Demonstrated Improvement of Prematurely Aged Skin by *TA-65® for Skin*

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Abstract:

Telomere attrition is the central hallmark of cellular senescence. TA-65® is a small molecule that has been shown to slow telomere attrition. The main objective of the current study is to evaluate cosmetic benefits of *TA-65® for Skin*, a cosmetic skin-cream that contains TA-65® in subjects with prematurely aged skin (photo-aged). Randomized, double-blind and placebo-controlled study was carried out on 35 photo-aged subjects for sixteen weeks. Clinical assessments were performed at baseline and following 4, 8 and 16 weeks of use. VISIA® complexion analysis system (Canfield Scientific, Fairfield, NJ) revealed that *TA-65® for Skin* may reduce pre-clinical damage and uneven pigmentation after eight weeks' application. Histological analyses of biopsies revealed that the expression of inflammatory cytokines and protease were declined whereas collagen level increased after 16 weeks' use of *TA-65® for Skin*, demonstrating the improvement of pre-maturely aged skin by *TA-65® for Skin.*

Introduction:

Repeated exposure of ultra violet light results in what is called photo-aging. In aging and photo-aging, human skin accumulates senescent keratinocytes and fibroblasts. Senescence not only limits replicative potential of cells but also fuels inflammation associated with aging and photo-aging (Lasry and Ben-Neriah 2015). Thus anti-senescence compounds have tremendous potential as novel therapeutics. TA-65® is a small molecule telomerase activator derived from the *Astragalus* plant. TA-65® has been developed for topical cosmetic applications and the proposed study is designed to test cosmetic skin improvement.

Objective:

The main objective of this study is to evaluate the cosmetic benefits of *TA-65® for Skin* in subjects with prematurely aged skin.

Methodology:

Randomized, double-blind, placebo-controlled study of 35 photo-aged subjects was carried out for sixteen weeks with clinical assessment performed at baseline and following 4, 8 and 16 weeks of use.

The subjects were randomly assigned to either placebo group or treatment group. The placebo group applied placebo cream, which is identically formulated and packed to that of *TA-65® for Skin*, but lacks the active ingredient, TA-65®.

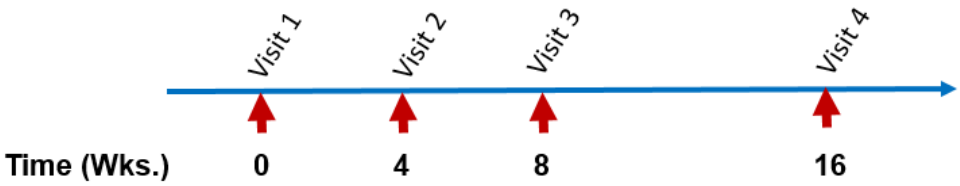

Figure 1: Visits at different time during study period

VISIA® complexion analysis system

VISIA® complexion analysis system (Canfield Scientific, Fairfield, NJ) has been used to objectively measure (Goldsberry, Hanke et al. 2014) the following parameters at all indicated visits (Figure 1): UV spots, wrinkles, brown spots and red areas.

Punch biopsy:

One subject from each group underwent punch biopsy procedure, wherein a small skin tissue were taken at the beginning and end of the trial (16 weeks). The biopsy samples (3mm) were sent to Development Engineering Sciences, LLC (Flagstaff, AZ) which analyzed the molecular markers suggestive of the skin's health by nuclear counting, histology and Real Time – Polymerase Chain Reaction (RT-PCR). Following parameters have been tested on the punch biopsies.

Nuclear counting:

The nuclear counting algorithm was used to count the number of cells present in the stratum basale and spinosum of the H&E stained sections. The algorithm was tuned as per Aperio's algorithm user guide for nuclear quantification. Images acquired from the Hamatsu Nanozoomer digital slide scanner were analyzed using Aperio algorithms.

Immunohistochemistry (IHC) analysis:

Collagen 1, elastin and filaggrin presence in the dermis and epidermis, respectively, were quantified using a color deconvolution algorithm from digitally-scanned IHC slides. Collagen 1 was analyzed at a depth of 200μm below the stratum basale, into the dermis. Elastin was analyzed at a depth of 200 μm above the hypodermis into the dermis. Filaggrin was measured in the stratum granulosum layer.

Real-time PCR (RT-PCR):

IL-6, IL-8, TNF-α, MMP-1 and MMP-12 were measured by RT-PCR. Samples were analyzed in triplicate to calculate fold change (increase or decrease) in gene expression.

Results and Discussion:

VISIA® complexion analysis system analyzed the following parameters: UV spots, wrinkles, brown spots and red areas.

There was a significant reduction (38%) in the mean UV score at 16 weeks of *TA-65® for Skin* application compared to placebo group (p=0.038) (Table 1 and Figure 2). The mean score of UV spots after 16 weeks' in placebo group and *TA-65® for Skin* were 236±30 and 147± 30 respectively.

