6%.

This study is the only study designed to analyze a possible relationship between composite LSR score and treatment efficacy. However, it has only looked at 2 randomized controlled trials to conduct its regression analysis. The effect of corticosteroid on IMB is not well understood. The mechanism of action of corticosteroid is multi-factorial, thus, we cannot accurately predict how it will interact with the neutrophil-mediated inflammatory response. It is unclear if this possible interaction will decrease LSR scores and thus decrease efficacy, or, as seen in previous studies, it will have no impact on efficacy.[20,21]

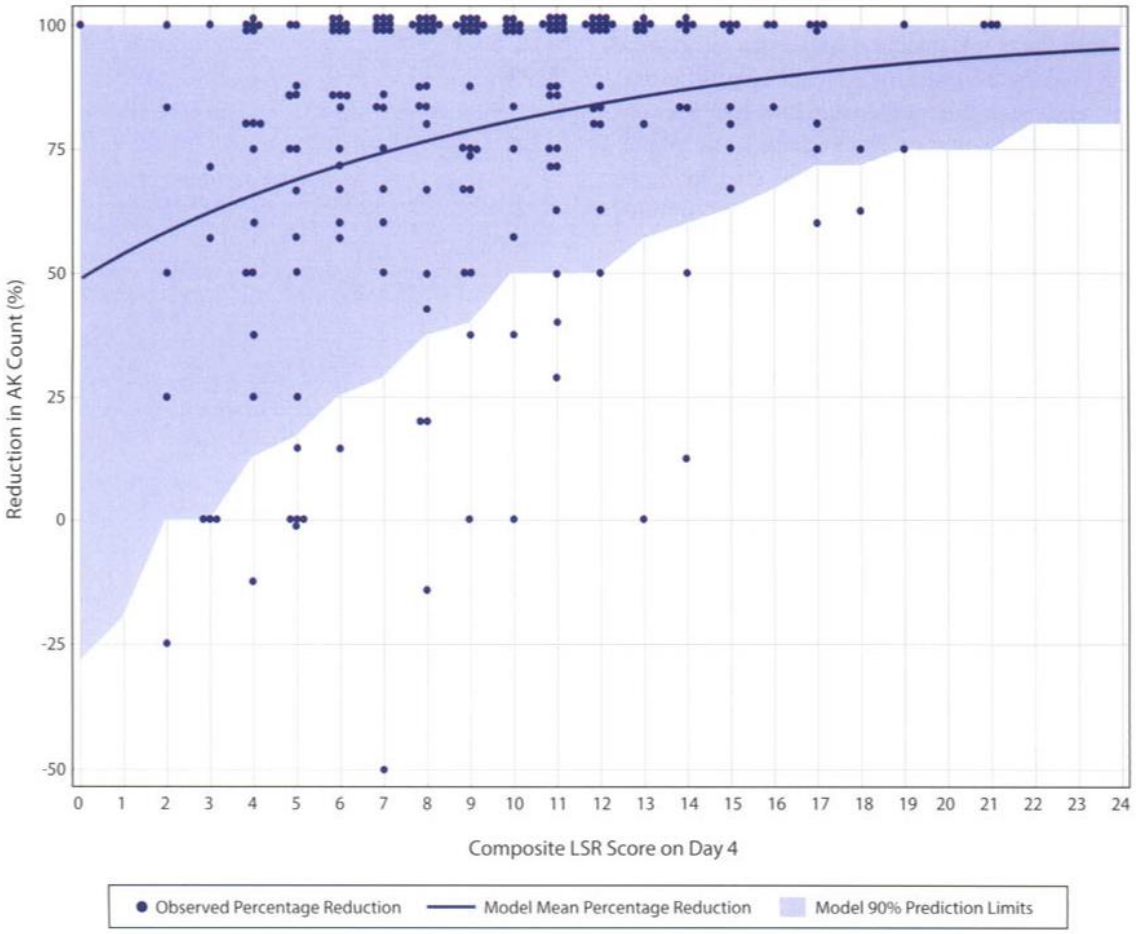

Figure 1. Expected percentage reduction from baseline for face-treated AKs based on regression analysis of AK counts at day 57 and composite LSR Score on day 4.

2.2.5 Role of Combination Therapy with Corticosteroid

A limited number of studies have investigated the role of combination therapy with corticosteroids to reduce AK treatment-induced LSR. In the past 40 years, a handful of studies have conducted randomized controlled trials to examine the effect of corticosteroids on first-line AK field therapies, such as fluorouracil, cryotherapy, PDT, and IMB.

In dermatology practice, the application of topical corticosteroids 15 minutes after 5-fluorouracil cream has been shown to be helpful in reducing the inflammatory response.[37] Breza et al. was the first study to examine the impact of triamcinolone on fluorouracil-induced inflammation.[38] In a randomized, intra-individual, trial at the Veterans Administration Hospital in Miami, patients with moderate to severe AKs (n=19) were treated on both sides of the face with 1% fluorouracil in propylene glycol. This acted as the control, while one side of the face was chosen as the experimental side, in which three different interventions were used. Group 1 (n=5) had treatment with 0.4% triamcinolone acetonide dissolved into the 1% fluorouracil solution of propylene glycol. Group 2 (n=5) had 0.5% triamcinolone acetonide cream applied 10 to 15 minutes after the solution dried and Group 3 (n=5) had 0.1% triamcinolone acetonide cream likewise applied 10 to 15 minutes after the solution dried. During the initial four weeks of the study, two physicians judged the degree of redness, pruritus, dryness, irritation, inflammation, and crusting on each side of the face, and determined that Group 1 and 2 had noticeably suppressed inflammation. Findings were reported as none, left side greater than right, right side greater than left, or equal on both sides. The physicians were not blinded, the sample size was limited, and there was not an independent, quantitative grading scale used to assess the reduction of inflammation. However, this was the first study that attempted to study the impact of corticosteroids on reducing LSRs induced by AK therapies. It also showed that triamcinolone of higher potency may be more effective in treating inflammation without affecting the efficacy of treatment.

Two studies examined the efficacy of clobetasol propionate to reduce inflammation caused after cryotherapy. In a double blinded, randomized, placebo-

controlled trial, Hindson et al. studied 19 patients with basal cell carcinoma (BCC) and 18 patients with viral warts of the hands.[18] Patients were randomly assigned to application of clobetasol propionate or an ointment base immediately after cryotherapy. The degree of inflammation was estimated by measuring the volume of the lesions before and after cryotherapy via a mold formed by alginate dental cement. Results showed that 24 hours after cryosurgery there was a reduction in the percentage volume increase for clobetasol propionate versus ointment group in BCC patients (44.81± 21.95 vs 145.83 ± 44.72; $p<0.001$). Similar results were seen in patients with viral warts (53.50 ± 50.20 vs 183.0 ± 126.0; $p<0.02$).

There are several limitations to this study. Firstly, there is no evidence showing that alginate dental cement volume measurements are an accurate way of determining inflammation. Secondly, patients with BCCs got a local injection of 0.1% lignocaine prior to cryotherapy and it is unclear what impact, if any, receiving anesthetic caused on reducing inflammation. The average size of the lesions prior to treatment was not revealed in the study, so it is unclear if smaller lesions were chosen predominantly for the clobetasol propionate group while the ointment base received larger lesions that may cause more erythema after cryotherapy. Although this study was conducted on patients with BCC and warts, it is the first study to examine the benefit of corticosteroids on reducing inflammation caused by cryotherapy.

