

# A Novel, Volumizing Cosmetic Formulation Significantly Improves the Appearance of Target Glabellar Lines, Nasolabial Folds, and Crow's Feet in a Double-Blind, Vehicle-Controlled Clinical Trial

Patricia K. Farris MD,<sup>a</sup> Brenda L. Edison BA,<sup>b</sup> Ronni L. Weinkauff PhD,<sup>b</sup> Barbara A. Green RPh MS<sup>b</sup>

<sup>a</sup>Department of Dermatology, Tulane University School of Medicine, New Orleans, LA

<sup>b</sup>NeoStrata Company, Inc., Princeton, NJ

## ABSTRACT

Facial lines and wrinkles are caused by many factors including constant exposure to external elements, such as UV rays, as well as the dynamic nature of facial expression. Many cosmetic products and procedures provide global improvement to aging skin, whereas injectable therapies are frequently utilized to diminish specific, target wrinkles. Despite their broad availability, some patients are unwilling to undergo injectables and would benefit from an effective topical option. A noninvasive option to volumize target wrinkle areas could also extend benefits of commonly used cosmetic anti-aging products. To this end, a two-step formulation containing the novel, cosmetic anti-aging ingredient, N-acetyl tyrosinamide, was developed for use on targeted wrinkle areas. The tolerability and efficacy of the serum plus cream were tested for 16 weeks in women with moderate facial photodamage on predetermined wrinkle areas (glabellar lines, nasolabial folds, under eye lines, and lateral canthal (crow's feet) wrinkles) in a single-center, randomized, double-blind, vehicle-controlled, clinical trial. Seventy women (47 Active group, 23 Vehicle group) completed the study. Digital photography, clinical grading, ultrasound and self-assessment scores confirmed improvement to wrinkle areas. The topical cosmetic formulation was statistically superior ( $P < 0.05$ ) to its vehicle in visually improving nasolabial folds, glabellar lines, crow's feet, and under eye wrinkles and in reducing pinch recoil time. Both the test formulation and its vehicle were tolerated well. The novel, two-step cosmetic formulation reduced the appearance of wrinkles and increased skin elasticity thus providing an effective anti-aging option for target wrinkle areas. This study suggests that in addition to its use as monotherapy for reducing targeted lines and wrinkles this cosmetic formulation may also serve as an adjuvant to injectable therapies.

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## INTRODUCTION

Facial rhytides arise due to diminished production and increased breakdown of dermal matrix components, including collagen, glycosaminoglycans (GAGs), and elastin, as a result of natural aging and photodamage, coupled with repeated facial movement. Improvement of global photoaging symptoms can be achieved with effective, topical cosmetic formulations which typically address a wide range of skin concerns including dyspigmentation, laxity and surface lines. Physician intervention can provide significant, targeted improvement to wrinkle areas utilizing injectable treatments and a 3-dimensional approach of muscle relaxation, volume restoration and recontouring.<sup>1</sup> However, despite their mainstream use in dermatology, injectable therapies may not be ideal for every patient seeking wrinkle reduction. For example, injectables are not completely without risks or discomfort and can be challenging to use on certain areas of the face. In addition, research has confirmed the existence of a needle-averse population and determined that less than 10% of the target population for botulinum toxin A (BoNTA) injections receives treatment.<sup>2</sup> Furthermore, the nascent anti-aging patient may be apprehensive to adopt injectables as first-line, anti-wrinkle therapy. The availability of a noninvasive, clinically-proven, topical formulation that reduces the appearance of target lines

and wrinkles may prove useful as a cosmetic treatment and potential alternative or adjunct to injectables for certain patients.

