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Randomized Double-Blind, Placebo-Controlled Study of a Supplement and Serum on Grey Hair

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ABSTRACT

Worldwide, 74% of men and women between the ages of 45 and 65 have some grey hair. Currently, there is no treatment that has been shown in a clinical trial to repigment or slow the development of grey hair. In this study, we investigated the efficacy of a topical and supplement regimen (To The Root® serum and Not Today Grey supplement (Two Lines Inc.). A total of 173 men and women aged 28 to 65 years, with 5–25% grey hair, completed the study. Participants were randomized to include at least 40 subjects in each of the 4 groups: combination (supplement + serum), supplement (supplement + placebo serum), serum (serum + placebo supplement), and placebo (placebo supplement + placebo serum). There was a statistically significant and clinically meaningful reduction in the number of grey hairs in the group that received the combination of supplement and serum. This group had a 9.2% reduction in grey hair at week 12 (P=0.034 vs baseline) and a 13.0% reduction at week 24 (P=0.016 vs baseline). The placebo group had 9.7% increase (worsening) in the mean number of grey hairs from baseline to week 24. The comparison of the combination group vs the placebo group at week 24 showed a significant difference (P=0.004). These results were confirmed by photo grading of grey hair (P=0.002, week 24 vs baseline) and by subject observation of grey hair (P=0.002 vs baseline). These results represent synergistic effects of the proprietary blends in the oral supplement and the patented topical serum.

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INTRODUCTION

Greying of hair, also called canities or achromotrichia, is part of the natural aging process. It has been reported that worldwide, 74% of men and women between the ages of 45 and 65 have some grey hair.[1] Greying typically begins in the mid-30s for Caucasians, the late 30s for Asians, and the mid-40s for Africans.[2-3] The molecular mechanisms behind hair greying are complex and involve several factors, including dietary deficiencies, melanin production, oxidative stress, genetics, DNA damage, mitochondrial dysfunction, inflammation, and hormonal changes.[4-5] Unlike skin, where melanin production is constant, the production of pigment in hair halts in the catagen p,hase and it is completely absent in the telogen phase.[5-6] studies have observed in both ex vivo and human models that reduction of oxidative stress in the hair follicle can result in repigmentation.[7]

Currently, there is no treatment that has been shown in a clinical trial to slow the development or repigment grey hair.[8] Many people, especially women, dye their hair at home or at a salon to mask grey hair. Hair coloring remains the most popular cosmetic approach to manage grey hair.[5] According to a survey conducted by the American Academy of Dermatology in 2022, 75% of women and 12% of men in the United States reported using hair dye products, with a significant portion opting for at-home application, and this hair color market is forecasted to grow to USD 12.7 billion by the year 2033.[9] There have been concerns about the safety of hair dyes and some studies show that use of dyes is associated with a higher incidence of cancer.[10-11] The hair care products investigated in this research study are marketed products: To The Root® serum and Not Today, Grey supplement. These marketed products are rich in vitamins, minerals, antioxidants, and botanical extracts along with unique proprietary blends (proprietary blend in supplement and patented Mela-9® Complex in serum), carefully selected to deliver a multi-pronged approach to slow the progression of grey hair and aid in repigmentation of grey hair. Vitamins B6 (pyridoxine) and B9 (folate) are essential for maintaining a healthy body,[12] vitamin B5 (pantothenic acid) assists with healthy cell turnover and reduced inflammation,[13] and selenium is an antioxidant beneficial for reducing free radicals in the body.[14] The test products also contain vitamin B12 and Polygonum multiflorum (Fo-Ti) that protect melanocytes from oxidative stress and assist with melanin production.[15-16] Other ingredients include vitamin D and copper, which have been reported to protect hair from premature greying.[17]

The objective of this study was to investigate the effect of a combination of marketed hair care products (serum + topical) vs placebo on slowing the progression of grey hair and aiding its repigmentation. To our knowledge, this is the first randomized, controlled, double-blind clinical trial on the novel use of a combination of an oral supplement and a topical serum on grey hair.