To investigate the impact of clobetasol propionate on cryotherapy-induced erythema on normal skin, Humphreys et al. developed a smaller, single-blinded, intra-individual, randomized controlled trial, using 10 patients.[19] These patients applied cryotherapy on both arms, but applied 0.05% clobetasol propionate only on one. Both

arms were occluded with dressing for 4 hours to increase the penetration of the treatment. Independent evaluators measured erythema at 24 and 48 hours using a reflectance instrument that obtains an erythema index, measured via the blood content of the dermis. Results showed a significant reduction in the mean change in erythema among patients that applied 0.05% clobetasol propionate after cryotherapy compared to those with cryotherapy alone at 24 hours (40 ± 10 versus 85 ± 20; $P<0.05$) and 48 hours (82 ± 16 versus 137 ± 20; $P<0.05$). This trial was conducted with a small sample size and as a result it is difficult to evaluate the external validity of this study. Additionally, the reliability of the erythema index used to evaluate inflammation is unknown.

More recently, Wiegell et al. conducted a randomized controlled trial to evaluate the effect of clobetasol propionate treatment just before and after PDT treatment for AKs to reduce inflammation without compromising efficacy.[20] Post-treatment erythema is a major side effect of PDT and this prevents its usage in large areas.[39] This intra-individual study looked at 22 patients with AKs in the face and scalp and randomized them to combination therapy with clobetasol propionate 15 minutes before and after treatment, or to monotherapy with PDT alone.

Erythema was measured subjectively using a visual 4-point scale by blinded investigators the day after PDT treatment. It was also measured objectively using a skin-reflectance meter that measures skin remittance at 558 nm and 660 nm and calculates the content of melanin and hemoglobin in the skin. Erythema was scored on a scale from 0 to 100 and the mean value from measurements at five different sites was used for statistical analysis. Erythema was measured three times: before the application of topical steroid and lesion preparation, just after red LED illumination, and the day after PDT

treatment. The primary outcome measure of the study was increased erythema measured by the skin-reflectance meter, 1 day after treatment compared with baseline.

Results showed that clobetasol propionate significantly reduced erythema 24 hours after treatment in the corticosteroid-receiving PDT lesions compared to PDT alone (48.4 vs 52.8; p =0.007). The total erythema increase from baseline was only 7 points in the combination group versus 16 points in the monotherapy group (P=0.012). At a 3-month follow-up a total of 22 new AK lesions had developed in the monotherapy group versus 21 in the combination therapy (P = 0.58), suggesting that steroid treatment did not affect efficacy.

Erlendsson et al. is the only randomized controlled trial that has looked at the effect of clobetasol propionate after IMB treatment.[21] In a blinded, intra-individual, randomized controlled clinical trial looking at 21 patients with Grades I to III AKs on the face or scalp, two areas were treated with 0.015% IMB daily for three days. One area was randomized to receive topical 0.05% clobetasol propionate twice daily for 4 days. Assessments included LSR (0-24; days 1, 4, 8, 15, 57), pain (0-10) and pruritus (0-3; days 1-15), AK clearance (days 15, 57), and cosmetic outcome (0-3; day 57).

Control and treatment groups had similar LSR scores at day 4, prior to clobetasol propionate initiation (IMB 9.95 vs IMB+ clobetasol propionate 9.52; P = 0.285). Clobetasol propionate application was performed from days 4-7; day 8 results showed LSR between the two groups was not significant (IMB 6.81 vs IMB+ clobetasol propionate 6.81; P = 0.939). LSRs returned to baseline in both groups (IMB 0.67 vs IMB+ clobetasol propionate 0.38; p=0.250) by week 2. Pain was mild to moderate in patients and peaked at day 3 (IMB 2.6 vs IMB+ clobetasol propionate 2.9; p = 0.500) and

declined gradually thereafter. Pruritus peaked on day 7 (IMB 1.0 vs IMB+ clobetasol propionate 1.2; p = 0.312). There was no difference found in pain between the two groups. However, pruritus on day 9 was greater in the combination group (0.8 vs 1.1; p=0.042). AK clearance between monotherapy and combination therapy at week 8 was similar (86% vs 86%; p =0.991).

IMB is generally used in AKs grade I and II, but this study also looked at the efficacy of IMB in Grade III hyperkeratotic AKs. Results of all AK grades were combined and presented together so it is unclear how IMB-induced LSR differed in patients with Grade III AKs.[40] The study demonstrated that although the application of a glucocorticoid after finalized IMB treatment does not alleviate IMB-induced LSR, pain, or pruritus, the treatment does exert a therapeutic effect on all AK severity grades.

A limitation of Erlendsson et al. includes their short follow-up time as they did not analyze the efficacy and safety of the treatment after 2 months. Additionally, the study was designed to have enough power to detect a relative reduction in LSR scores, but was not powered to assess efficacy between outcomes.

2.2.6 Reviews of Studies Analyzing Possible Confounding Variables

Since this is an intra-individual study there are limited confounding variables. Baseline demographics of the patients will be analyzed for age, gender, race, ethnicity, geographic location, Fitzpatrick skin type, history of prior skin cancer, or previous AK treatments..[23] Lebwohl et al. found that majority of patients with widespread AK damage were Fitzpatrick I or II, approximately half had a history of skin cancer, and more than 75% had received prior cryotherapy.[23] AK characteristics such as mean AK lesion count

and location of lesions on the face will also be evaluated.[31] The size and location of the AKs can potentially impact the degree of irritation caused by IMB and thus the ability of the corticosteroid to influence this irritation.[34] If the severity of LSRs is related to selective absorption by abnormal skin, the degree of absorption into the abnormal AKs may be another differentiating factor.[31]

2.3 Review of relevant methodology

2.3.1 Study Design and Setting

Multiple studies have assessed the efficacy and safety of ingenol mebutate using an intra-individual randomized controlled trial.[21,33,38,41] This has several advantages because it reduces confounding and inter-patient variability.[3] Our study will focus on participants chosen from the West Haven VA as this includes a representative population of patients that generally develop AKs.[38,42] As seen in Erlendsson et al. and Moggio et al. randomization of lesions receiving clobetasol propionate will be done via consecutively numbered, closed, nontransparent envelopes containing a computer-generated allocation.[21,34] Based on our hypothesis that patients without corticosteroids will experience greater LSRs, it will likely be difficult to blind patients, so we will conduct a single-blinded study.[23,26]

2.3.2 Selection Criteria

Inclusion criteria will include patients with the presence of four to eight clinically typical, visible, and discrete AKs within a 25 cm^2 contiguous field on the face.[21,23,31] Goldenberg et al. showed that LSRs are less severe on the scalp versus the face.[31]

Lebwohl et al. demonstrated the complete clearance was greater on the face (47.3% vs 4.1%; P < .001) compared to the scalp (22.8% vs 2.0%; P = .001).[23] Thus, to reduce confounding, our proposed study will only evaluate patients with AKs on the face.

The median age of patients in randomized controlled trials looking at IMB is generally between 60-75, because elderly patients are more likely to get AKs.[20,31] Prevalence in the southern hemisphere is thought to be 60% in individuals over the age of 40 years.[30] Thus, our selection criteria will include patients above the age of 40, which would effectively represent 80% of the veteran population living in Connecticut.[42] There are no well-controlled studies of IMB gel in pregnant patients, therefore female subjects must be of either non-childbearing potential, post-menopausal, or use some form of contraception.[34] All patients must have the ability to follow trial instructions and written informed consent must be obtained prior to any trial-related procedures. Agreement from the subjects must allow photographs of the selected treatment area to be taken and used as part of the study data package.