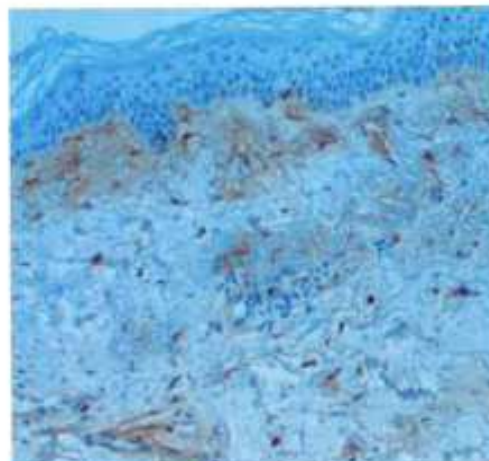
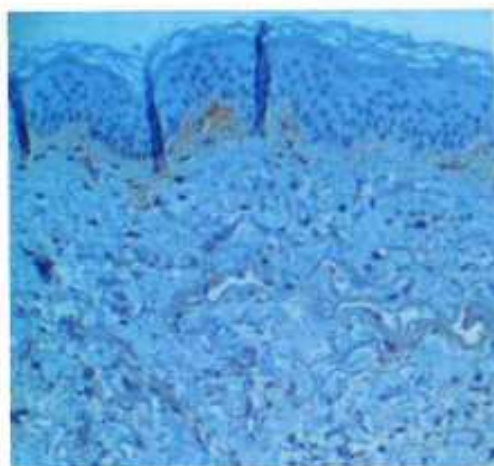
A two-step, volumizing formulation has been developed for application to target lines and wrinkles. The product contains a new cosmetic anti-aging ingredient, N-acetyl tyrosinamide, which increases matrix volume to plump and reduce the appearance of facial lines. An objective clinical study was conducted to evaluate the safety and effectiveness of this system in reducing the appearance of target wrinkle areas including glabellar lines, nasolabial folds, lateral canthal (crow's feet) wrinkles and under eye lines and wrinkles.

### Formulation Strategy Incorporating a Novel Volumizing Ingredient

The study products were developed to maximize the benefits of the amino acid compound, N-acetyl tyrosinamide. Independent research of this compound by dermatologist, Eugene J. Van Scott, MD and dermatopharmacologist, Ruey J. Yu PhD OMD was conducted under occlusion to exaggerate penetration and demonstrated a rapid increase in forearm skin thickness.<sup>3</sup> Later, this compound was evaluated in several in vitro and in vivo models



**FIGURE 1.** Differences in pro-collagen (brown cytoplasmic stain) between an untreated skin specimen (left) and a skin specimen treated with N-acetyl tyrosinamide (right, after 8 weeks).



and was shown to favorably impact important dermal matrix markers and provide measurable increases in skin thickness with corresponding anti-aging effects. Specifically, N-acetyl tyrosinamide stimulated collagen production in aged human dermal fibroblast cells<sup>4</sup> and increased hyaluronic acid in human dermal fibroblast and chondrocyte (cartilage) cells in vitro.<sup>5</sup> Topical application to human skin in vivo with N-acetyl tyrosinamide alone demonstrated a statistically significant increase in forearm skin thickness after 8 weeks of application, as measured by digital calipers. Corresponding histological assessment of skin biopsies showed stimulation of pro-collagen and GAGs, supporting earlier in vitro findings. (Figure 1) Application of N-acetyl tyrosinamide alone to target facial lines and wrinkles (glabellar, crow's feet, nasolabial folds) demonstrated noticeable visual improvements thus supporting its use as a noninvasive, cosmetic volumizing agent. (Figure 2) In separate testing, this compound was found to be non-photosensitizing, non-phototoxic, non-irritating, non-allergenic and safe for use around the eyes and with contact lenses.<sup>6</sup>

The test products were formulated as a two-step system in order to mimic the occlusive methodology used in the early development work of N-acetyl tyrosinamide.<sup>7</sup> Step 1 is a light serum packaged in a tube with roller ball to facilitate direct application to target wrinkle areas. It contains the cosmetic benefit ingredients, N-acetyl tyrosinamide, N-acetyl hydroxyproline and a low concentration of glycolic acid to aid in delivery. N-acetyl hydroxyproline has been shown to increase skin barrier function<sup>8</sup> and is used as an amino acid source for collagen. The Step 2 Finishing Complex is a lightly occlusive cream that is applied directly over Step 1. It contains complementary matrix enhancing ingredients including N-acetyl glucosamine, triethyl citrate plus palmitoyl oligo and tetra peptides (Matrixyl<sup>®</sup>, Sederma Inc.). N-acetyl glucosamine has been shown to increase hyaluronic

acid in human dermal fibroblast cells in vitro, and to increase skin thickness and improve the appearance of fine and coarse wrinkling in vivo.<sup>9</sup> Triethyl citrate has been shown to increase collagen production in human dermal fibroblast cells in vitro.<sup>9</sup>