MATERIALS AND METHODS

The supplement tested in this study (Not Today, Grey) contains a proprietary blend of Fo-Ti root, black sesame seed extract, PABA (para-aminobenzoic acid) and a combination of antioxidants, vitamins, and minerals. The placebo capsules contained inert ingredients and had the same appearance as Not Today, Grey. Subjects were instructed to take a dose of two capsules of their assigned test product with a meal at approximately the same time daily. The serum tested in this study (To The Root®) contains a patented Mela-9® Complex which includes a combination of antioxidants and a clinically effective peptide.[18] The placebo serum contained inert ingredients and had the same appearance as To The Root®. Subjects were instructed to use the serum twice daily on the target count area.

Study Participants

Men and women 18 to 65 years of age, who had not dyed their hair for at least 1 month prior to baseline and who agreed to refrain from dyeing the hair in the target area until study completion, were scheduled for eligibility screening at the clinic. Prospective subjects who met eligibility according to protocol inclusion and exclusion criteria were enrolled in the study and were assigned subject IDs. Using a computer-generated randomization list, enrolled subjects were assigned to one of four groups (cells):

- Combination: Serum and Supplement
- Supplement: Supplement and Placebo Serum
- Serum: Serum and Placebo Supplement
- Placebo: Placebo Supplement and Placebo Serum

Clinical evaluations were conducted in the clinic at visit 1 (baseline), visit 2 (week 12), and visit 3 (week 24); compliance checks were conducted over the phone at weeks 6 and 18, with a follow-up check over the phone at week 26.

Live Grading of Grey Hair by Expert Graders

The expert graders assessed each subject's number of grey hairs on the Clinical Count Area at baseline and weeks 12 and 24. The Clinical Count Area (2 cm x 2 cm (4cm^2)) was selected on the crown/top of head or on the right temple area (representing approximately 0.667% of a typical head of hair which is 600 cm). The locations of the target grey area and clinical count area were documented on a diagram at baseline (measured from the front hairline) for each subject to ensure that subsequent assessments were performed on the same location. The expert grader used the following scale (only whole numbers allowed) to assess grey hair in the count area: 7= 31 or more grey hairs/cm^2, 6 = 26–30 grey hairs/cm^2, 5 = 21–25 grey hairs/cm^2, 4 = 16-20 grey hairs/cm^2, 3 = 11—15 grey hairs/cm^2, 2 = 6–10 grey hairs/cm^2, 1 = 1–5 grey hairs/cm^2. Prospective subjects qualified if they had grey hair score of 2–6 (where 2 = 6–10 grey hairs/cm^2 and 6 = 26–30 grey hairs/cm^2) in the clinical count area, with grey hair covering ≤ 25% of the overall head. The grader counted the number of grey hairs in the 4 cm^2 clinical count area and divided the number of grey hairs by 4 to obtain the average number of grey hairs in a 1 cm^2 area, to which the 1–7 scale was applied.

Standardized Global Photography and Photo Grading

The standardized global photographs were taken at baseline and weeks 12 and 24 and used for Investigator global assessment (IGA) to capture changes in appearance of hair density and other hair quality attributes. Digital images were taken of each subject's target area using the Canfield Hair Rig Imaging System (Canfield Imaging Systems) and a Canon EOS 7D DSLR camera with 28–75 mm lens under visible lighting. Prior to photography, each subject's hair was parted down the center (front to back) and combed. Each subject's head was positioned on a head stand [with chin rest and forehead rest] at an angle of 60° to capture hair color with top of scalp toward the camera at fixed distance. Images were captured by a professional photographer. A total of 2 images, 1 taken at an angle of 0° and 1 taken at an angle of 45° or 60° (at the photographer's discretion) were taken from each subject at each time point. At each photography visit, a color standard (CasMatch Color Mire chip from Bear Medic Corporation) was photographed prior to beginning each day's photography, and RGB color intensity was measured to make sure lighting was consistent. Because only one of the two sites (Dallas, Texas) had the imaging instrument, standard photos were not captured from all subjects. The standard photographs were used for photo grading by investigator or designee to evaluate hair density on the target area and to rate the changes in each subject's hair on the target area, using a -1 to 1 scale (-1 = less compared to baseline, 0 = same as baseline, and 1 = more compared to baseline) at weeks 12 and 24. The standard photographs also were used for grading other attributes like hair volume, shine, count, and overall health using the IGA scale similar to previous reports.[19]

Subject Global Assessment (SGA)

The subjects answered 7 questions on a scale of +3 to -3 using baseline as reference with +3 being the most favorable response, 0 indicating no change and -3 being the most unfavorable response. The questions were designed to assess changes in grey hair amount and overall hair number, texture, shine, strength, and softness at weeks 12 and 24.