There was a significant reduction in the mean scores of wrinkles after 4 weeks (21%; $p<0.05$) and 8 weeks (29%; $p<0.05$) of *TA-65® for Skin* application compared to baseline. The mean scores at baseline, 4 weeks and 8 weeks were 24±1.7, 19±0.8 and 17±2 respectively (Table 1). Another independent study conducted at Essex Testing Clinic Inc., likewise, show reduction in wrinkles following the application of *TA-65® for Skin*. Taken together, the objective analyses from the current study and Essex study indicate that *TA-65® for Skin* can effectively reduce facial wrinkles.

There was a significant reduction in the mean scores of brown spots after applying *TA-65® for Skin* for 8 weeks (31%; $p<0.05$) and 16 weeks (70%; $p<0.05$). The mean scores at baseline, 8 weeks and 16 weeks were 100±22, 69±19 and 30±12, respectively (Table 1 and Figure 2).

There was a significant reduction in the mean scores of red areas after applying *TA-65® for Skin* for 8 weeks (30%; $p<0.05$) and 16 weeks (19%; $p<0.05$). The mean scores at baseline, 8 weeks and 16 weeks were 73±8, 51±5 and 59±3, respectively. Another independent study conducted at Essex unveils similar benefits: the erythema score significantly reduced following the daily application of *TA-65® for Skin*. Taken together, both objective analyses indicate that *TA-65® for Skin* can effectively decrease red areas of the skin (Table 1).

Table 1: Cosmetic effects of *TA-65® for Skin*

	Mean	SEM*	*p*-value**
UV spots (after 8 weeks)			
Placebo	236	30	
TA-65® for Skin	147	30	0.05
Wrinkles (*TA-65® for Skin*)			
Baseline	24	1.7	
After 4 weeks	19	2.8	0.04
After 8 weeks	17	2	0.003
UV spots (*TA-65® for Skin*) left side			
Baseline	204	34	
After 4 weeks	171	33	0.03
After 8 weeks	141	26	0.002
Brown spots (*TA-65® for Skin*)			
Baseline	100	22	
After 4 weeks	88	21	0.01
After 8 weeks	69	19	0.02
After 16 weeks	30	12	0.001
Red areas (*TA-65® for Skin*)			
Baseline	73	8	
After 8 weeks	51	5	0.01
After 16 weeks	59	3	0.05

***SEM denotes standard error of mean**

**** *P*-values are derived from comparing the placebo group with *TA-65® for Skin* (Unpaired t-test), or by comparing the values at baseline with that of the indicated time points (Paired t-test).**

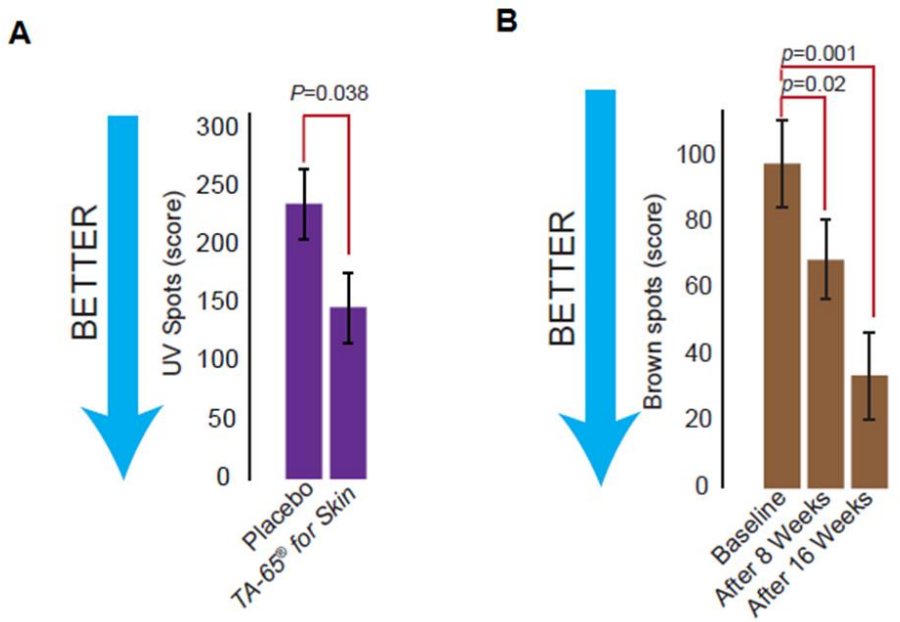

Figure 2: Cosmetic benefits of *TA-65® for Skin.* *TA-65® for Skin* reduced UV spots by 38% compared to placebo group (A) and reduction in uneven pigmentation by 31% after 8 weeks and further reduced by 70% after 16 weeks compared to baseline (B) p-values are estimated by the t-tests.

