

**Greystone Final Report
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The Efficacy of a Novel Topical MMP Regulator in
Aging Skin**

STUDY NUMBER: DCS-95-04

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SPONSOR: Greystone

PRODUCT: MMP Inhibitor

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STUDY TITLE: The Efficacy of a Novel Topical MMP Regulator in Aging Skin

**Signatures of the noted individuals ensures that all designated persons have agreed
this version of the report is final:**

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1. PROTOCOL SYNOPSIS

Title of Study:	The Efficacy of a Novel Topical MMP Regulator in Aging Skin
Study Period:	6 weeks with a 4 week extension
Test Product, Dose and Mode of Administration:	MMP Regulator A thin layer applied topically 2 times daily for 6 weeks, with a study extension to 10 weeks
Comparative therapy:	Vehicle 1 without MMP Regulator Vehicle 2 without MMP Regulator and selected botanicals
Placebo:	None
Objective:	To evaluate the efficacy of a novel topical MMP regulator in facial aging.
Design:	<p>Subjects in this double-blinded study will be randomized to apply a topical MMP regulator twice daily for 6 weeks plus a 4 week extension to one side of the face and vehicle 1 or vehicle 2 twice daily for 6 weeks to the opposite side of the face plus a 4 week extension</p> <p>Clinical evaluations will be performed at:</p> <p>Baseline Week 2 Week 4 Week 6 Week 10 extension <i>(There is an allowance for an unscheduled visit at any time if necessary.)</i></p>
Study Population:	Subjects 25-65 years of age with a diagnosis of mild to moderate facial aging as assessed by the dermatologist investigator. Subjects may also have other associated facial dermatoses such as rosacea, sensitive skin, eczema, acne, etc.
Number of Subjects:	40 subjects using placebo 1 and active on split face 10 subjects using placebo 2 and active on split face

Inclusion Criteria:	<ol style="list-style-type: none"> 1. Females 2. Age: 25 to 65 years. 3. Subjects must have mild to moderate facial aging. 4. Subjects must be in general good health as determined from a medical history. 5. Subjects must be willing not to change any of their skin care products or facial cosmetics for the duration of the study. 6. Subjects must read and sign the informed consent form after the nature of the study has been fully explained. 7. Subjects who are using hormones or oral contraceptives must have used them for more than 3 months prior to study enrollment and must not discontinue their use during the study.
Exclusion Criteria:	<ol style="list-style-type: none"> 1. Subjects with known allergies or sensitivities to ingredients contained in the test products. 2. Subjects who are required to spend excessive time in the sun (i.e. lifeguards, other outdoor workers). 3. Subjects who are pregnant or nursing or planning to become pregnant during the course of the study. 4. Subjects who are currently participating or have participated within the last 4 weeks in any other clinical study (i.e., dermal patch, use tests, investigational drug or devices, etc.). 5. Subjects viewed by the investigator as not being able to complete the study.
Endpoints:	<p><u>Efficacy:</u> The efficacy endpoints are the investigator objective and subject subjective ordinal assessment of aging improvement, as well as analysis of photographs, TEWL, corneometry, and profilometry.</p> <p><u>Safety:</u> The incidence of all adverse events reported during the study.</p>
Measures:	Investigator aging assessments consisting of periorbital wrinkling, erythema, pigmentation irregularities, skin roughness, scaling, and itching will be performed at Weeks 0, 2, 4, 6, and at the 10 week extension. Photography, noninvasive TEWL, corneometry, profilometry, and subject assessments will be conducted at Baseline and at Weeks 0, 2, 4, 6, and the 10 week extension.
Statistical Methods:	<p><u>Primary Efficacy Criterion:</u> Statistically significant ($p=0.05$ or less) improvement in facial aging as assessed by the dermatologist investigator favoring active over vehicle.</p> <p><u>Secondary Efficacy Criterion:</u> Statistically significant ($p=0.05$ or less) improvement in aging as assessed by the subjects favoring active over</p>

	<p>vehicle.</p> <p><u>Tertiary Efficacy Criterion:</u> Statistically significant ($p=0.05$ or less) improvement in profilometry, TEWL, corneometry, or photography favoring active over vehicle.</p> <p><u>Safety:</u> The safety endpoint is the incidence of all adverse events reported during the study will be summarized by treatment group.</p>
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2. STUDY VISIT SCHEDULE