Research Endpoints

The primary efficacy endpoint was the change from baseline in the scaled grey hair count at week 24, as determined by clinical counting of grey hair. Secondary efficacy endpoints were photo grading to assess quality of hair (density count, volume, shine, and overall health) and subject's self-assessment of hair attributes.

Statistical Analysis

All data were reviewed before analysis to ensure accuracy and completeness. Frequency analyses and cross tabulations were performed to ensure data accuracy and consistency. Mixed model, sign test, or Wilcoxon signed rank test was applied where appropriate. The results of the statistical tests, including *P*-values, were reported with corresponding confidence intervals to provide a measure of

precision and reliability, with graphical representations provided where applicable. All statistical tests were one-tailed at significance level alpha=0.05.

Continuous variables were summarized using descriptive statistics, including sample size (N), mean, standard deviation (SD), median, minimum, and maximum values. Categorical variables were presented as frequencies and percentages. Statistical analysis was conducted using SAS software version 9.4 (SAS Institute). Subjects who missed the final visit were excluded from the data analysis.

RESULTS

One hundred and seventy-three subjects were enrolled in the study, of whom the majority (72.8%) were females. There were at least 40 subjects in each of the four test groups, with similar demographics in each group as summarized in Table 1. The mean age of the population was 49.2 +/- 7.3 years. The demographically diverse pool of research participants included those who were White/Caucasian, Black/African American, Asian, multiracial, native Hawaiian or other Pacific Islander, and American Indian or Alaska native.

Clinical Counting of Grey Hair

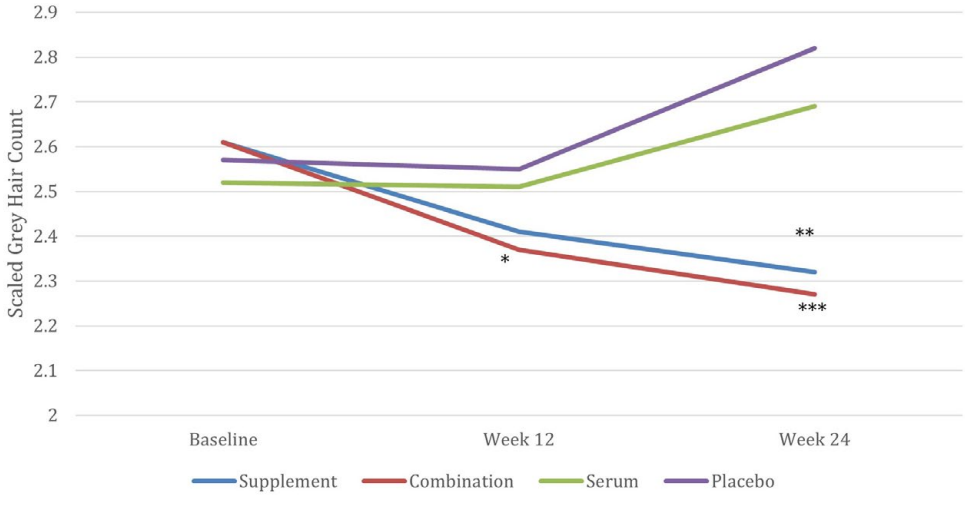
Subjects in the combination treatment group (taking the supplement and applying the serum) showed a progressive decrease in grey hair count (scaled) at week 12 and week 24 (Figure 1 and Table 2). The decrease from baseline was -9% (2.37 vs 2.61, *P*=0.034) and -13% (2.27 vs 2.61, *P*=0.016) at week 12 and week 24, respectively. In contrast, subjects in the placebo group showed an increase at week 24 (+10%). Comparing at week 24, the reduction of grey in the combination group to the increase in grey in the placebo group showed a mean difference of -0.59, a statistically significant difference (*P*=0.004). At 24 weeks, 88% of subjects in the combination group had fewer grey hairs or no change; only 12% had more grey hair (Table 3). In contrast, 35%

TABLE 1.