## MATERIALS AND METHODS

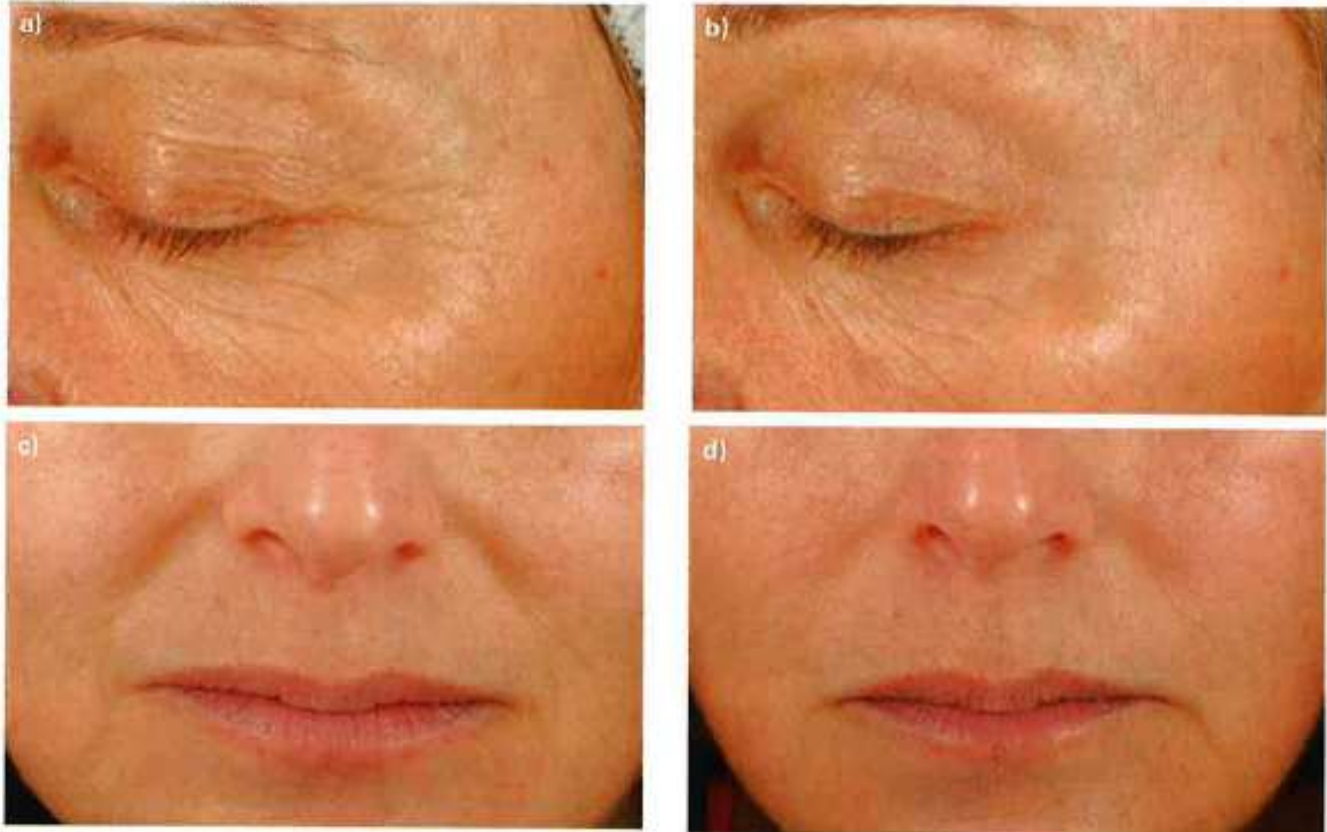
### Study Design

This was a single-center, double-blind, randomized, vehicle-controlled, 16-week clinical study. The protocol was reviewed and approved by a local institutional Review Board, and all subjects had provided written informed consent before enrollment.

Caucasian women aged 40 to 65 years old, with moderate facial photodamage, defined as a score of 4 to 6 on a 0 to 9 modified Griffith's scale<sup>10</sup>, on at least one area including glabellar lines, nasolabial folds, and/or lateral canthal (crow's feet) wrinkles were eligible for enrollment. In addition, subjects had no known allergies to skincare products, or skin/eye conditions, or uncontrolled chronic diseases that could interfere with evaluations. Other exclusions to participation included: current use of medications for skin or eye conditions; routine use of anti-aging topical products, including prescription retinoids within 6 months; use of hydroxyacids, retinol and other anti-aging cosmetics within 2 months; and/or cosmetic procedures within 6 months.

