A Novel Macrophage-Activating Gel Improves Healing and Skin Quality After CO_2 Laser Resurfacing of the Chest

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BACKGROUND After laser resurfacing, it is imperative that an appropriate postoperative regimen is followed for optimal wound healing. There is currently no consensus about which agents should be used.

OBJECTIVE To evaluate the safety and efficacy of a novel macrophage-activating gel in a Phase 2B trial to be used after fractionated ablative laser resurfacing of the chest.

MATERIALS AND METHODS Forty-two adults who received fractionated CO_2 laser resurfacing of the chest were randomized (active or placebo) for 5 consecutive days after procedure. Skin quality at baseline and follow-up was assessed by a blinded evaluator using the Fitzpatrick–Goldman Wrinkle Scale. Subject satisfaction with skin healing and quality was also assessed.

RESULTS At 28 days according to the Fitzpatrick–Goldman Wrinkle Scale, 85% of subjects achieved an improvement of at least 33% for the active group versus 50% in the placebo group (absolute difference 35%; $p = .04$). Similarly, 75% of subjects achieved an improvement score of at least 33% in elastosis in the active group versus 35% in the placebo group at 28 days (40% absolute difference; $p = .011$).

CONCLUSION This study confirms the potent effects of the novel macrophage-activating gel for optimization of skin healing and quality after laser resurfacing of the chest.

Fractionated ablative resurfacing is a proven therapeutic option to improve the appearance of ultra-violet-induced photodamage, rhytidosis, dyspigmentation, and suboptimal skin texture.[1,2] Postoperative skin care is critical in promoting optimal wound healing and cosmetic outcome after laser therapy.[3]

A number of serums, gels, and creams based on varied technologies including growth factors, herbal healing agents, stem cells, and other molecules have been used after procedure[4–12]; however, there is currently no gold standard agent because of the lack of rigorous clinical data supporting most of these topical substances.[13]

TR-987 0.1% gel (Tissue Repair Limited, Sydney, Australia) is a drug candidate that has been designed to activate pattern recognition receptors on immune cells developed during evolution to defend against pathogenic microorganisms. Using the principle of biologic pattern recognition, the hydrogel uses a highly purified insoluble glucan "skeleton" of a yeast cell that exposes the antigenic regions of the yeast glucan to macrophages as a decoy.

When recognizing a perceived threat, a genetically coded pattern recognition process through stimulation of toll-like receptor 2 and dectin-1 membrane receptors is triggered on wound macrophages, and signaling pathways meant to combat the threat are activated.[14,15]

Although the evolutionary purpose of these mechanisms is to defend against a pathogenic threat, they may also be leveraged as powerful stimuli for tissue repair and regeneration, leading to enhanced wound healing.

In this prospective, Phase 2B double-blind, placebo-controlled, randomized clinical trial, the authors assess the safety and efficacy of TR-987 0.1% gel in improving skin quality outcomes after fractional CO_2 laser resurfacing of the chest.

Methods

Clinical Study Design

This double-blind, randomized, placebo-controlled clinical trial was institutional review board–approved. Forty-two otherwise healthy subjects were enrolled. Subjects were between the ages of 45 to 65 years with Fitzpatrick skin types between I-III and baseline chest wrinkling class of II or greater and elastosis score of 6 or greater on the Fitzpatrick–Goldman classification of wrinkling and degree of elastosis Fitzpatrick–Goldman Wrinkle Scale

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(FGWS) (see **Supplemental Digital Content 1**, Table S1, http://links.lww.com/DSS/B164).

Fractionated Ablative Laser Resurfacing of the Chest

All subjects underwent a standardized fractionated CO_2 laser resurfacing treatment to the chest. Sixty minutes before laser resurfacing, topical anesthesia consisting of a compounded 7% lidocaine and 7% tetracaine in a petrolatum base was applied. The settings for the CO_2 laser resurfacing (ActiveFX, Lumenis, Yokneam, Israel) were fluence of 80 mJ/cm^2 and pattern 3/size 6/density 3.

After the treatment, a cool sterile dilute vinegar compress (1 tablespoon vinegar in 1 cup of sterile water) was applied to the chest followed by a sterile water rinse. After the rinse, postoperative gel (TR-987 0.1% active gel or placebo gel) was applied to the chest.