Demographic Distribution of the Subjects Enrolled in the Study

N (Number of Subjects)	All Subjects		Combination Gp: St + Sr		Suppl Gp: St + PSr		Placebo Gp: PSt + PSr		Serum Gp: PSt + Sr	
	173		41		41		49		42	
Age (years)										
Mean	49.2		49.3		48.9		49.7		48.6	
Standard deviation	7.3		7.2		7.3		7.2		7.7	
Minimum	28		31		32		28		30	
Median	50.0		51.0		50.0		51.0		49.0	
Maximum	65		65		60		60		60	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Sex										
Female	126	(72.8)	30	(73.2)	29	(70.7)	37	(75.5)	30	(71.4)
Male	47	(27.2)	11	(26.8)	12	(29.3)	12	(24.5)	12	(28.6)
Ethnicity										
Hispanic or Latino	33	(19.1)	13	(31.7)	6	(14.6)	6	(12.2)	8	(19.0)
Not Hispanic or Latino	140	(80.9)	28	(68.3)	35	(85.4)	43	(87.8)	34	(81.0)
Race										
American Indian or Alaska Native	1	(0.6)	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.4)
Asian	18	(10.4)	3	(7.3)	6	(14.6)	5	(10.2)	4	(9.5)
Black or African American	47	(27.2)	10	(24.4)	7	(17.1)	18	(36.7)	12	(28.6)
Native Hawaiian or other Pacific Islander	1	(0.6)	0	(0.0)	0	(0.0)	1	(2.0)	0	(0.0)
White or Caucasian	101	(58.4)	28	(68.3)	27	(65.9)	23	(46.9)	23	(54.8)
Multiracial	3	(1.7)	0	(0.0)	1	(2.4)	1	(2.0)	1	(2.4)
Other	2	(1.2)	0	(0.0)	0	(0.0)	1	(2.0)	1	(2.4)
Fitzpatrick skin type										
I	3	(1.7)	0	(0.0)	2	(4.9)	0	(0.0)	1	(2.4)
	All Subjects		Combination Gp: St + Sr		Suppl Gp: St + PSr		Placebo Gp: PSt + PSr		Serum Gp: PSt + Sr	
II	39	(22.5)								
III	60	(34.7)								
IV	34	(19.7)								
V	33	(19.1)								
VI	4	(2.3)								

FIGURE 1. Clinical grading - changes in grey hair over 24 weeks.



Combination and supplement N=41 at all time points, serum N=42 at baseline and week 24 and N=41 at week 12, placebo N=49 at all time points. *P=0.034 vs baseline; **P=0.032 vs baseline and P=0.005 vs placebo; ***P=0.016 vs baseline P=0.004 vs placebo.

of those who received placebo had more grey hair at 24 weeks (Table 2). The actual count of grey hair in the target area was improved in the combination group (34.38 at week 24 and 34.95 at week 12 vs 35.11 at baseline), whereas the count in the placebo group increased from 38.29 at baseline to 42.31at week 12 and to 47.81 at week 24. The differences between treatment groups at weeks 12 and 24 were statistically significant, P=0.014 at week 12 and P=0.002 at week 24.

The subjects in the supplement group also showed progressive reduction in grey hair, 7.7% and 11.1% at week 12 and 24 respectively, but the statistical significance (P=0.032) of changes in grey at week 24 vs baseline was less than that in the combination group (P=0.016).

The subjects in the serum group had little difference in grey hairs at week 12 or 24, but the number of subjects in this group who experienced more greying was only 16% at week 24 vs 35% in those who received placebo.

TABLE 2.

Scaled Hair Counts at Baseline and Weeks 12 and 24.

	Scaled Grey Hair Count Baseline (Mean±SD)	Scaled Grey Hair Count Week 12 (Mean±SD)	Scaled Grey Hair Count Week 24 (Mean±SD)	Week 12 Change vs Baseline	Week 24 Change vs Baseline
Combination	2.61±1.02 N=41	2.37±1.34 N=41	2.27±1.34 N=41	-9.2%*	-13.0%***
Supplement	2.61±1.12 N=41	2.41±1.22 N=41	2.32±1.42 N=41	-7.7%	-11.1%**
Placebo	2.57±0.87 N=49	2.55±1.08 N=49	2.82±1.29 N=49	-0.8%	+9.7%
Serum	2.52±0.77 N=42	2.51±1.23 N=41	2.69±1.37 N=42	-0.4%	+6.7%

*P=0.034 vs baseline, **P=0.032 vs baseline and P=0.005 vs placebo, ***P=0.016 vs baseline and P=0.004 vs placebo.