IMB gel has not been well studied in certain populations so patients will not be enrolled if they had a recent transplantation, are immunosuppressed, have other severe systemic infections, or Olsen's grade III AK.[34,43] Patients with known allergies to any molecule in IMB or corticosteroids will also be excluded.[44,45] As seen in Moggio et al. patients will be excluded if they have had any prior field therapy, including IMB, for their AKs within a period of 6 months.[3,34] Areas within 5 cm of an incompletely healed wound or within 10 cm of a suspected BCC or SCC will not be included in the study.[23] Additionally, as seen in Erlendsson et al. and Lebwohl et al., we will exclude patients who recently used medications or treatments that could interfere with study results (e.g.,

topical medications, artificial tanners, immunomodulating agents, cytotoxic drugs, UVB phototherapy, corticosteroids, or an oral retinoid).[21,23]

2.3.3 Intervention and Method of Administration

The safety in IMB is most commonly evaluated by LSR.[1,4,31,34] Of note, the FDA has recently reported several cases of severe allergic reactions and herpes zoster associated with the use of IMB.[29] There are also reports of severe eye injuries that have occurred with incorrect IMB usage, involving patients accidentally transferring IMB from the hands to eyes or lips via cosmetic application or insertion of contact lenses.[29] No clinical IMB trial has reported these adverse effects in patients. Although our study will not focus on these side effects, we will include detailed information regarding these safety issues on our patient consents so participants are aware and know how to avoid them. Instructions on home application of treatment and importance of proper hand wash will be presented both on paper and verbally. Researchers and patients will apply a one unit-dose tube of 0.015% IMB gel to cover the chosen 25cm^2 areas and let it dry for 15 minutes before applying corticosteroids.

Topical corticosteroids can cause local side effects such as epidermal thinning, dermal striae, atrophy, telangiectasia, purpura, and tachyphylaxis.[15] Systemic side effects such as suppression of HPA axis, growth stunting in children, and Cushing's syndrome have been reported, but are rare. The systemic effects of topical corticosteroids depend on how they are absorbed through the skin and the pharmacokinetics and potency of the corticosteroid chosen.[44] Absorption can increase depending on factors such as steroid occlusion, application site, skin integrity, and application frequency.[12] Adverse effects

increase when potent steroids are applied over large areas. This study will attempt to reduce these effects by applying a thin layer of clobetasol propionate 0.05% ointment, without occlusion, to a small ($25cm^2$) treatment allocated area once a day[21,22] for a maximum duration of 8 days, thus limiting overall systemic absorption and side effects.

2.3.4 Outcome

Primary Outcome: Local Skin Reaction at day 4 and AK clearance at day 57

Rosen et al. developed an objective and quantitative scale that includes six typical LSRs: erythema, flaking/scaling, crusting, swelling (edema), vesiculation/pustulation, and erosion/ ulceration. They are measured from a scale of 0-4, for a cumulative sum of 24. There are accompanying photographs that correlate the severity of LSR to the numerical scale. This scale has been verified by the Australian College of Dermatologists, shows good inter-observer grading concordance, and has been used in several studies to characterize LSRs after IMB application.[4,31,36,46]

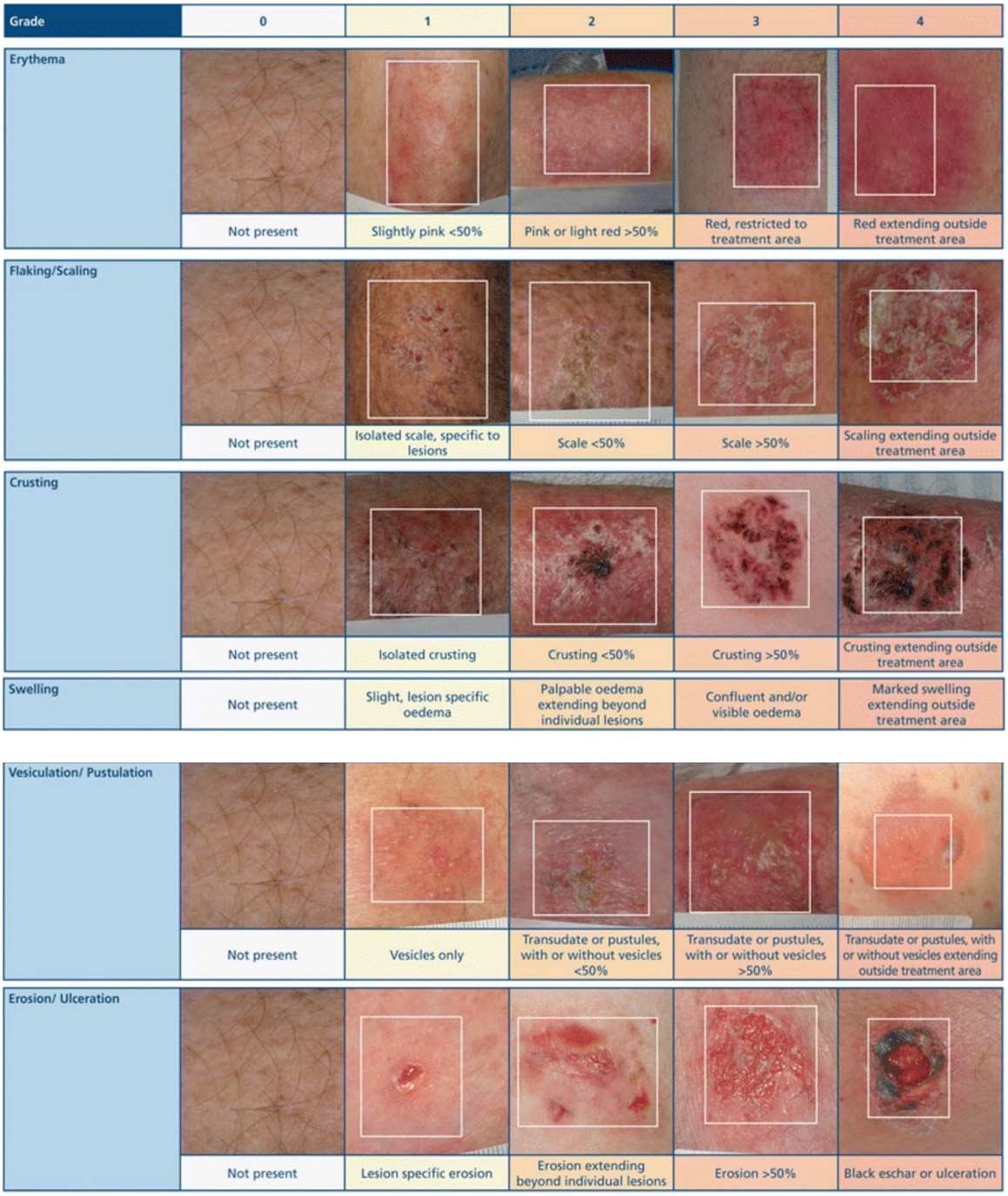

Figure 2. The LSR grading scale is a quantitative scale for the evaluation of LSRs arising from topical ingenol mebutate treatment.

Jim et al., showed that the absolute reduction in LSR scores was dependent on the day 4 composite score.[40] A total of 220 patients were treated for AKs on the face, and 56 patients were treated for AKs on the scalp. A simple regression model showed that the composite LSR score on day 4 can be used to predict the week 1, 2, 4, and 8 composite LSR scores and is a significant predictor in the resolution of LSRs. The importance of assessing the LSR severity on the 4th day has also been established in other studies.[23,26,27] Thus, our study will also use the mean LSR composite score on day 4 as the main data point to evaluate safety in the two groups and predict overall outcomes.

Several studies have established the 57th day of treatment as an endpoint for AK clearance.[21,23,46] Our study will emulate these data and measure a reduction of 75% or more in the number of clinically visible and dermoscopy AKs in the target treatment area at week 8.

