TA-65® for Skin Reduces the Appearance of Aging Effects by Increasing Firmness and Reducing Wrinkles and Redness

Toni F. Miller, Ph.D.
Essex Testing Clinic Inc., 799 Bloomfield Ave., Verona NJ 07044
Study sponsored by TA Sciences, Inc., 420 Lexington Ave., New York, NY 10170

Abstract
TA-65® for Skin is a topical cream that has been developed as a novel product containing the active ingredient TA-65®, which is a pure molecule extracted from the Chinese herb, *Astragalus*. TA-65® has been extensively studied both experimentally and clinically for the impact on health and lifestyle. *TA-65® for Skin* has been tested in a pilot clinical study for its impact on skin's function. The study suggests that *TA-65® for Skin* improves skin's function by increasing firmness, reducing wrinkles and erythema.

Introduction
Skin undergoes age-related decline in regenerative capacity resulting in compromised homeostatic imbalance and severe skin conditions (Pillai, Oresajo et al. 2005). Senescence, a state of replicative arrest, has been shown to increase with age in dermal fibroblasts and epidermal keratinocytes in human (Dimri, Lee et al. 1995). Senescence limits replicative potential of cells and fuels inflammation in aging (Campisi 2013).

Telomeres, the TTAGGG DNA sequences at the ends of chromosomes, act as a mitotic clock and prevent from aberrant proliferation (Chan and Blackburn 2004). Telomeres undergo shortening with each cell division, and critically short telomeres result in cellular senescence and aging (Chan and Blackburn 2004, Buckingham and Klingelhutz 2011). Telomeres are maintained by the enzyme telomerase which adds telomeric sequences to the chromosome (Buckingham and Klingelhutz 2011).

In somatic cells, the telomerase gene is repressed resulting in telomere shortening and senescence. When introduced into cells, telomerase helps maintain or lengthen their telomeres and allow cells to continue growing in a normal manner, suggesting that telomerase activation could be of therapeutic importance in combating aging. Furthermore, increasing evidence suggest that telomerase plays pivotal role in maintaining skin's function (Buckingham and Klingelhutz 2011).

A small molecule derived from root extracts of Astragalus has been identified in a screen based on its ability to up regulate telomerase activity in neonatal human keratinocytes (TA-65®, TA Sciences Inc.). TA-65® has been shown to rescue short telomeres and decrease the senescent cells. Telomerase activation and studies of TA-65® *in vitro*, in mice and humans as a nutritional supplement have been reported (Bernardes de Jesus, Schneeberger et al. 2011, Harley, Liu et al. 2011). TA-65® has also been shown to increase wound healing in vitro. In the current study, topical formulation of TA-65® (*TA-65® for Skin*) is investigated as a novel product for skin aging. The pilot study suggests significant improvement in skin firmness, reduction in fine lines and wrinkles and reduction in erythema following the use of *TA-65® for Skin*.

Objective
The objective of this study was to determine the effect of *TA-65® for Skin* on the appearance of Crow's feet fine lines/wrinkles, skin firmness and the appearance of red/inflamed skin in a panel of 54 female subjects, aged 35-60 years, after 2, 4, 8 and 12 weeks' application of *TA-65® for Skin* or other skin care formulations.

Methodology
This study was conducted in compliance with U.S. Code of Federal Regulations (CFR), Title 21 and informed consent was obtained from each subject. Fifty-four (54) female subjects, aged between 36 and 60 were enrolled in this 12 weeks' study.

Twenty subjects were instructed to apply *TA-65® for Skin* twice daily for 12 weeks and were instructed to report to the Testing Facility following 2, 4, 8 and 12 weeks of product use for visual evaluations, instrumental measurements and digital photographs. Additionally, at the final visit, subjects were required to respond to a questionnaire. Evaluations of efficacy were based on a comparison of baseline vs. each observation period for each product individually.