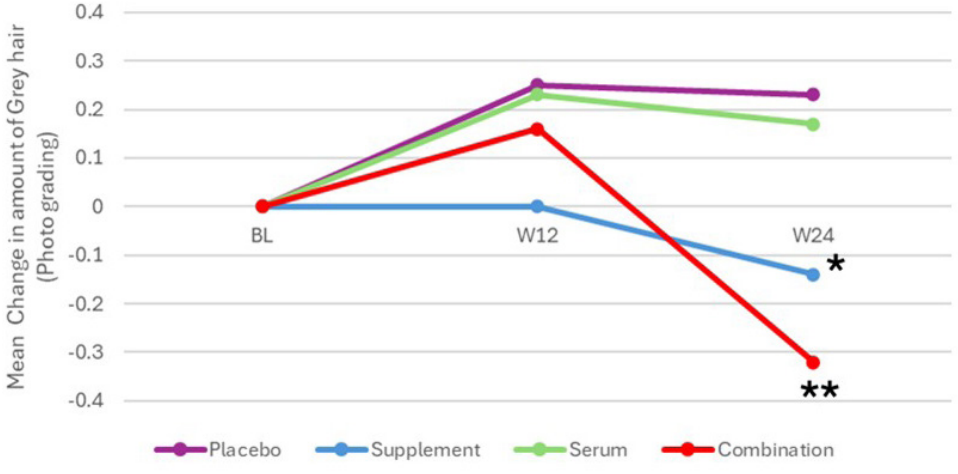
TABLE 3.

Percent of Subjects With Grey Hair Changes at Week 24.

	% of Subjects With More Grey Hair at Week 24	% of Subjects With No Change in Grey Hair at Week 24	% of Subjects With Fewer Grey Hair at Week 24	% of Subjects With Fewer or No Change in Grey Hair at Week 24
Combination	12 (12)	50 (49)	38 (39)	88
Supplement	12 (14)	48 (49)	38 (37)	85
Placebo	33 (35)	45 (45)	22 (20)	65
Serum	17 (16)	67 (67)	17 (17)	83

Combination and supplement N=41 at all times, serum N=42 at baseline and week 24 and N=41 at week 12, placebo N=49 at all times.

FIGURE 2. Photo grading - changes in grey hair over 24 weeks.



Combination, N=31 at all time points, supplement, N=28 at baseline and week 24 and N=27 at week 12, serum N=30 at all time points, placebo N=44 at all time points.
*P=0.031 vs placebo, **P=0.021 vs baseline, P=0.002 vs placebo.

FIGURE 3. Photographic images of three subjects at baseline and week 24. Subjects from the combination group are shown in the left panel and those from the placebo group in the right panel.