Secondary Outcome: Pain, pruritus, and AK clearance at 1 year

Our secondary outcomes will measure differences in pain and pruritus scores as these are the most common side effects reported after the usage of IMB.[21,27] There are limited studies specifically stating days when application site pain and pruritus were analyzed, so we will follow Erlendsson et al. and measure pain (0-10) and pruritus (0-3) using a VAS from days 0-15. Patients will record pain and pruritus using a VAS log at home and they will also be asked about this during office visits.[21] In order to establish long term efficacy, we will also analyze AK clearance at 12 months.[25,26]

Adherence:

Our study will use a patient adherence survey and verbal questioning to assess medication compliance. Studies have consistently shown that patient-reported adherence is higher than objective measures of adherence.[47] Measurements using Medication Event Monitoring System cap to measure overall adherence can be used as a more objective measure of adherence. However, this tool will not tell us how the medication was used, when it was applied, to what it was applied, and how much was applied to each area.[48]

Sample Size and Statistical Significance

Many intra-individual studies have calculated the sample size using a significance level of 0.05 and a power of 80%.[20,21,33,34] We have two primary hypotheses to assess improvement in safety and non-inferiority in efficacy between combination therapy and monotherapy. It is not reasonable to calculate our sample size based on a superiority test since testing a non-inferiority hypothesis will require a larger sample size.[49] Thus, we will be using a two-sided one-sample t-test to calculate our sample size. Erlendsson et al. reported the LSR for IMB group was 9.95 before application of clobetasol propionate.[21] We will calculate our sample size based on a standard deviation of 4 and a 2.0 difference in relative reduction, which corresponds to 20% reduction after treatment.[21] Using the Power Analysis and Sample Size software and a two-sided one-sample t-test with alpha=0.05, we calculated a sample size of 34 **(Appendix A)**.

Our second hypothesis is testing non-inferiority in efficacy between the treatment and control group. Garbe et al. established a 15% -55% difference as significant for AK

clearance, and Moggio et al. established a non-inferiority margin of 20%.[26,34] Since no study looking at corticosteroid combination therapies has established a non-inferiority margin, we will extrapolate data from these two studies. We used McNemar's test to determine a sample size of 34 patients will have 80% power to detect difference in paired proportions of 26% - 34% between two arms. We will use this sample size to evaluate our primary outcome of AK clearance. To ensure adequate sample size, we will account for a 5% lost to follow-up, which brings our sample size up to 36.[21]

2.4 Conclusion

In conclusion, multiple studies have hypothesized that the inflammatory response generated by IMB is caused by neutrophilic infiltration.[36] There are various theories on the role of corticosteroids on neutrophils, however several studies have shown that it works to reduce inflammation by preventing the transmigration of neutrophils to the inflammatory loci.[5,13] Although several studies have analyzed the impact that corticosteroids have in reducing erythema associated with AK field therapies, only one study has previously looked at the impact of combining glucocorticoids with IMB to study its efficacy and safety. This will be the first study done in the United States to study the application of clobetasol propionate before, during, and after IMB treatment and analyze the impact on LSR reduction and long term efficacy. By conducting this study, we will understand whether the immunosuppressive properties of corticosteroids will counteract the immune-stimulating effects of IMB and render it ineffective, or if the inflammatory response can be curbed by corticosteroids without affecting its efficacy.

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CHAPTER III: STUDY METHODS

3.1 Study Design

We will conduct a single-centered, intra-individual, single-blinded, randomized control trial. The investigators will be blinded to the intervention, however, participants will not be blinded due to the nature of the study.

3.2 Study Population and Sampling

Our study population will be patients of the Dermatology Clinic at the West Haven VA. A convenience sample of veterans aged 40 and above that meet the inclusion criteria will be chosen as participants.

3.2.1 Inclusion Criteria

Patients aged at least 40 with the presence of four to eight clinically typical, visible, and discrete AKs within a 25 cm^2 contiguous field on the face or scalp. Female subjects must be of either non-childbearing potential, post-menopausal, or have a confirmed clinical history of sterility (e.g. hysterectomy). Women must consent to using highly effective methods of contraception defined as abstinence, vasectomized partner, an intrauterine device, or oral contraceptives. All patients must have the ability to follow trial instructions, and written informed consent must be obtained prior to any trial-related procedures. Subjects must allow photographs of the selected treatment area to be taken and used as part of the study data.

3.2.2 Exclusion Criteria

Excluded patients will include those with recent transplantation or immunosuppression, other severe systemic infections, Olsen's grade III AK and/or invasive tumors within the treatment area, recent use of medications or treatments that could interfere with study results (e.g., topical medications, artificial tanners, immunomodulating agents, cytotoxic drugs, UVB phototherapy, corticosteroids, or an oral retinoid), known allergies to any molecule in IMB or clobetasol propionate, pregnancy or lactation, prior topical treatment for AKs within a period of 6 months, likelihood of poor compliance, or an inability to fully consent to the study. Areas within 5 cm of an incompletely healed wound or within 10 cm of a suspected BCC or SCC will also be excluded.

3.3 Subject Protection and Confidentiality

This study will require approval by the Institutional Review Board (IRB) and Human Investigation Committee (HIC) at West Haven VA where subjects will be recruited and the study conducted **(Appendix B)**. In accordance with HIPPA Privacy Rule all participant records, photographs, and identifiers will be protected. All patients will be assigned a unique code that will serve as their identifier throughout the course of this study and protect participant confidentiality. All electronic records and patient information will be password protected and encrypted on computers. Access to patient records will be provided to the dermatology team directly involved in the care of the patient. Any physical records or paper consents will be stored in a locked cabinet at the WHVAMC Building One and will be shredded once the data analysis is complete.

3.4 Recruitment

Recruitment will be directed towards all veterans aged 40 and above who are patients in the dermatology clinic at the West Haven VA. Recruitment flyers **(Appendix C)** will be posted in the dermatology clinic for patients. A letter to the dermatologist team at the West Haven VA will be sent out asking for their participation in the study and their help in recruiting subjects **(Appendix D)**. Trained research personnel at the site will identify potential study participants established by their diagnosis of AK. Consent for participation will be obtained before subjects get assessed for eligibility based on the inclusion criteria.

3.5 Study Variables and Measures

Two symmetrical contralateral areas of 25 cm^2, harboring a similar (4-8) number of AKs, will be selected in an individual. Through randomization these areas will get assigned monotherapy or combination therapy. Randomization will be done using consecutively numbered, closed, nontransparent envelopes, which will contain a computer-generated allocation.

The control for this study is monotherapy with 3 days of 0.015% IMB gel application to the allocated areas on the face or scalp. The intervention for this study is 0.05% clobetasol propionate ointment. Patients will apply a thin application of 0.05% clobetasol propionate ointment to the assigned treatment area in addition to the standard 3 days of 0.015% IMB gel. They will wait 15 minutes after IMB application to apply the clobetasol propionate. The first application of clobetasol propionate will be applied by

the researchers on day 0. The intervention area will receive clobetasol propionate both before, during, and after 0.015% IMB gel therapy.

The primary outcomes will be LSRs on day 4 and AK clearance efficacy on day 57. LSRs, which will be recorded quantitatively via photographic guides and a well-defined LSR grading scale. It will include erythema, flaking or scaling, crusting, swelling, vesiculation or pustulation, and erosion or ulceration. The scale will range from 0 to 4 with higher numbers indicating greater severity. The mean composite LSR score will be the average of the composite LSR scores of the treated AKs and it will be recorded at each office visit. AK clearance efficacy will be defined as more than 75% visual and dermoscopy clearance at day 57.

Secondary outcomes will assess the long-term follow-up of AK clearance at 12 months. Application site pain (0-10) and pruritus (0-3) are commonly reported as adverse effects of IMB and will also be assessed from days 0-15. Patients will record pain and pruritus using a VAS at home and they will also be asked about this during office visits **(Appendix E)**.

3.5.1 Blinding of Intervention

Investigators that are blinded to the treatment allocation will be assessing patients during follow-up office visits to determine their LSR scores. Patients cannot be blinded because IMB therapy will induce LSR and according to our hypothesis clobetasol propionate will decrease inflammation on the treatment area.