	Week 0	Week 2	Week 4	Week 6	Week 10(ext)
Informed Consent	X				
Medical History	X				
Subject Eligibility Determined	X				
Assess Concurrent Medications	X	X	X	X	X
Investigator Aging Assessment	X	X	X	X	X
Corneometry, TEWL	X	X	X	X	X
Subject Assessment	X	X	X	X	X
Photography	X	X	X	X	X
Profilometry	X			X	X
Adverse Event Assessment		X	X	X	X

3. INTRODUCTION

Aging of the facial skin is a ubiquitous condition ultimately affecting all women worldwide. It is due to extrinsic and intrinsic factors. It appears that both extrinsic and intrinsic aging are due to the secretion of metalloproteases (MMP) such as collagenase (collagenase-1, MMP-1) and gelatinase (gelatinase A, MMP-2).¹ Collagenases cleave the triple helical domain of fibrillar collagens at a neutral pH and are produced following less than 10 minutes of UVA radiation exposure. The collagenase breaks down the fibrillar

¹ Singer CF, Marbaix E Lemoine P, Courtoy PJ, Eeckhout Y: Local cytokines induce differential expression of matrix metalloproteinases but not their tissue inhibitors in human endometrial fibroblasts. Eur J Biochem 1999;259(1-2):40-5

collagens present in the dermis resulting in the appearance of wrinkles that contribute to an aged facial appearance.

4. STUDY OBJECTIVE

The study objective was to evaluate the efficacy of a topical MMP regulator in facial skin aging.

5. STUDY DESIGN OVERVIEW

This was a 6-week double-blind, split-face, vehicle-controlled randomized study with a four week extension. One group of 20 subjects applied the vehicle 1 to the left face and the active to the right face. The second group of 20 subjects applied the active to the left face and the vehicle 1 to the right face. A third group of 5 subjects applied the active to the left face and vehicle 2 to the right face. And, a fourth group of 5 subjects applied the vehicle 2 to the left face and the active to the right face. Both products were applied twice daily. Subjects had mild to moderate aging as assessed by the dermatologist investigator. Investigator and subject visual facial assessments, noninvasive assessments and photography were performed at all evaluation time points (weeks 0, 2, 4, 6 and the 10 week extension).

6. STUDY POPULATION

6.1 NUMBER OF SUBJECTS

40 female subjects 25-65 years of age who met the inclusion and exclusion criteria were enrolled. An additional 10 subjects were added to test a second vehicle formulation.

6.2 INCLUSION CRITERIA

The following items represented the inclusion criteria:

1. Females
2. Age: 25 to 65 years.
3. Subjects must have mild to moderate facial aging.
4. Subjects must be in general good health as determined from a medical history.
5. Subjects must be willing not to change any of their skin care products or facial cosmetics for the duration of the study.
6. Subjects must read and sign the informed consent form after the nature of the study has been fully explained.
7. Subjects who are using hormones or oral contraceptives must have used them for more than 3 months prior to study enrollment and must not discontinue their use during the study.

6.3 EXCLUSION CRITERIA

The following represented the exclusion criteria:

1. Subjects with known allergies or sensitivities to ingredients contained in the test products.
2. Subjects who are required to spend excessive time in the sun (i.e. lifeguards, other

- outdoor workers).
3. Subjects who are pregnant or nursing or planning to become pregnant during the course of the study.
 4. Subjects who are currently participating or have participated within the last 4 weeks in any other clinical study (i.e., dermal patch, use tests, investigational drug or devices, etc.).
 5. Subjects viewed by the investigator as not being able to complete the study.

6.4 CONCOMITANT MEDICATIONS & RESTRICTIONS

All subjects remained on any oral or topical medications unchanged in type or dose during the 6-week study period and the 4-week extension period.