The chest area was selected because it enabled a larger area to be evaluated more accurately for improvements in skin quality which was considered more appropriate to assess skin and wrinkling scores. Although the chest may not heal as robustly as the face after fractional ablative laser resurfacing because of the relative reduced density of sebaceous glands, presumptive stem cells, and other factors, this technique can still be applied safely and effectively to this area as long as pulse energy, density, and other laser parameters are adjusted accordingly.

The treatment area was standardized by defining the anatomical boundaries of the chest which extended superiorly to the clavicles, laterally to the nipple line, and then tapering inferiorly in a V shape along natural contours to the mid-sternum. Some variation was included at the discretion of the laser physician to optimize aesthetic outcome.

Study Intervention

After ablative laser resurfacing of the chest, 42 subjects were randomized to receive the TR-987 0.1% active gel or the placebo gel consisting of the vehicle without the active compound. Study staff applied a full tube (30 g) daily at study visits for a total of 5 applications. Once these series of applications were completed, all subjects were transitioned to routine standard of care consisting of daily gentle cleansing and application of 1% dimethicone ointment.

Evaluation of Efficacy, Safety, and Satisfaction

Subjects were followed daily after the CO_2 resurfacing procedure until complete reepithelialization of the treated skin occurred. A blinded evaluator rated erythema, edema, exudate, crusting, and percentage healing of the treated area daily using a standardized 5-point scale. Subjects were also asked daily to evaluate pain, itching, tightness, oozing, and crusting on a standardized 10-point visual analog scale.

A blinded evaluator assessed skin quality across wrinkling and elastosis at baseline, Day 28, and Day 104 using the validated 3- and 9-point FGWS. Although the FGWS is not validated specifically for chest wrinkles, it has been used in the literature as a standard scientific tool for the assessment of cutaneous wrinkling and elastosis.[16,17] This scale was chosen (as opposed to the Fabi–Bolton scale which was also developed by the authors' group) because of its more granular nature and the ability of the FGWS to assess both wrinkling and elastosis, 2 elements of cutaneous photodamage that can specifically be addressed with the treatment modality used in this article. The Fabi–Bolton scale does not allow for the evaluation of cutaneous elastosis.

At Day 28 and Day 104, subjects rated their satisfaction in terms of healing and overall cosmetic result on a standardized 5-point scale. Blinded evaluator rating of erythema, crusting, and percentage healing and subject evaluation of pain, itching, tightness, oozing, and crusting also occurred at Day 28 and Day 104.

All digital images were taken under identical settings using the stationary Canfield Vectra 3D Imaging System to ensure comparability and to eliminate slight variations in lighting, position, and camera angle that are unavoidable with standard 2D photography.

Statistical Methods

Descriptive statistical methods were used to summarize the data from this study which included number of subjects (n), mean, median, SD, minimum, and maximum for continuous data, and frequencies and percentages for categorical data. All data collected during the study were reported and analyzed.

Skin quality end point analysis (difference between groups regarding subjects achieving a reduction in elastosis of $\geq$3 points and reduction in wrinkling $\geq$1 point) at both 28 and 104 days was conducted using χ^2 tests. The primary end point was the proportion of patients achieving $\geq$3-point reduction in elastosis at 28 days; the remaining secondary end points (reduction in wrinkling $\geq$1 point at 28 days; reduction in elastosis of $\geq$3 points at 104 days; and reduction in wrinkling $\geq$1 point at 104 days) were tested statistically using a simple serial gatekeeping procedure per the statistical analysis plan before data analysis.

Unless specified otherwise, all statistical testing was two-sided and performed using a significance (alpha) level of 0.05.

Intent-to-Treat Population

The intent-to-treat population was all randomized subjects, regardless of actual treatment.

Modified Intent-to-Treat Population

The modified ITT (MITT) population was all randomized subjects who completed the study for at least 28 days after randomization (the "completer population"), according to the original treatment assignment group.

The analysis presented will focus on the MITT group which removes only 2 patients who were lost to follow-up and could not be contacted after the randomization visit.