3.5.2 Assignment of Intervention

At the time of enrollment at least two symmetrical AK sites will be identified on the patient to undergo randomization. Each patient will also receive a unique identifier code using a computer generated randomization program to de-identify their personal information. A thin application of 0.05% clobetasol propionate ointment will be applied on the chosen AKs in office on day 0. Patients will be given a 15g tube of 0.05% clobetasol propionate ointment to take home and continue application once a day until day 7. Veterans will be provided with written instructions and treatment supplies required for the study **(Appendix F).**

3.5.3 Adherence and Safety

Adherence will be monitored via self-administered patient medication logs **(Appendix G)**. Patients will also be verbally questioned about their medication compliance during clinic visits. Patients will be reminded by investigators to administer appropriate clobetasol propionate and IMB doses at each appointment. If major adverse effects occur as a result of corticosteroids or IMB, individual patient safety will be assessed to determine if the patient should continue the trial.

3.6 Data Collection

There will be two independent investigators that will evaluate the LSRs, take photographs, and assess patient pain and pruritus using VAS Scales during each office visit.

Initial Assessment: Day 0

During office visits, patients will be identified by the residents working at the West Haven VA Dermatology Clinic. During the initial assessment meeting patients will be given information about the research study and their eligibility for the study will be determined. Researchers will review the patient's electronic medical record to ensure they have not received topical treatment for their AKs in the past six months, and that they meet criteria for participation. Once patients meet the inclusion and exclusion criteria they will have photographs taken of the AK lesions at baseline and then clobetasol propionate will be applied to the allocated area.

Follow-up assessment: Day 1, 4, 7, 15, 57 and 12 months

On day 1 patients will return for their first IMB application to both the control and treatment areas. Researchers will demonstrate the proper application of IMB so patients can apply the product appropriately at home. During each follow-up visit two blinded investigators will take photographs and conduct LSR assessment. We will also review patient's daily log of pain and pruritus and assess it again during office visits via the VAS method. At day 57 overall lesion clearance will be dichotomized into PR (partial resolution of at least 75% visible or dermoscopy AKs) or NR (no resolution or resolution less than 75%). We will study the long term efficacy of treatment by having the patients return at 12 months.

Table 1. Follow-up Assessments

	Screening/ Day 0	Day 1	Day 2	Day 3	Day 4	Day 5-6	Day 7	Day 15
Office Visits	x	x			x		x	x
Photographs	x	x			x		x	x
Informed Consent	x							
Corticosteroid Application	x	x	x	x	x	x	x	
Ingenol Mebutate Application		x	x	x				
Pain & Pruritus Measured	x	x	x	x	x	x	x	x

3.7 Sample Size Calculation

Using the Power Analysis and Sample Size software we determined that using a two-sided t-test with alpha=0.05, gives us a sample size of 34 **(Appendix A)**. This will provide us 80% power to detect a relative reduction in LSR score of 2.0 (SD 4.0), which corresponds to a 20% reduction after treatment. Using McNemar's test and the sample size of 34 patients, we will have 80% power to detect differences in paired proportions of 26% - 34%. To ensure adequate sample size, we will account for a 5% lost to follow-up, which brings our sample size up to 36.

3.8 Analysis

The study will use intention-to-treat analysis with statistical significance considered for p-values < 0.05. Primary outcomes such as the mean composite LSR scores will be assessed utilizing the quantitative scale created by Rosen et al. AK clearance will be dichotomized into PR and NR. Pain and pruritus will be measured using a VAS. Wilcoxon signed-rank test will be used to analyze ordinal values such as LSR, pain, and pruritus. McNemar's test will be used to analyze dichotomous variables such as AK clearance.

3.9 Timeline and Resources

Recruitment, randomization, data collection, and data analysis will be completed for this study within two years. Recruitment period will begin January 2018 and will continue until October 2018. Data collection and data analysis will be continuous during that period and this will allow us enough time to conduct a 12 month follow-up. Due to the patient volume at the West Haven Dermatology Clinic we are not anticipating difficulty obtaining the 36 patients required for the study.

The West Haven VA will have a designated primary investigator responsible for oversight of the trial. The Principal Investigator of this study will be Dr. Suguru Imaeda and the Co-Principal Investigator will be Shreya Amin, PA-SII. Two dermatology residents will be the researchers at the site and will be responsible for identifying and screening potential participants, obtaining informed consent, collecting baseline and follow-up data, and performing any additional tasks that may be required during the trial. Two separate, blinded residents will be the investigators responsible for evaluating LSR,

pain, and pruritus scores during each visit. Data analysis can occur after eight weeks of treatment application and will continue until December 2020. The Yale School of Medicine will provide funding and resources for the study.

CHAPTER IV: CONCLUSION

4.1 Advantages and Disadvantages

There are a number of strengths to this proposed study design. The intra-individual study design is a great advantage because it minimizes confounding factors by allowing individuals to be compared only to themselves. This prevents inter-patient variability in the response to IMB or corticosteroids. Randomization of sites getting treatment allocation is determined by a computer, which prevents selection bias by the researchers.

Conducting research at a single center, such as the West Haven VA, significantly limits the demographics of the population studied. At the time of publication there are 213,420 veterans living in the state of Connecticut, 60% of them are over the age of 60, 88% of them are white, and 8% are female.[1] However, fair-skinned males with high cumulative sun exposure are typically the patients that suffer from AKs so this is representative of the study population. Thus, this should not affect the external validity of the study and its generalizability to patients suffering from widespread actinic damage. Also creating a single-centered study with a representative population prevents variability in the timing, delivery, and assessment of study interventions.

Convenience sampling will ensure that there is enough study population, however because of high patient volume at the West Haven Dermatology Clinic starting the study in January may skew the number of patients recruited in the winter months and limit the number of patients in the summer. Also, it is difficult to blind the subjects because of the nature of this study, which might result in subject bias. However, the short duration of application time will most likely prevent any crossover from occurring.

A disadvantage of this intervention includes its complicated application course. IMB is favored over other field therapies because of its short treatment course of 3 days. Applying corticosteroid before and after IMB can provide additional treatment burden for patients that may end up reducing overall adherence. Non adherent patients, or those with minimal social support may be less willing to complete the study or follow-up at 12 months after their lesions have resolved. However, through proper patient education and explanation of the effectiveness of corticosteroids to reduce LSR and improve IMB tolerability, patients may be satisfied with the treatment.[2]

Studies analyzing the impact of 0.015% IMB gel on the scalp have shown decreased efficacy and reduced LSR scores compared to the face.[3,4] As a result, our study is analyzing combination therapy only on the face where LSRs are reported to be more adverse. Excluding patients with scalp AKs is advantageous because it reduces potential confounders, however it limits the scope of our study. Further research can investigate the effect of combination therapy specifically looking at scalp AKs.

There are conflicting data about the effect of corticosteroids on IMB neutrophil-mediated inflammation. If combination therapy with a Class I corticosteroid, such as clobetasol propionate, has decreased efficacy compared to IMB alone at day 57 or month 12, further studies can look at combination with a lower potency corticosteroid. Previous research combining triamcinolone 0.5% cream and 5-fluorouracil demonstrated reduction of inflammation without impact on efficacy, so the impact of triamcinolone with IMB can also be investigated.

4.2 Clinical and/or Public Health Significance

LSRs are common adverse effects reported in patients using IMB for the treatment of AKs. Studies have shown LSR severity cannot be predicted before starting the treatment. While corticosteroids have been successfully used to reduce erythema caused by other AK field therapies, there are limited studies analyzing its use with IMB. Currently more research needs to be conducted to investigate the role of concurrent clobetasol propionate application with IMB to reduce LSRs. Studies have shown AK clearance improves with higher IMB concentrations.[5] However, patients are hesitant to apply IMB with higher concentrations over large treatment areas as this induces more inflammation,[6] which can potentially lead to increased cosmetic effects. If this study can show the success of corticosteroids in reducing LSR without affecting efficacy, we can expand the role of IMB to areas greater than 25 cm^2. Providers can also prescribe higher concentrations without worrying about treatment safety and tolerability. Additionally, for patients that suffer severe LSRs, pain, and pruritus using 0.015% IMB gel for face lesions, this combination therapy may provide significant relief, leading to improvement in cosmetic results and patient satisfaction.