7. CLINICAL ASSESSMENTS AND RATING SCALE DEFINITIONS

7.1 CLINICAL MEASURES

Subjects were evaluated by collecting a variety of observations at Weeks 0, 2, 4, and 6 of the study, as well as the 10-week extension. The same trained evaluator at each study facility performed all visual assessments whenever possible. The parameters to be evaluated were:

1. Overall Aging Assessment: Each side of the face was evaluated separately for aging based on the following 5-point ordinal grading scale: 0=None, 1=Slight, 2=Mild, 3=Moderate, 4=Severe.
2. Investigator Visual Evaluations: Periorbital wrinkling, erythema, pigmentation irregularities, skin roughness, scaling, and itching were graded using the 5-point scale below.

5-point grading scale

- 0 = None
1 = Slight
2 = Mild
3 = Moderate
4 = Severe

3. Subject Assessment: Subjects completed a questionnaire at each visit regarding stinging, skin roughness, and skin wrinkling.
4. Noninvasive Assessments: Subjects had TEWL measurements and pin probe corneometry performed at each visit from the right and left forehead. Profilometry was performed from both cheeks at baseline and at week 6 and at the week 10 extension.
5. Digital Photography: Subjects had digital photography performed at

baseline and weeks 0, 2, 4, and 6 of the study. Photographs were also obtained at the week 10 extension visit. The digital photography consisted of frontal, right, and left facial images of the subject with the head placed in a three-point restraining mount.

8. EFFICACY MEASURES

8.1 SUBJECT COMPLIANCE

Subjects applied the products 2 times daily, morning and evening. Compliance was determined from the diary sheets. Review of the diary sheets indicated excellent subject compliance such that no data required disqualification.

9. FINAL SUBJECT STATUS

A study termination form was completed for each study subject who receives study product. All subjects completed week 6. 4 subjects in the active/vehicle 1 group elected to not complete the week 10 extension and 2 subjects in the active/vehicle group elected not to complete the week 10 extension.

10. STUDY MEDICATION

10.1 DOSAGE AND FORMULATIONS

The study products consisted of an active MMP regulator as compared to vehicle 1 without the MMP regulator and vehicle 2 without the MMP regulator and the selected botanical additives.

10.2 STORAGE AND ACCOUNTABILITY OF STUDY PRODUCT

The study product was stored at room temperature in a locked, limited access area at the study site. All bottles of both study active and vehicle were returned by all subjects. Selected samples were returned to the sponsor and the remainder of the returned product and unused vehicle was destroyed. All unused active was returned to the study sponsor.

11. ADVERSE EVENTS

11.1 ADVERSE REACTIONS

No serious adverse reactions occurred, however many subjects reported both eye stinging and facial stinging associated primarily with the active. This data was captured and is reported under the data analysis. The sponsor was notified of the stinging associated with study products after the week 2 visit and it was elected by the investigator and the sponsor to complete the study despite the incidence of stinging.

12. STATISTICAL METHODS

The statistical evaluation of the data was performed by Dermatology Consulting Services utilizing a Mann Whitney nonparametric two-tailed paired test.

12.1 SAMPLE SIZE RATIONALE

A sample size of 40 subjects was chosen by the sponsor as sufficient to obtain the split face needed for evaluation of the active versus the vehicle 1. At the request of the sponsor, 10 additional subjects were added to evaluate the active versus vehicle 2.

12.2 RANDOMIZATION PROCEDURES

Patients were assigned with a computer generated randomization schedule to apply the active or the vehicle 1 or 2 to the right or left face.

12.3 SIGNIFICANCE LEVEL

Significance was defined at the p=0.05 or less level based on a two-sided test.

12.4 COMPARABILITY OF STUDY GROUPS AT BASELINE

Patient demographic and baseline evaluations were compared to determine that the 4 groups were properly balanced. Statistical analysis revealed equal balancing of subject findings within the 4 groups.

12.5 PROTOCOL MODIFICATIONS

Several protocol modifications were made based on the wishes of the sponsor. The study was extended to add 10 subjects to test an additional vehicle 2 without the botanical additives. Additionally, the study was extended to 10 weeks to allow for collection of additional data.

12.6 AUDITS/INSPECTIONS

The sponsor audited the study at the week 10 visit.

13. RESULTS

The raw data tables and statistical analyses are attached as Excel data sheets for each of the parameters analyzed: investigator assessment active/vehicle 1, investigator assessment active/vehicle 2, subject assessment active/vehicle 1, subject assessment active/vehicle 2, noninvasive assessments, and photography assessments. An unblind sheet is also attached.