Results

Subject Demographics

Forty-two subjects were enrolled in the study; all were women. **Supplemental Digital Content 2**, Table S2, http://links.lww.com/DSS/B164 shows the baseline values for age, Fitzpatrick skin type, wrinkling, and elastosis scores. Subjects enrolled in the study were all female and of Caucasian descent and ranged in age from 45 to 67 years, with a mean age of 57 years. All subjects were in overall good health without uncontrolled preexisting conditions and were considered to be good candidates for laser resurfacing of the chest.

Two subjects were lost to follow-up at Days 10 and 12 and were uncontactable after the initial procedure. Subsequent analysis was performed based on MITT as described above.

Skin Quality Results

The validated 3- and 9-point FGWS was used to evaluate the effectiveness of the TR-987 0.1% gel in optimizing skin quality after procedure versus placebo gel. Both test and control regimens promoted safe and effective healing of the chest skin after the procedure. Summary data of all MITT patients are provided in **Supplemental Digital Content 3**, Table S3, http://links.lww.com/DSS/B164.

The proportion of subjects achieving ≥3-point reduction in elastosis between baseline (randomization) and 28 days was 75% in the TR-987 group (15/20), and 35% in the placebo group (7/20), an absolute difference of 40% ($p = .011$) (Figure 1). The active group at Day 28 demonstrated over double the effectiveness (40% absolute difference and 114% percentage difference) in delivering patients a 33% improvement in elastosis (i.e., ≥3-point reduction in elastosis over a 9-point scale). This was statistically significant ($p < .011$).

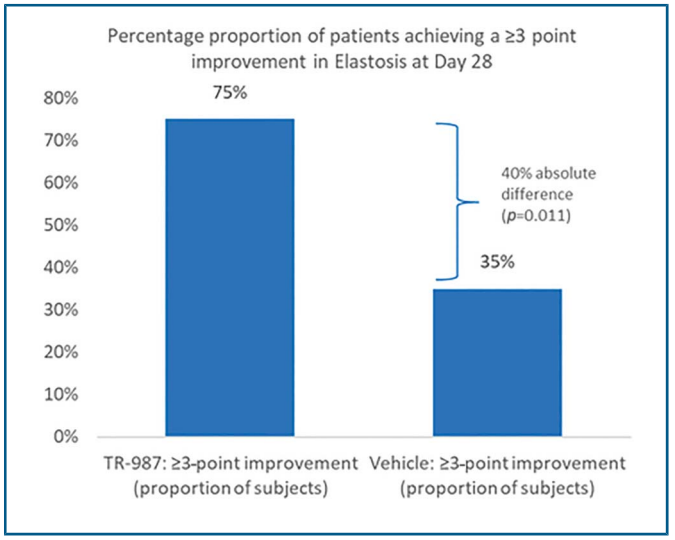

Figure 1. The proportion of patients within each of the TR-987 and vehicle groups who achieved a ≥3-point improvement in elastosis scores between baseline and Day 28 (Fitzpatrick–Goldman classification). A χ^2 test for unadjusted proportions was used to determine significance expressed as a p value.

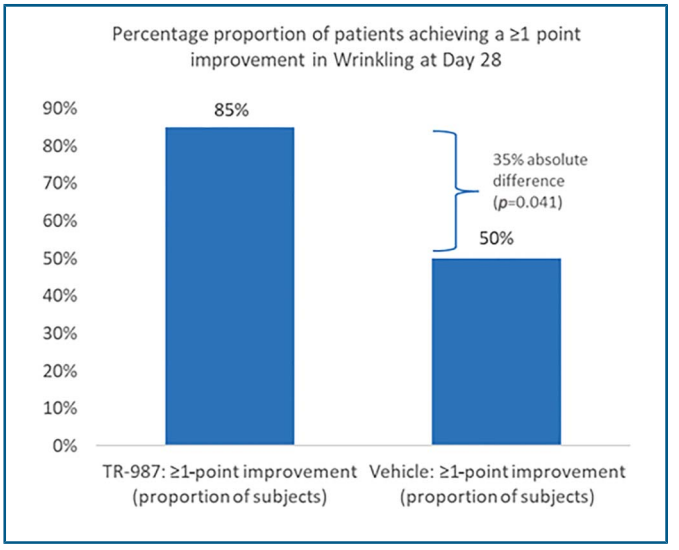

Figure 2. The proportion of patients within each of the TR-987 and vehicle groups who achieved a ≥1-point improvement in wrinkling score between baseline and Day 28 (Fitzpatrick–Goldman classification). A χ^2 test for unadjusted proportions was used to determine significance expressed as a p value.