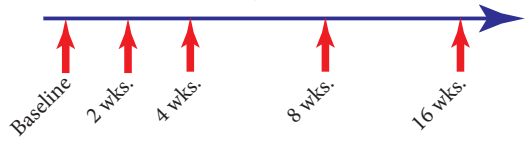

Figure 1: *Study visits*

Subjects reported to the Testing Facility for the baseline visit. A Cutometer® measurement was taken on the face to measure skin's firmness. Digital photographs were taken of the face of each subject to determine changes in Crow's feet fine lines/wrinkles and redness. Digital imaging was done using Visia CR® and ImagePro® software was used to analyze the changes in Crow's feet fine lines/wrinkles. Additionally, evaluation of irritation was conducted for safety purposes.

Results and Discussion
Safety
A trained technician evaluated the face of each subject for irritation at baseline and after 2, 4, 8 and 12 weeks. The mean score of irritation was zero for all products tested in this study, which indicate that *TA-65® for Skin* does not cause any irritation on the face of any subject at any time during the study.

Efficacy
Evaluation of Skin Firmness
Compared to the baseline measurements, there were improvement in the mean percent of the skin firmness. The *TA-65® for Skin* significantly improved the skin firmness by 42%, 93%, 101%, 89% after 2, 4, 8 and 12 weeks of daily application of *TA-65® for Skin* ($p<0.05$)(Table 1). A total of 89%, 83%, 83% and 83% of the subjects showed improvement after 2,4,8 and 12 weeks of *TA-65® for Skin* use (Table 1).

Time	*Mean score ± S.D*	*p-value*	*Mean % change from baseline*	*% of subjects with improvement from baseline*
Baseline	0.405 ± 0.159	-	-	-
Week 2	0.517 ± 0.135	0.002	42.2%	89%
Week 4	0.603 ± 0.090	<0.001	93.3%	83%
Week 8	0.629 ± 0.070	<0.001	100.6%	83%
Week 12	0.591 ± 0.082	<0.001	89.4%	83%

Table 1: *Evaluation of skin firmness by Cutometer®. Statistically significant differences from baseline, p≤0.05*

Evaluation of Crows' Feet Fine Lines/Wrinkles

Compared to baseline, there were improvement in the mean percent of the Crow's feet fine lines/wrinkles. The mean percent improvement are 10.6%, 7.6%, 7.3% and 18.1% respectively at 2, 4, 8 and 12 weeks (Table 2). The improvements were statistically significant at 12 weeks (Table 2).

A total of 68%, 79%, 67%, and 78% of the subjects showed improvement from baseline after 2, 4, 8 and 12 weeks of *TA-65® for Skin* use, respectively (Table 2).

Time	*Mean score ± S.D*	*p-value*	*Mean % change from baseline*	*% of subjects with improvement from baseline*
Baseline	1524.4 ± 1702.4			
Week 2	1043.0 ± 430.1	0.145	-10.6%	68%
Week 4	1107.9 ± 706.4	0.169	-7.6%	79%
Week 8	1024.5 ± 446.2	0.108	-7.3%	67%
Week 12	929.8* ± 438.4	0.002	-18.1%	78%

Table 2: *Evaluation of Crow's Feet Fine Lines/Wrinkles by VISA CR® digital imaging. *Statistically significant differences from baseline, p ≤0.05.*

Evaluation of Skin Redness

Compared to baseline, there were improvement in the mean percent of the skin redness. The mean percent improvement are 1.3 %, 4.5 %, 5.9% and 8.6% respectively at 2, 4, 8 and 12 weeks (Table 3). The decrease in redness were statistically significant at week 4, 8 and 12 (p<0.05).

A total of 58%, 63%, 72%, and 89% of the subjects showed improvement from baseline after 2, 4, 8 and 12 weeks of *TA-65® for Skin* use, respectively (Table 3).