14. DISCUSSION

Each of the data sets is discussed separately below.

1. Investigator Assessment Active/Vehicle 1

This data set was particularly challenging due to the excellent moisturizing characteristics of vehicle 1. Statistical superiority ($p=0.015$) of the active over vehicle 1 was achieved at week 6 in the overall investigator assessment. The data sets were well balanced as indicated by the excellent matching of the skin characteristics at baseline. Little difference between the active and vehicle 1 groups could be appreciated at week 2, probably due to the moisturizing benefits that were present in both comparative products that typically manifest after 14 days of use. There was a trend for the investigator to prefer the active product both at week 2 and week 4. This trend became statistically significant at week 6, but the trend was lost at week 10. Subjects reported much less stinging at week 10 and a noticeable change in the active product smell and appearance. Could the active agent have degraded in the 10-week product? Also, the vehicles began to thicken and perhaps a more concentrated moisturizer benefit was delivered to the skin surface. The weather cooled dramatically between weeks 6 and 10, which may have also influenced the study by increasing the apparent moisturizing effect of vehicle 1.

2. Investigator Assessment Active/Vehicle 2

Vehicle 2 did not perform as well as vehicle 1, thus allowing better separation from the active. Again, the groups were well balanced at baseline, but only 10 subjects were present in this group accounting for the challenging statistics. There was a clear preference for active over vehicle 2 at all time points that reached statistical significance in terms of skin scaling ($p=0.041$) and skin roughness ($p=0.045$) at week 10. The overall evaluation almost reached statistical significance ($p=0.065$), but this was prevented by the limited data points as this arm of the study was powered too low.

3. Subject Assessment Active/Vehicle 1

The subjects were unable to distinguish any skin benefits in terms of improvement in skin wrinkling and roughness between the active and vehicle 1. This may have been due to the excellent moisturizing qualities of both products that the subjects could not discern. However, there was a statistically significant increase in skin stinging for the active over vehicle one at all time points (weeks 2, 4, 6, 10) with the biggest difference occurring at week 6.

4. Subject Assessment Active/Vehicle 2

The subjects were unable to distinguish any difference in terms of improvement in skin wrinkling and roughness between the active and vehicle 2. There was no statistically significant difference between active and vehicle 2 in terms of stinging. However, the active did receive higher stinging scores than vehicle 2. This may be due to the small number of subjects enrolled in this arm of the study.

5. TEWL Assessment Active/Vehicle 1

This data revealed that both the active and vehicle 1 were excellent moisturizers that did not damage cutaneous barrier function.

6. TEWL Assessment Active/Vehicle 2

This data revealed that both the active and vehicle 2 were excellent moisturizers that did not damage cutaneous barrier function.

7. Corneometry Assessment Active/Vehicle 1

The data demonstrated that skin hydration was not altered by either the active or vehicle 1.

8. Corneometry Assessment Active/Vehicle 2

This data demonstrated that skin hydration was not altered by either the active or vehicle 2.

9. Replica Analysis

The replicas were analyzed by CuDerm Corporation. The data that was returned was quite extensive. The instruction sheets for interpretation are attached to this report, as are the analyzed replica parameters. The data is broken into two sets labeled N and P. N represents the appearance of the major wrinkles around the eyes characterized as crow's feet and the P represents the appearance of the minor fine lines around the eyes. N stands for normal and P stands for parallel to indicate the direction of the light as it illuminates the specimen to digital image analysis.

The data has been presented at each evaluation time point consisting of baseline, week 6, and week 10 for both the active vs. placebo 1 and active vs. placebo 2. The data charts comparing active vs. placebo 1 at week 6 show a statistically significant value ($P=0.021$) in terms of wrinkle breadth, but the smaller number in the placebo 1 column indicates that smaller wrinkles were produced by the placebo 1 over the active. This is also the case for the one statistically significant point in number of wrinkles at week 10 where placebo 1 again outperformed the active. There were no statistically significant points for placebo 2, which is expected since placebo 1 outperformed placebo 2 in all other analyses.