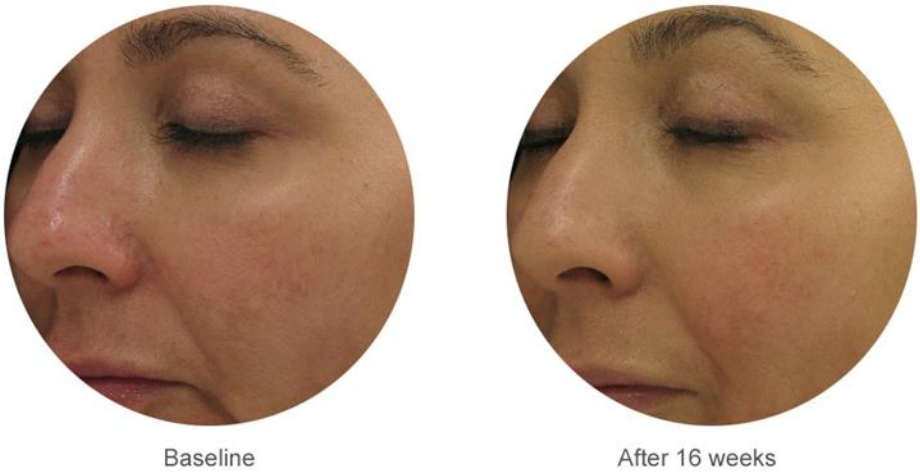

Figure 3: Representative image showing the cosmetic efficacy of *TA-65® for Skin.* Daily application of *TA-65® for Skin* for 16 weeks improves uneven pigmentation.

Histopathology Findings:

Histopathology comparisons between active vs. placebo treatment sites at the 16 week time-point did not reveal any noticeable differences with respect to acanthosis, spongiosis, chronic inflammation, hyperkeratosis, epidermal mononuclear infiltration, or dermal edema. No trend change existed for prominence of any of these characteristics between active and placebo treated samples. Representative histopathology is shown in figure 4.

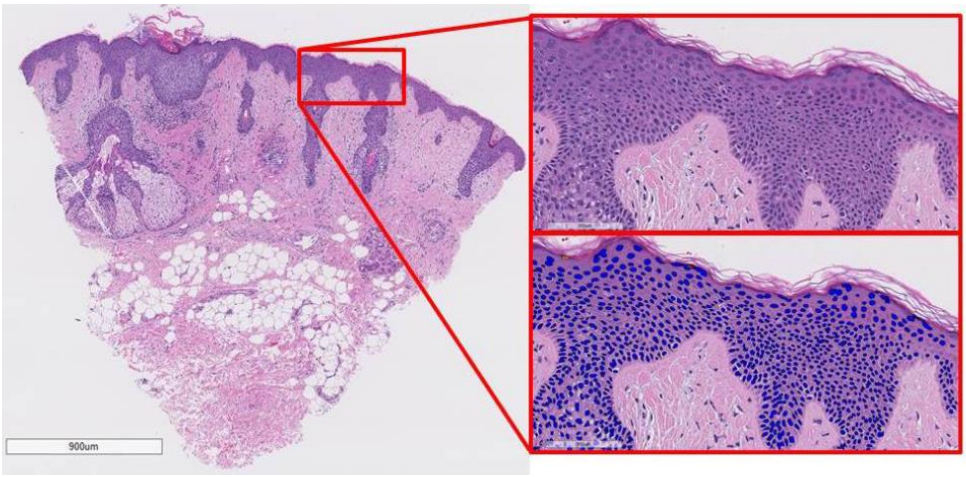

Figure 4A: H&E staining of punch biopsy at baseline in *TA-65® for Skin* group. No acanthosis, spongiosis, chronic inflammation, hyperkeratosis, epidermal mononuclear infiltration or dermal edema were observed at baseline. Left panel is the low magnification (scale bar = 900μm) and right panel is the high magnification (scale bars = 100μm).