Enrolled subjects were randomized in a 2:1 ratio to use either the two-step formulation containing anti-aging benefit ingredients (Active group), or their vehicles (Vehicle group). Twice a day for 16 weeks, subjects cleansed their faces with their regular cleanser and those in the Active group treated target wrinkle areas with Step 1 Activator followed by Step 2 Finishing Complex directly over the Step 1 Activator (NeoStrata<sup>®</sup> Skin Active Line Lift Step 1 Activator and Step 2 Finishing Complex). Those

**FIGURE 2.** Volumizing effect of topical N-acetyl tyrosinamide on crow's feet at baseline **a)** and after 8 weeks **b)**, and nasolabial folds at baseline **c)**, and after 6 weeks **d)**.



in the Vehicle group applied vehicle formulations of Step 1 and Step 2 to targeted wrinkle areas as above. The Vehicle formulations were devoid of the anti-aging benefit ingredients and were packaged identically to match the Active products. In addition, all subjects applied a bland day cream SPF 20 in the morning and bland night cream in the evening to provide all-over moisturization and sunscreen protection. There were no anti-aging benefit ingredients in the day and night-time moisturizers.

### Efficacy and Safety Assessments

Subjects were evaluated at baseline (week 0) and week 4, week 8, week 12, and week 16 after starting study treatment.

#### Visual Grading of Efficacy and Safety Parameters (Weeks 0, 4, 8, 12, and 16)

At each visit, a trained clinician graded glabellar lines, nasolabial folds, under eye lines and wrinkles, and crow's feet using a modified Griffiths' scale (GS) from 0 (none) to 9 (severe) with 0.5 grade increments. In addition, nasolabial folds were graded using a modified Wrinkle Severity Rating Scale (WSRS), a validated scale used to grade the effects of injectable wrinkle fillers on nasolabial folds, ranging from 1 (absent) to 5 (extreme) with 0.5 grade increments.<sup>11</sup> Objective

skin irritation (erythema, dryness/scaling), subjective irritation (burning, stinging, itching, tingling, tightness/dry feeling) and adverse events were recorded.

#### Pinch recoil/Elasticity (Weeks 0, 4, 8, 12, and 16)

Pinch recoil measurements on the lateral side of the eye area were collected at each visit. Briefly, the skin was pinched and held for approximately 2 seconds and then released. The time it takes for the skin to return to its original conformation was recorded to the nearest 100<sup>th</sup> of a second. A decrease in pinch recoil time is associated with an improvement in skin firmness/elasticity.

#### Skin Thickness (Weeks 0 and 16)

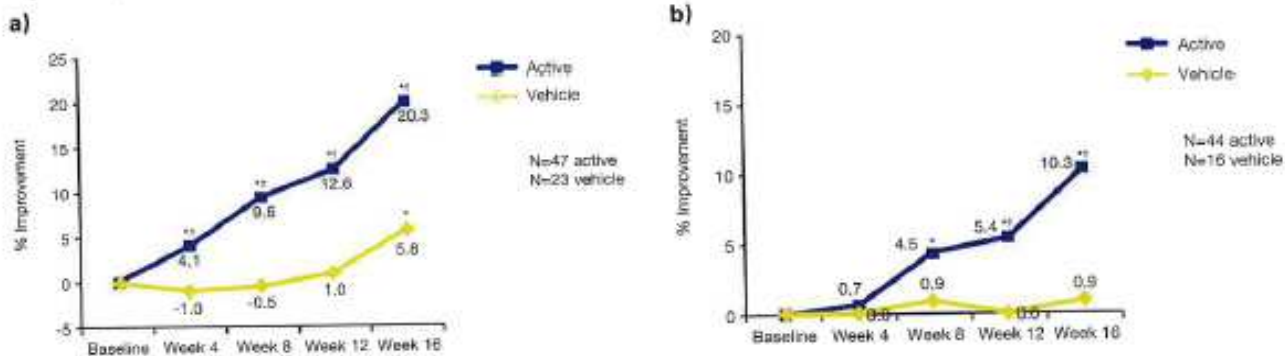
Skin density in the lateral canthal area was imaged via ultrasound on half of the subjects in each group as selected by the Investigator. An increase in dermal density was interpreted as a thickening of the tissue, resulting from deposition of matrix components.