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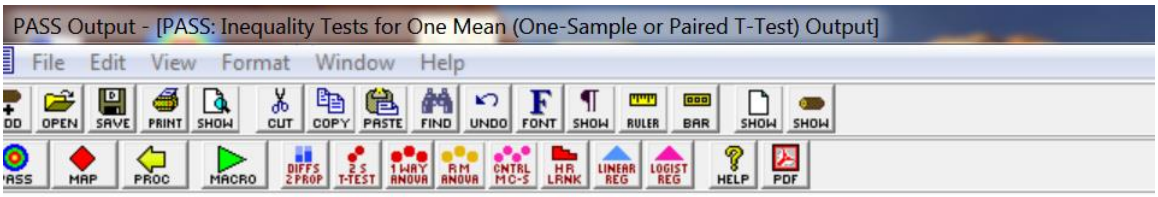
APPENDIX A: Sample Size

Sample size Calculation Using Primary Endpoint as 4-day LSR Score



One-Sample T-Test Power Analysis

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Numeric Results for One-Sample T-Test

Null Hypothesis: Mean0=Mean1 Alternative Hypothesis: Mean0<>Mean1
Unknown standard deviation.

Power	N	Alpha	Beta	Mean0	Mean1	S	Effect Size
0.80778	34	0.05000	0.19222	0.0	2.0	4.0	0.500
0.81711	23	0.05000	0.18289	0.0	2.5	4.0	0.625
0.82505	13	0.05000	0.17495	0.0	3.5	4.0	0.875

References

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Zar, Jerrold H. 1984. Biostatistical Analysis (Second Edition). Prentice-Hall. Englewood Cliffs, New Jersey.

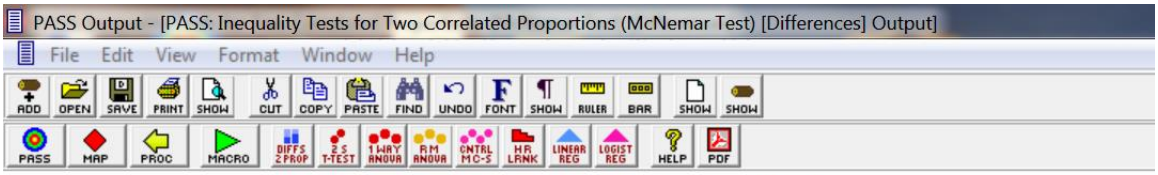
Report Definitions

Power is the probability of rejecting a false null hypothesis. It should be close to one.
N is the size of the sample drawn from the population. To conserve resources, it should be small.
Alpha is the probability of rejecting a true null hypothesis. It should be small.
Beta is the probability of accepting a false null hypothesis. It should be small.
Mean0 is the value of the population mean under the null hypothesis. It is arbitrary.
Mean1 is the value of the population mean under the alternative hypothesis. It is relative to Mean0.
Sigma is the standard deviation of the population. It measures the variability in the population.
Effect Size, |Mean0-Mean1|/Sigma, is the relative magnitude of the effect under the alternative.

Summary Statements

A sample size of 34 achieves 81% power to detect a difference of -2.0 between the null hypothesis mean of 0.0 and the alternative hypothesis mean of 2.0 with an estimated standard deviation of 4.0 and with a significance level (alpha) of 0.05000 using a two-sided one-sample t-test.

Sample size Calculation Using Non-Inferiority Test



McNemar Test Power Analysis

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Numeric Results for Two-Sided Test

Power	N	P10	P01	Difference (P10-P01)	Proportion Discordant	Odds Ratio	Alpha	Beta
0.80000	34	0.020	0.280	-0.260	0.300	0.072	0.05000	0.20000
0.80000	34	0.048	0.352	-0.304	0.400	0.136	0.05000	0.20000
0.80000	34	0.080	0.420	-0.341	0.500	0.189	0.05000	0.20000

References

Schork, M. and Williams, G. 1980. 'Number of Observations Required for the Comparison of Two Correlated Proportions.' Communications in Statistics-Simula. Computa., B9(4), 349-357.

Machin, D., Campbell, M., Fayers, P., and Pinol, A. 1997. Sample Size Tables for Clinical Studies, 2nd Edition. Blackwell Science. Malden, MA.

Report Definitions

Power is the probability of rejecting a false null hypothesis. It should be close to one.
N is the number of pairs in the sample.
P10 is the proportion of pairs in cell 1,2 of the 2x2 table.
P01 is the proportion of pairs in cell 2,1 of the 2x2 table.
Difference is the difference between proportions parameter under the alternative hypothesis.
Proportion Discordant is the total of P10 and P01.
Odds Ratio is the value of this parameter under the alternative hypothesis.
Alpha is the probability of rejecting a true null hypothesis. It should be small.
Beta is the probability of accepting a false null hypothesis. It should be small.

Summary Statements

A sample size of 34 pairs achieves 80% power to detect an odds ratio of 0.072 using a two-sided McNemar test with a significance level of 0.05000. The odds ratio is equivalent to a difference between two paired proportions of -0.260 which occurs when the proportion in cell 1,2 is 0.020 and the proportion in cell 2,1 is 0.280. The proportion of discordant pairs is 0.300.

APPENDIX B: HIC

CONSENT FOR PARTICIPATION IN A RESEARCH PROJECT

200 FR. 1 (2016-2)

YALE UNIVERSITY SCHOOL OF MEDICINE – YALE-NEW HAVEN HOSPITAL: WEST HAVEN VETERANS AFFAIRS

Study Title: Effect of Corticosteroid with Ingenol Mebutate on Local Skin Reaction for Actinic Keratosis Treatment
Principal Investigator: Dr. Suguru Imaeda

Invitation to Participate and Description of Project

You are invited to participate in a research study designed to look at the ability of corticosteroids to reduce the adverse effects cause by actinic keratosis treatment by ingenol mebutate. You have been asked to participate because you have been diagnosed with Grade I or II actinic keratoses, have not treated the lesions in the past 6 months, are above the age of 40, and are able to consent in English. We will be recruiting approximately 36 patients from the West Haven VA to participate in this study.

In order to make an informed decision about whether or not you wish to participate in this research study we will review the risks and benefits of this study. This consent form gives you detailed information about the research study, which a member of the research team will also discuss with you and answer any remaining questions you may have. This discussion should go over all aspects of this research: its purpose, the procedures that will be performed, any risks of the procedures, and possible benefits. Once you understand the study, you will be asked if you wish to participate; if so, you will be asked to sign this form.

Description of Procedures

If you agree to participate in this study, we will first obtain information about your previous actinic keratosis treatments to make sure that it doesn't interfere with our study. This may require us to obtain your medical information through your electronic medical record.

Once deemed eligible, you will be asked to apply a thin layer of ingenol mebutate for 3 days to the chosen lesions on the face and corticosteroid for 8 days to just the assigned treatment area. A computer-generated allocation will indicate which AKs get assigned to combination therapy with clobetasol propionate treatment and ingenol mebutate and which ones get assigned to just the ingenol mebutate therapy. The first application of corticosteroid and the next application of both corticosteroid and ingenol mebutate will be

done in office. After that the applications will be done by you at home and we will require you to fill out an adherence log to determine how you are applying the medication.

In addition, you will need to return to the West Haven VA for follow-up visits at Day 1, Day 4, Week 8, and 12 months. At each follow-up appointment you will bring your adherence log. You will be asked questions regarding your adherence, and photographs of the study sites will be taken. You will also be asked to grade your pain and pruritus using a visual analog scale.

A description of this clinical trial will be available on *http://www.ClinicalTrials.gov*, as required by U.S. Law. This Web site will include summary of the results, but will not have any information that can identify you. You can search this Web site at any time.