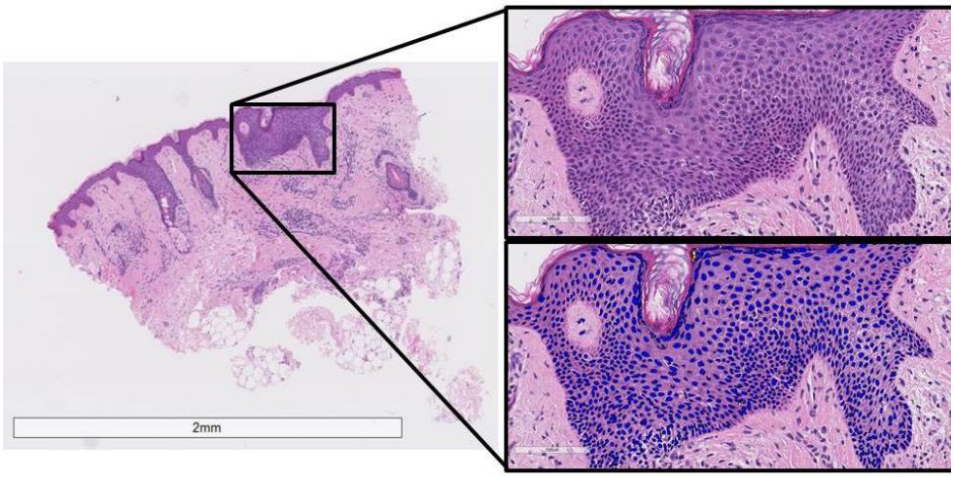

Figure 4B: H&E staining of punch biopsy after 16 weeks of *TA-65® for Skin* use. No acanthosis, spongiosis, chronic inflammation, hyperkeratosis, epidermal mononuclear infiltration or dermal edema were observed with 16 weeks use of *TA-65® for Skin*. Left panel is the low magnification (scale bar = 900μm) and right panel is the high magnification (scale bars = 100μm).

Immunohistochemistry and RT-PCR:

Expression of pro-inflammatory molecules IL-6, TNF-α and MMP-12 increased in the skin treated with placebo cream, whereas application of *TA-65® for Skin* reduced them within 16 weeks (Figure 5A). A 2 fold reduction in the IL-α and TNF-α and 3 fold reduction in the MMP-12 were observed after 16 weeks' use of *TA-65® for Skin;* in placebo group there is a 10 fold increase in IL-6, 1 fold increase in TNF-α and 7 fold increase MMP-12. No improvement in the molecular markers IL-8 and MMP-1 were observed following the application of *TA-65® for Skin*.

Collagen I is a structural protein found in the dermis. It provides the framework to the skin and represents the major extracellular matrix protein within the integument. Figure 5B shows that the treatment of TA-65® for Skin increases the level of collagen by 57% after 16 weeks. No improvement in the molecular markers elastin and filaggrin were observed following the application of *TA-65® for Skin*.

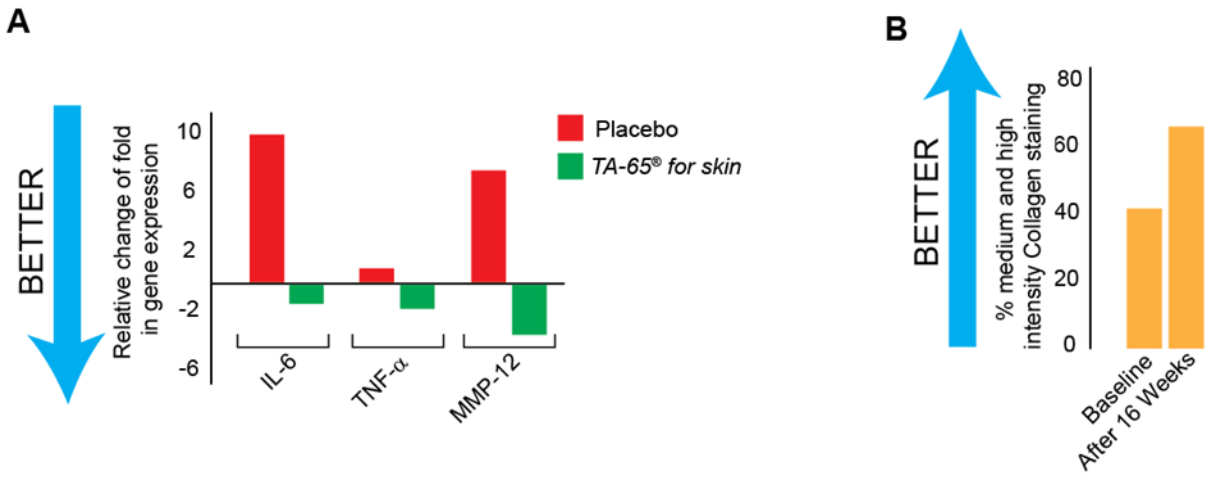

Figure 5: Expression of inflammatory cytokines and protease declines after 16 weeks' use of *TA-65® for Skin*. IL-6 and TNF-α declines by 2 fold, and MMP-12 declines by over 3 fold after 16 weeks' use of *TA-65® for Skin* (A). Collagen level increases by 57% after 16 weeks' use of *TA-65® for Skin* compared to baseline (B).

References:

Goldsberry, A., C. W. Hanke and K. E. Hanke (2014). "VISIA system: a possible tool in the cosmetic practice." J Drugs Dermatol **13**(11): 1312-1314.

Lasry, A. and Y. Ben-Neriah (2015). "Senescence-associated inflammatory responses: aging and cancer perspectives." Trends Immunol.