The proportion of subjects achieving ≥1-point reduction in wrinkling between baseline and 28 days was 85% in the TR-987 group (17/20), and 50% in the placebo group (10/20), an absolute difference of 35% ($p = .041$) (Figure 2). The active group at Day 28 demonstrated a 70% percentage difference over control in delivering patients a 33% improvement in wrinkling (i.e. ≥1-point reduction in wrinkling over a 3-point scale). This was statistically significant ($p < .041$).

Although there were statistically significant and meaningful differences observed at Day 28 in terms of accelerated skin quality improvements as measured by elastosis and wrinkling, by Day 104, elastosis changes were comparable between active and vehicle groups. Although there remained some differences in wrinkling at Day 104, these were not statistically significant.

At 104 days after procedure, the proportion of subjects achieving ≥3-point reduction in elastosis between baseline was 55% in the active group (11/20), and 50% in the placebo group (10/20), a difference of 5% ($p = .75$).

At 104 days, the proportion of subjects achieving ≥1-point reduction in wrinkling between baseline and 104 days was 90% in the TR-987 group (18/20), and 65% in the placebo group (13/20), an absolute difference of 25%. This was not statistically significant, although post hoc analysis indicated significance may be achieved with powering the study with double the patient numbers.

Wound Healing Results

Physician-assessed end points that were examined included percent healing, erythema, edema, crusting, and exudation.

Wound closure was measured by percent healing with wound closure assessed at 100% when complete wound

epithelialization occurred. No substantial differences were observed in percent healing rates between the groups, with all subjects achieving complete wound closure defined as 100% wound epithelialization by Day 10 to 12.

For physician-assessed parameters, the data indicate that for erythema, the active group showed slightly higher results from Day 4 to Day 8 compared with the placebo group, but after Day 8, the active arm showed improved erythema over the control arm.

The active arm seemed to demonstrate higher crusting scores over the control arm by Day 14, although the differences were not considered to be clinically significant. There were no apparent differences observed in the results for edema, exudation, and percent healing.

The subject-assessed end points of pain, itching, and tightness, at 14 days, all seemed similar for both groups, whereas the active arm seemed to record superior scoring between Days 3 and 10. The subject-assessed end points of pain, itching, and tightness, at 14 days, showed lower subject scoring in the TR-987 group. Subjects' experience with the gel on these measures seemed superior over the control with more meaningful differences between the groups observed in itching. Regarding oozing, the active arm recorded superior scoring between Days 3 and 10 with subjects assessing no differences between the groups after Day 10. Statistical analysis was not undertaken for any of these measures because it was clear no statistical differences emerged between the groups.

Adverse Events

No differences were observed between the placebo and active arms in terms of adverse events. Two patients exhibited mild infections that resolved with antifungal and corticosteroid treatments, respectively. One patient exhibited dermatitis, which was resolved with an antifungal treatment. Aside from these, there were no adverse events considered outside the normal course of healing, or deemed to be attributable to TR-987, that occurred during this study. No deaths, serious adverse events, or other significant adverse events occurred during this study.

Discussion

The clinical study confirms statistically significant skin quality improvements from the blinded physician scoring of elastosis and wrinkling at 28 days from the use of the TR-987 0.1% gel after fractionated ablative laser resurfacing of the chest. The magnitude of the differences recorded was clinically and aesthetically meaningful. The gel seems to improve the skin quality outcomes of the underlying procedure as measured by elastosis and wrinkling on a percentage basis of between 70% and 114% for achieving a greater than 33% improvement in elastosis and wrinkling.

Measures associated with other healing parameters, including complete wound epithelialization, seemed relatively consistent between the groups, although on some measures, the active gel seemed to deliver some meaningful benefits, including reduced pain, itching, oozing, and tightness. By contrast, the degree of crusting seemed slightly greater in the active arm, although by Day 12, both groups resolved to consistent levels. Erythema seemed elevated during the first 8 days in the active arm but was then diminished compared with the placebo arm across Days 10 to 12.