#### Digital Photography (Weeks 0, 4, 8, 12, and 16)

Digital photography was captured by a single medical photographer using standardized lighting conditions. Subjects' faces were placed in a facial-positioning device to ensure reproducibility at subsequent visits. A color chart was incorporated into



**FIGURE 3.** Improvement in **a)** under eye lines and wrinkles and **b)** nasolabial folds over 16 weeks in the Active group vs the Vehicle group. \*Significantly better than baseline ( $P < .05$ ). \*\*Significantly better than vehicle ( $P < .05$ ).



each image to ensure consistent lighting. Images were untouched and were cropped to show changes in targeted areas.

#### Self-Assessment (Weeks 0, 4, 8, 12, and 16)

Self-perceived efficacy and tolerability were collected using self-assessment questionnaires.

#### Statistical Analysis

All clinical data were collected via electronic data capture. The primary outcome evaluated the efficacy scores for glabellar lines, nasolabial folds, under eye lines and wrinkles, and crow's feet at each time point between the Active group and Vehicle group. Scores within each treatment were also compared to baseline condition. Both between group and within treatment group comparisons were conducted using t-tests. Pinch recoil times were compared between groups using the Wilcoxon Rank Sum test. All statistical comparisons were performed at  $P < .05$  level of significance. Self-assessment questionnaires were tabulated.

#### RESULTS

A total of 70 women completed study treatment (47 in the Active group and 23 in the Vehicle group). The mean ages of the subjects were 55 years in the Active group and 59 years in the Vehicle group. The number of subjects in each graded category varied based upon meeting the minimum inclusion criteria (grade of 4-6 on 0-9 scale) for the particular graded parameter. The population size for each parameter is as follows: glabellar lines  $n=30$  Active,  $n=14$  Vehicle; under eye lines and wrinkles  $n=47$  Active,  $n=23$  Vehicle; crow's feet  $n=37$  Active,  $n=20$  Vehicle; nasolabial folds  $n=44$  Active,  $n=16$  Vehicle.

The Active group was statistically superior ( $P < .05$ ) to its Vehicle on all clinically-graded targeted line and wrinkle parameters with improvement as early as week 4 and continued improvement through week 16. Participants in the Active

group showed significantly more improvement versus participants in the Vehicle group using the GS scale for under eye wrinkles as early as 4 weeks (20.3% vs 5.8%; Figure 3a), crow's feet beginning at 8 weeks (17.8% vs 7.9%), and glabellar lines at 16 weeks (7.7% vs 1.3%). Nasolabial folds showed statistically greater improvement versus Vehicle using the WSRS beginning at week 12 (10.3% improvement vs 0.9%; Figure 3b). Pinch recoil time also significantly improved over Vehicle group by the end of treatment (10.1% vs 5.7%).

Clinical photography demonstrated improvements in periorcular fine lines and wrinkles, glabellar lines, and nasolabial folds (Figures 4a, 4b, 5, and 6). Ultrasound imaging demonstrated improvement in dermal density that corresponds to the visual changes seen in clinical photographs (Figures 4c,d).