Risks and Inconveniences

Risks of Clobetasol Propionate:

Risks currently associated with topical corticosteroids are minimal and are listed below.

- Central nervous system: Localized burning (5% to 40%), numbness of fingers (<2%), intracranial hypertension (children; systemic effect reported with topical corticosteroids)
- Dermatologic: Stinging of skin (<2% to 5%), pruritus (<2% to 3%), eczema (pruritus hiemalis: 2%), xeroderma (≤2%), erythema (<2%), folliculitis (<2%), skin atrophy (<2%), skin fissure (<2%), telangiectasia (<2%), atrophic striae (children)
- Endocrine & metabolic: Adrenal suppression, Cushing's syndrome, glycosuria, growth suppression, HPA-axis suppression, hyperglycemia
- Local: Local irritation (1%), local pain (1%)
- Respiratory: Upper respiratory tract infection (8%), nasopharyngitis (5%), streptococcal pharyngitis (1%)
- Prevalence of positive contact allergy to topical corticosteroids is between 0.2% to 6%. Contact allergy is suspected in patients with worsening symptoms or lack of expected improvement in conditions otherwise responsive to topical corticosteroids.

Risks of Ingenol Mebutate:

There is a greater than 10% risk of:

- Dermatologic: Erythema (92% to 94%), desquamation (≤90%), exfoliation of skin (≤90%), crusted skin (74% to 80%), swelling of skin (face/scalp: 79%; trunk/extremities: 64%), localized vesiculation (face/scalp: ≤56%; trunk/extremities: ≤44%), pustules (face/scalp: ≤56%; trunk/extremities: ≤44%),

dermal ulcer (≤32%), skin erosion (≤32%), application site pain (face/scalp: 15%, trunk/extremities: 2%)

Risk between 1% to 10%:

- Central nervous system: Headache (face/scalp: 2%)
- Dermatologic: Application site pruritus (8%), application site irritation (trunk/extremities: 4%), skin infection (face/scalp: 3%; at application site)
- Ophthalmic: Periorbital edema (face/scalp: 3%)
- Respiratory: Nasopharyngitis (trunk/extremities: 2%)

Frequency not defined:

- Ophthalmic: Conjunctivitis, eyelid edema, eye pain
- According to a FDA Safety Alert on August 21, 2015 there have cases of anaphylaxis, conjunctivitis (chemical-induced), corneal injury (burn), eye injury, herpes zoster, pigmentation alteration (application site), scarring (application site), and severe hypersensitivity (includes allergic contact dermatitis)

➢ Eye problems, including severe eye pain, swelling or drooping of your eyelids, corneal burn, redness, swelling and irritation inside the eye, or swelling around your eyes can happen if ingenol mebutate gel gets in your eyes. To avoid getting any of the ingenol mebutate gel into or around the eyes, it is important that you wash your hands well with soap and water after each application. If you accidentally get ingenol mebutate gel in your eyes, flush them with large amounts of water and get medical care as soon as possible.

Benefits

Benefits of this study include the potential improvement in local skin reactions and other side effects produced by the treatment of actinic keratosis with ingenol mebutate. Since all patients will be receiving the standard of care, ingenol mebutate, clearance of actinic keratoses is expected to be seen in all participants. We hope the results of this study will aid in the general advancement of scientific knowledge related to this subject.

Economic Considerations

Thank you for your participation in this clinical trial. While there is no financial incentive for your participation, all topical therapies used for this study will be provided to you free of charge. All follow-up appointments will also be provided at no cost. However, please be aware that if you see a medical provider for other reasons than this study, you will still be responsible for any co-pays required by your insurance company.

Treatment Alternatives/Alternatives

Ingenol mebutate is currently a FDA approved first line field therapy for the treatment of actinic keratosis. The current treatment options for lesion directed actinic keratosis include surgery, cryotherapy, dermabrasion. Other field therapies for wide spread actinic damage includes 5-flurouracil, imiquimod, and photodynamic therapy.

Confidentiality

Any identifiable information obtained for the study will remain confidential and will only be disclosed with your permission or as required by U.S. or State law. Examples of information we are legally required to report includes abuse of a child or elderly person, or certain reportable diseases. When you enroll in the study, a unique identifier code will be randomly assigned to you, and your name will not be used in the study or data analysis.

All records with your information will be stored on encrypted, password protected computers. Information about your study participation will be entered into your Computerized Patient Record System (CPRS). Once placed in your CPRS, these results are accessible to all of your providers who participate in the CPRS system. Information within your CPRS may also be shared with others who are appropriate to have access to your CPRS (e.g. health insurance company, disability provider.)

Representatives from the Yale Human Research Protection Program, the Yale Human Investigation Committee (the committee that reviews, approves, and monitors research on human subjects) may inspect study records during internal auditing procedures. However, these individuals are required to keep all information confidential.

In addition, paper files that are generated will be stored in a locked cabinet and destroyed after the completion of the study. You will have the right to view and request a copy of photographs taken during follow-up visits. These will be erased after the completion of the study in 2 years. When the results of the study are published, or discussed in conference, information about your identity will not be revealed until your consent if obtained.

In Case of Injury

West Haven Veterans Affairs does not provide funds for the treatment of research-related injury. If you are injured as a result of your participation in this study, treatment will be provided. You or your insurance carrier will be expected to pay the costs of this treatment. No additional financial compensation for injury or lost wages is available. You do not give up any of your legal rights by signing this form.

Voluntary Participation and Withdrawal

Participating in this study is voluntary and you are free to choose not to take part in this study. Refusing to participate will not result in a penalty or loss of benefits to which you are otherwise entitled (such as your health care outside the study, the payment for your health care, and your health care benefits). However, you will not be able to enroll in this research study or receive any of the treatment therapies.

If you do become a subject, you are free to stop and withdraw from this study at any time during its course. To withdraw from the study, you can call a member of the research team at any time and tell them that you no longer want to take part. This will cancel any follow-up appointments.

The researchers may withdraw you from participating in the research if necessary. Examples include becoming pregnant, developing a skin cancer near the site of research, any non-compliance to treatment, or experiencing severe side effects as a result of treatment.

Withdrawing from the study will involve no penalty or loss of benefits to which you are otherwise entitled. It will not harm your relationship with your own doctors or with any medical staff at the West Haven Veterans Affairs Medical Center.

When you withdraw from the study, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others until the end of the research study, as necessary to insure the integrity of the study and/or study oversight.

Questions

We have used some technical terms in this form. Please feel free to ask about anything you don't understand and to consider this research and the consent form carefully – as long as you feel is necessary – before you make a decision.

Authorization

I have read (or someone has read to me) this form and have decided to participate in the project described above. Its general purposes, the particulars of my involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

Name of Subject: _____

Signature:_____

Relationship:_____

Date:_____

_____ _____

Signature of Principal Investigator Date

or

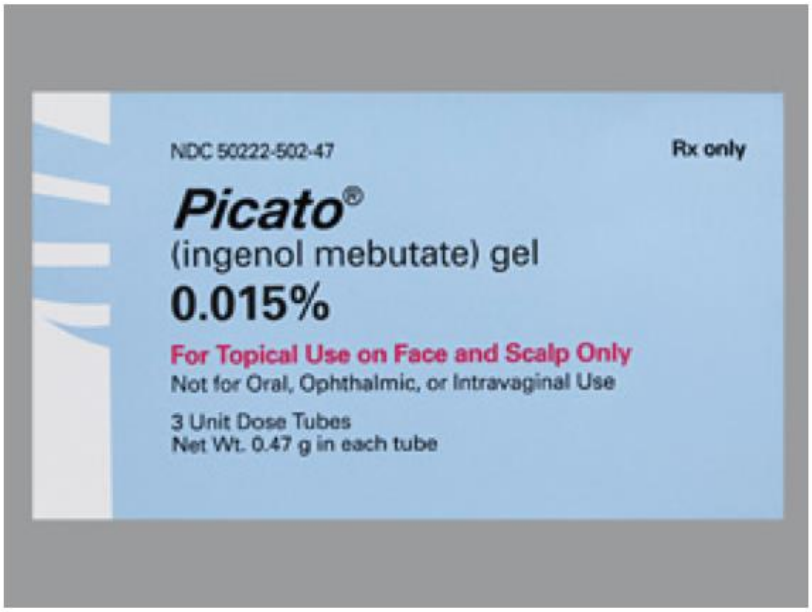
_____ _____

Signature of Person Obtaining Consent Date

If you have further questions about this project or if you have a research-related problem, you may contact the Principal Investigator, Shreya Amin, 347-610-3803. If, after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at 203-432-5919. If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203) 785-4688.