The P analysis of the replicas failed to reveal any statistically significant endpoints. This too might be expected based on the subject and investigator assessments. In a good study, the subjective and objective data is consistent.

10. Photography Analysis

This analysis of the baseline versus the week 10 photographs of both sides of the face to compare the active with vehicle 1 revealed no statistically significant difference. There was also no statistically significant difference between vehicle 2 and the active. The photographs were consistent and illustrative, but were hard to evaluate in a blinded fashion.

15. RECOMMENDATIONS

Based on the data analysis, I would make the following recommendations:

1. It appears that the MMP regulator active underwent some degradation during the 10-week run time of the study. Both a darkening of the color of the active and an odor were noted as the subjects used the product. I believe that the genestein in the active was oxidizing accounting for both the color change and the odor. This means that more and better antioxidants should be incorporated into the formulation. This hypothesis could be easily verified by analyzing a freshly made sample and comparing it to the contents of the tubes that were returned by the study subjects for ingredient content and auto-oxidation products.
2. The MMP regulator active also thickened during the course of the study. I would recommend the incorporation of additional humectants to maintain the water content of the lotion and prevent the increased viscosity. The humectant that I

- would recommend is glycerin to both improve the moisturizing ability of the product and minimize the water loss.
3. Both vehicle 1 and vehicle 2 experienced thickening. This is expected based on the comments that were made under point 2 indicating the need for increased product humectancy.
 4. The active product and the vehicles did not dispense well from the supplied packaging. The orifice was too small for adequate dispensing and the thickened product eventually became impossible to removed from the semirigid plastic container. I would recommend a metal tube with a small orifice to allow removal of the entire contents from the container and also to minimize oxygen contact and prevent oxidation.
 5. The active was responsible for the eye and facial stinging experienced by the subjects. This was verified by the statistical analysis. Additional formulation work should be done to determine to source of the stinging and eliminate this noxious sensory stimulus. In over-the-counter products, facial stinging is an undesirable characteristic.

16. CONCLUSIONS

In summary, I think this was a well performing product. The issues of sensory stinging and product stability can be easily overcome. The study was somewhat challenging because both vehicles were clearly actives in the study. Overall, vehicle 1 performed better than vehicle 2, based on the statistical analysis, as expected. Future studies should either use vehicle 2 for comparison or consider using a market leader moisturizer, such as Vaseline Intensive Care Lotion. This study design would highlight the combined benefit of the many individual ingredients contained in the active.

16.1.1 PRIMARY EFFICACY ENDPOINT

The primary efficacy endpoint was the statistically significant ($p=0.05$ or less) improvement in facial aging as assessed by the dermatologist investigator favoring active over vehicle. This efficacy endpoint was achieved at the end of week 6 in terms of overall investigator assessment for the active/vehicle 1 group. It was also achieved for the vehicle/active 2 group at week 10 for improvement in skin roughness and decreased skin scale.

16.1.2 SECONDARY EFFICACY ENDPOINT

The secondary efficacy endpoint was the statistically significant ($p=0.05$ or less) improvement in skin condition as assessed by the subjects showing a preference for active over vehicle. This efficacy endpoint was not achieved. There was a statistically significant increase in stinging with active over vehicle 1, however. This means that the active contained an ingredient that produced cutaneous stinging.

16.1.3 TERTIARY EFFICACY ENDPOINT

The tertiary efficacy endpoint was the statistically significant ($p=0.05$ or less) improvement in profilometry, TEWL, corneometry, or photography favoring active over vehicle. This endpoint was not met in terms of TEWL or corneometry. This is not unexpected since both the active and vehicle 1 and vehicle 2 were all excellent moisturizers.

Subject Image Legend
Zoe Diana Draelos, MD
Evaluation of Active on Skin Appearance

Subject 2, left face, baseline and week 6: good example of improved skin texture

Subject 15, right face, baseline and week 6: good example of improved skin color

Subject 21, left face, baseline and week 6: good example of improved crow's feet

Subject 37, right face, baseline and week 6: good example of improved crow's feet

Subject 38, right face, baseline and week 6: good example of improved crow's feet

Subject 39, left face, baseline and week 6: improve crow's feet and improved skin texture