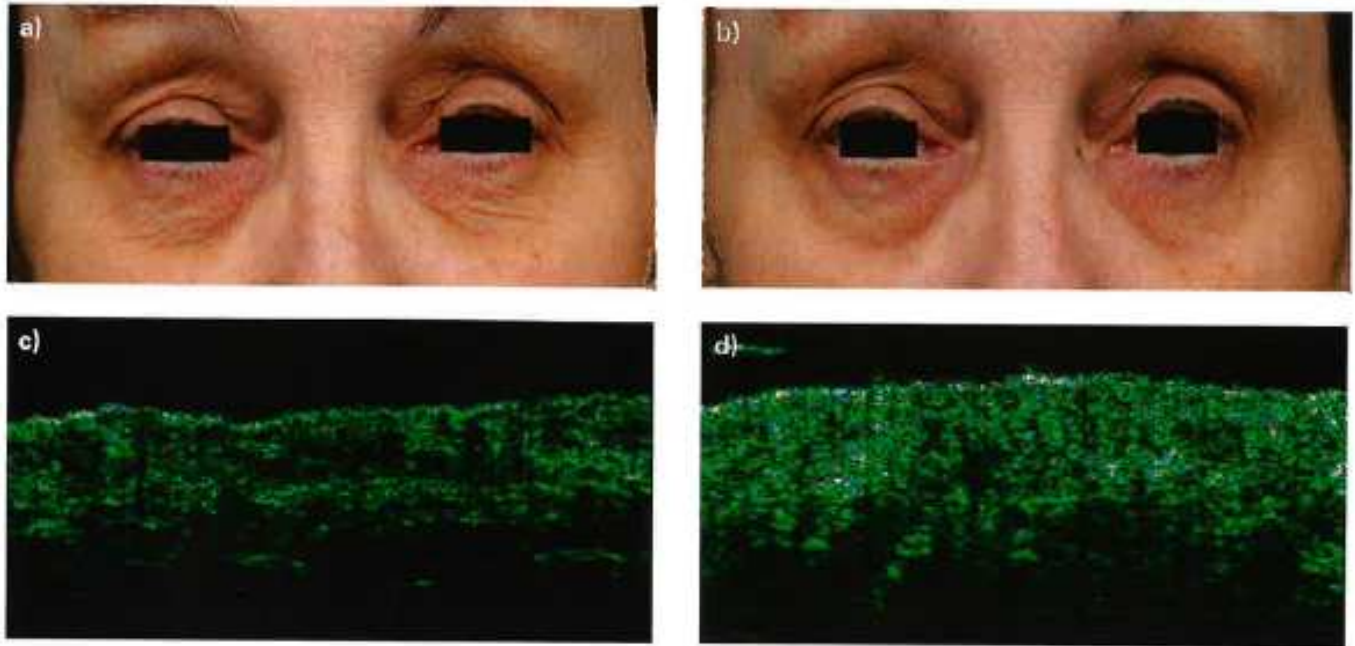
Self-assessment supported clinical grading. Study participants agreed that their lines and wrinkles were less noticeable and appeared to be filling and plumping, and they thought their skin looked and felt firmer.

All products were well tolerated throughout the 16-week study. There was one report in the Active group of moderate skin irritation in the crow's feet area and no reports of irritation in the Vehicle group.

#### DISCUSSION

There is a continuing desire to improve the visible signs of aging among patients and consumers. Many patients regularly use anti-aging cosmetics to help improve global signs of photoaging including mottled pigmentation, sallowness and surface roughness. A noninvasive, topical cosmetic formulation that can be applied directly to target wrinkle areas could provide added benefit for treating aging skin. The two-step, topical formulation containing the novel skin volumizing agent, N-acetyl tyrosinamide with other complementary, matrix-enhancing in-

**FIGURE 4.** Subject's improvement in under eye area lines and wrinkles observed in clinical photographs **a)**, baseline; **b)**, after 16 weeks use of Active products and in ultrasound images **c)**, baseline; **d)**, after 16 weeks use of Active products. Note the increased density of dermal matrix components in ultrasound images (increase in colored components).



**FIGURE 5.** Improvement in glabellar lines and under eye lines and wrinkles in a subject using the Active products for 16 weeks **a)**, baseline; **b)**, after 16 weeks.





**FIGURE 6** Volumized nasolabial folds in a subject using the Active products for 16 weeks **a)**, baseline; **b)**, after 16 weeks.



redients, demonstrated statistically significant clinical efficacy versus its vehicle control. The Active formulation reduced the appearance of targeted wrinkles including, glabellar lines, nasolabial folds, under eye wrinkles, and lateral canthal (crow's feet) wrinkles. Significantly greater improvement was shown with the Active group over the Vehicle control group starting as early as week 4 and continuing through week 16. In addition, the Active group increased skin firmness and elasticity significantly more than the Vehicle control. Ultrasound revealed improvements in dermal density that corresponded to clinically-graded eye area improvements.

The significant results achieved with use of the Active formulations versus the Vehicle control affirmed the clinical contribution of the cosmetic, anti-aging ingredients and sub-

stantiate the volumizing benefits of the noninvasive, topical formulation. The tested formulation is an effective, targeted wrinkle treatment option which can be used to augment the effects of all-over, anti-aging products. It is appropriate for a young patient population (eg, 30+) who may not be ready for injectable therapies, or for needle-shy patients who are seeking an alternative to injections. It can also be used to plump hard to inject treatment sites such as perioral rhytides and under eye lines and wrinkles. Furthermore, these results suggest the potential for complementary benefits with injectable therapies such as neurotoxins and dermal fillers by providing additional skin volumizing effects. The topical cosmetic volumizing formulation can be used alone as a targeted wrinkle reducer or adjunctively with injectable therapies for patients seeking wrinkle effacement.

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## AUTHOR CORRESPONDENCE

**Barbara A. Green**

E-mail:..... bgreen@neostrata.com