APPENDIX C: Patient Recruitment Flyer

Do you suffer from severe adverse effects from Ingenol Mebutate?



We are looking for individuals who suffer from local skin reactions after ingenol mebutate treatment for actinic keratoses. Please contact us if you are interested or have any questions!

Shreya Amin, PA-S
347-610-3803

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Shreya Amin, PA-S
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APPENDIX D: Letter to Dermatology Providers

To the Dermatology Team at the West Haven VA:

We are pleased to announce that we have recently received IRB approval to conduct an intra-individual randomized clinical trial to study the effect of corticosteroid with ingenol mebutate to reduce local skin reactions for actinic keratosis treatment.

We are currently recruiting patients seen at the West Haven Dermatology Clinic to participate in this clinical trial. Participation will be free and all medical treatments will be paid for in this study.

To be considered patients must be:

- Aged 40 or above with the presence of four to eight clinically typical, visible, and discrete actinic keratoses within a 25 cm^2 contiguous field on the face.
- Female subjects must be of either non-childbearing potential, post-menopausal, or have a confirmed clinical history of sterility (e.g. hysterectomy). Women must be willing to consent to using high effective methods of contraception defined as abstinence, vasectomized partner, an intrauterine device, or oral contraceptives.
- All patients must have the ability to follow trial instructions, agree to allow photographs to be taken as part of the study data analysis, and sign a written informed consent prior to any trial-related procedures.

Exclusion Criteria for this trial includes:

- Excluded patients will include those with recent transplantation or immunosuppression, other severe systemic infections, Olsen's grade III AK and/or invasive tumors within the treatment area, known allergies to any molecule in IMB or corticosteroids, pregnancy or lactation, prior topical treatment for AK within a period of 6 months, likelihood of poor compliance, or an inability to fully consent to the study. Areas within 5 cm of an incompletely healed wound or within 10 cm of a suspected BCC or SCC will also be excluded.

If you or a team member encounters a patient in the dermatology clinic who might fit the criteria for our study, we would be very grateful if you could enroll them in the study. Thank you for your help!

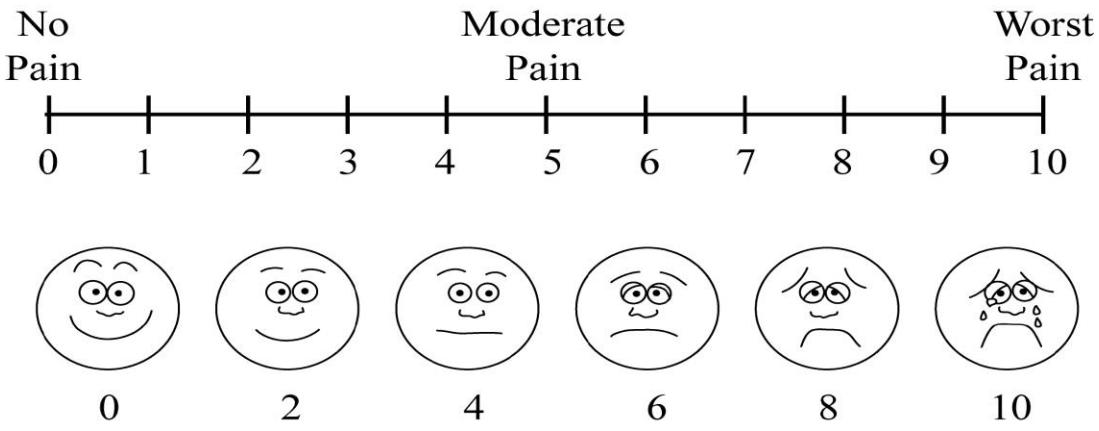
Sincerely,

Shreya Amin, PA-SII
Dr. Suguru Imaeda

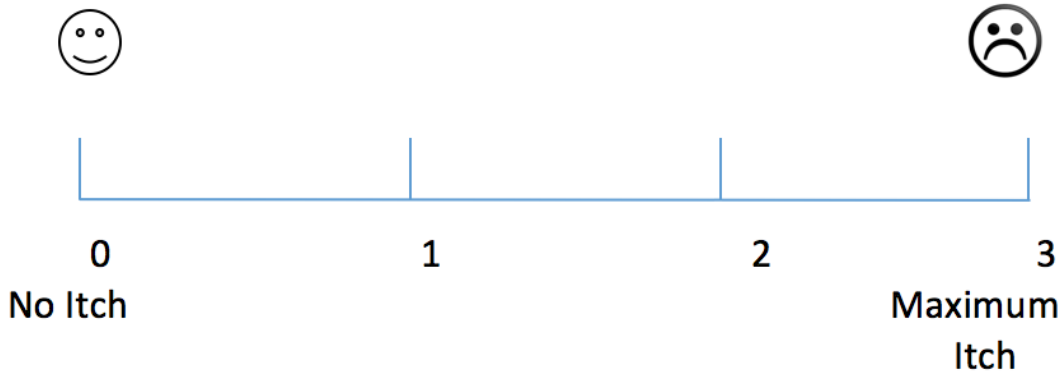
APPENDIX E: Patient VAS for Pain and Pruritus

Pain and Pruritus Log

Pain



Pruritus



Day	Date	Pain	Pruritus
0			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			

APPENDIX F: Patient Instructions for Ingenol Mebutate and Corticosteroids

Patient Instructions for Ingenol Mebutate and Clobetasol Propionate Usage

Thank you for participating in this clinical research trial. For the following 8 days please follow these suggestions regarding medication application.

Sunscreen Application:

- Apply at least SPF 15 every day to the face
- Wear protective clothing and use shade during peak sun hours from 10am to 3pm

Ingenol Mebutate Application:

- Apply ingenol mebutate topically to the two chosen areas on the face using a total of six one unit-dose tubes for three days. One unit-dose tube will cover ~5 cm x 5 cm (~25 cm^2 or ~2 inch x 2 inch).
- Spread evenly then allow gel to dry for 15 minutes
- Do not cover with bandages or occlusive dressings
- Wash hands immediately after applying and avoid transferring gel to any other areas
- Avoid washing or touching the treatment area for at least 6 hours, and following this period of time, patients may wash the area with a mild soap. Not for oral, ophthalmic, or intravaginal use
- Avoid application near or around the mouth, lips, or periocular areas

Clobetasol Propionate Application:

- Apply an even, thin coat of ointment only to the allocated treatment area on the face 15 minutes after ingenol mebutate application.
- Once ingenol mebutate is discontinued continue corticosteroid application for four more days.
- Do not cover with bandages or occlusive dressings
- Wash hands immediately after applying and avoid transferring ointment to any other areas
- Avoid application near or around the mouth, lips, or periocular areas

APPENDIX G: Patient Adherence Log

Patient Medication Log Patient ID: _____

	Date of Treatment	Administration of IMB (Y/N)	Administration of Corticosteroid (Y/N)
Day 1			
Day 2			
Day 3			
Day 4			
Day 5			
Day 6			
Day 7			

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