Overall healing despite the addition of the proinflammatory active to standard of care seemed relatively consistent in both groups, with both groups assessing a high score for overall healing satisfaction. The active arm delivered material differences in skin quality at Day 28, while achieving healing consistent with the placebo arm.

The authors hypothesize that TR-987 is activating or accelerating the tissue repair process because of its ability to stimulate a mild proinflammatory process. This process is characterized by an influx of macrophages into the wound

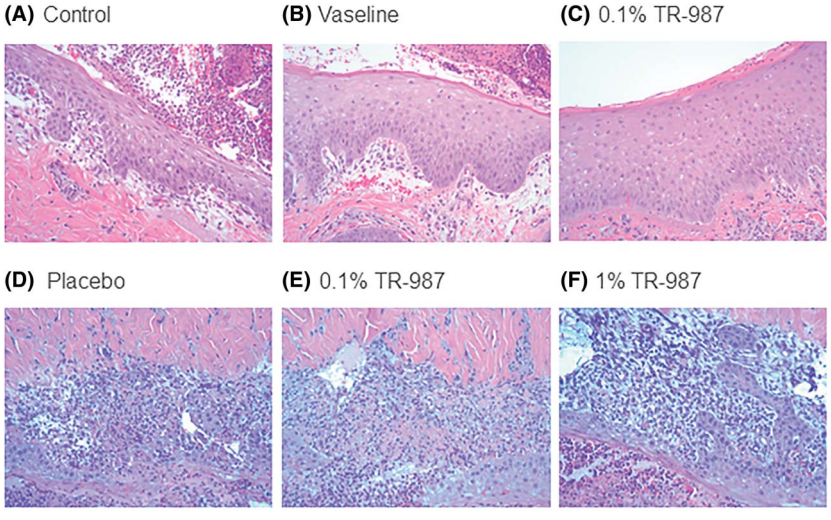

Figure 3. The most marked increase in epidermal thickness occurred at the 0.1% TR-987 test sites (A–C). Split-skin graft preparations demonstrated a greater regenerative response with TR-987® compared to placebo based on changes in the stratum lucidum and stratum corneum at day 5 of treatment (D–F).

space, followed shortly afterward by a spike in levels of proinflammatory cytokines such as TNF-β, that in turn attract other types of inflammatory cells. This is associated with initiation of the cascade of repair processes such as fibrogenesis, angiogenesis, and epithelialization.[4]

The results of this study suggest that the induction of proinflammatory activity by TR-987 into this damaged environment does not compromise the acute healing process but in contrast further improves the final skin quality outcome. Presumably, TR-987 achieves this positive effect by mildly stimulating wound macrophages, which act to enhance tissue regeneration, collagen production, and fibrosis. As a result, skin rejuvenation is accelerated, without the addition of any deleterious impacts from the proinflammatory agent. The physical properties of the drug product arising from the combination of the hydrogel base and proprietary β-glucan active ingredient may also contribute to the improved skin quality outcomes observed.[18–21]

There were no meaningful differences in elastosis observed at Day 104 between the groups; however, meaningful differences in wrinkling persisted at Day 104 from Day 28, which were not statistically significant. Post hoc statistical power analysis suggests that statistical significance at Day 104 in wrinkling could be achieved with double the subject numbers, and the positive longer-term results on wrinkling from the use of the TR-987 gel could be investigated further.

Data on the effects of TR-987 have also been documented in histological studies using animal models as well as Phase 1 and Phase 2 clinical data in chronic venous leg ulcers.[22,23]

In a Goettingen mini-pig model, histological examination of comparisons between TR-987 gel and placebo demonstrated that the most marked increase in epidermal thickness occurred at the 0.1% TR-987 test sites after daily administration for 5 days (Figure 3A–C). Subsequently, histological examination of split-skin graft preparations in the presence and absence of TR-987 demonstrated a greater regenerative response with TR-987 compared with placebo based on changes in the stratum lucidum and stratum corneum at Day 5 of treatment (Figure 3D–F).

TR-987 is the only topical drug in development with an insoluble β-glucan which is distinct from soluble β-glucans which are believed to invoke significantly less inflammatory response.[24]

Conclusion

In summary, this study demonstrates the potential of TR-987 0.1% gel to significantly improve skin quality outcomes after fractionated ablative laser resurfacing without any negative side effects, most likely through a unique immunomodulatory mechanism of action.

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