Time	*Mean score ± S.D*	*p-value*	*Mean % change from baseline*	*% of subjects with improvement from baseline*
Baseline	13.4 ± 2.6			
Week 2	13.1 ± 2.3	0.418	-1.3%	58%
Week 4	12.6 * ± 2.2	0.032	-4.5%	63%
Week 8	12.2* ± 2.1	0.003	-5.9%	72%
Week 12	11.8* ± 1.9	<0.001	-8.6%	89%

Table 3: *Evaluation of Skin Redness. *Statistically significant differences from baseline, p≤0.05.*

Evaluation of Irritation

There was no irritation on the face of subjects at any time during the study.

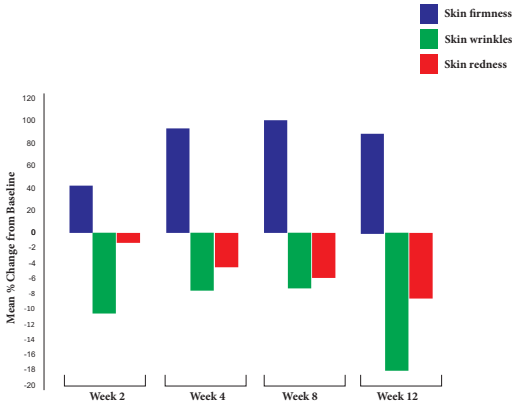

Figure 2: *Cosmetic efficacy of TA-65® for Skin*

Questions	Strongly agree or agree
This product reduces the appearance of Crow's feet fine lines.	83.3%
This product reduces the appearance of Crow's feet wrinkles.	77.8%
The signs of aging around my skin feel firmer.	83.3%
This product made my skin feel firmer.	94.4%
This product made my skin feel tighter.	88.9%
This product helped to reduce the appearance of red/ inflamed skin.	72.2%
Overall, my skin appears healthier since using the test product	88.9%
Overall, my skin appears younger since using the test product.	83.3%
I would purchase this product.	77.8%
I would recommend this product to a friend	77.8%

Table 4: *Subject Questionnaire summary.*

Subject Questionnaire

At the final visit (12 weeks), subjects responded to a questionnaire. The summary results are represented in the following table (Table 4). After 12 weeks of use, *TA-65® for Skin* was associated with high level of subject acceptance.

Conclusions

The clinical efficacy study revealed that *TA-65® for Skin* helped to improve the appearance of Crow's feet fine lines/wrinkles, increase skin firmness and reduce the appearance of red/inflamed skin when used twice daily for 12 weeks.

References

Bernardes de Jesus, B., K. Schneeberger, E. Vera, A. Tejera, C. B. Harley and M. A. Blasco (2011). "The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence." *Aging Cell* 10(4): 604-621.

Buckingham, E. M. and A. J. Klingelhutz (2011). "The role of telomeres in the ageing of human skin." *Exp Dermatol* 20(4): 297-302.

Campisi, J. (2013). "Aging, cellular senescence, and cancer." *Annu Rev Physiol* 75: 685-705.

Chan, S. R. and E. H. Blackburn (2004). "Telomeres and telomerase." *Philos Trans R Soc Lond B Biol Sci* 359(1441): 109-121.

Dimri, G. P., X. Lee, G. Basile, M. Acosta, G. Scott, C. Roskelley, E. E. Medrano, M. Linskens, I. Rubelj, O. Pereira-Smith and et al. (1995). "A biomarker that identifies senescent human cells in culture and in aging skin in vivo." *Proc Natl Acad Sci U S A* 92(20): 9363-9367.

Harley, C. B., W. Liu, M. Blasco, E. Vera, W. H. Andrews, L. A. Briggs and J. M. Raffaele (2011). "A natural product telomerase activator as part of a health maintenance program." *Rejuvenation Res* 14(1): 45-56.

Pillai, S., C. Oresajo and J. Hayward (2005). "Ultraviolet radiation and skin aging: roles of reactive oxygen species, inflammation and protease activation, and strategies for prevention of inflammation-induced matrix degradation - a review." *Int J Cosmet Sci* 27(1): 17-34.