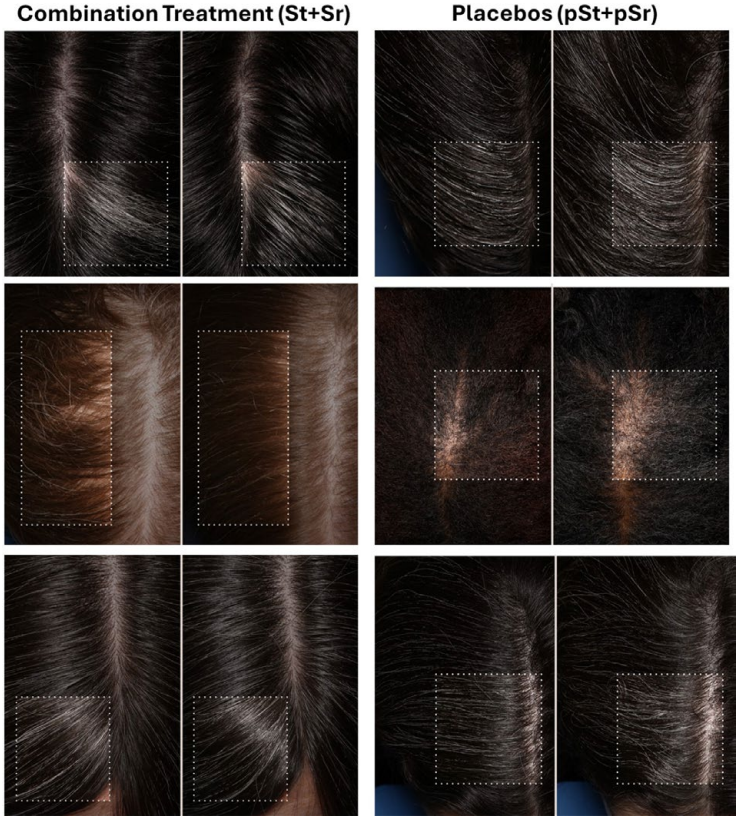
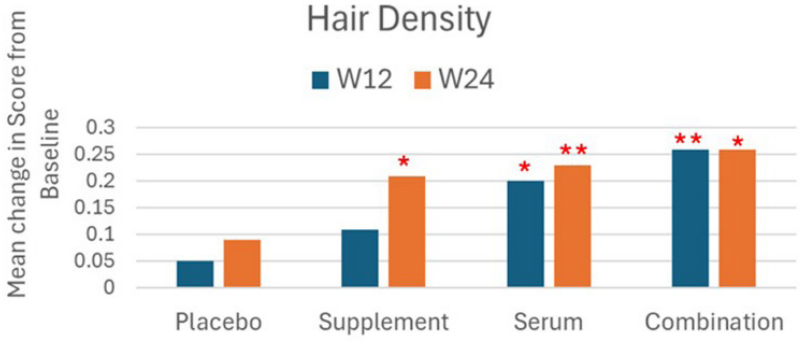
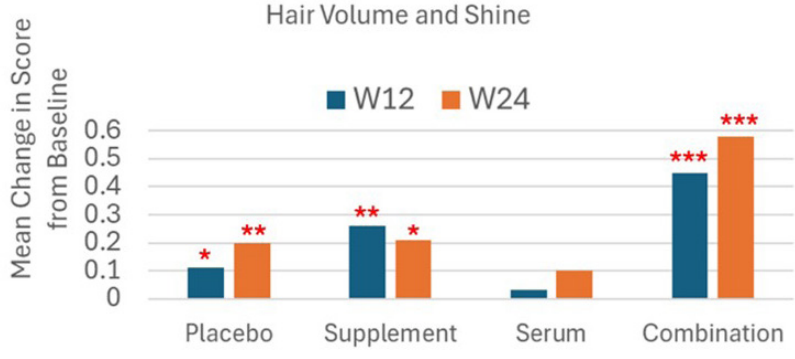


FIGURE 4. Changes in other hair attributes assessed by photo grading, hair density (A), hair volume and shine (B), total amount of hair (C), overall hair quality (D).

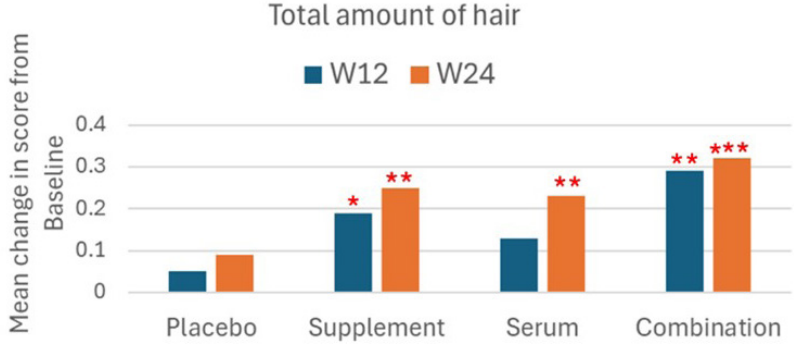
(A)



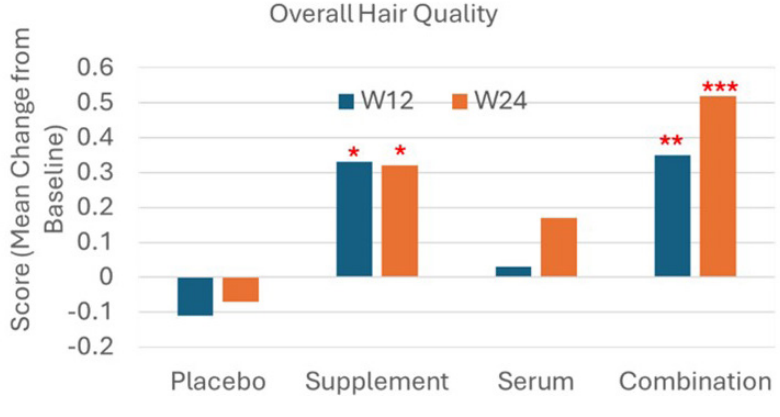
(B)



(C)



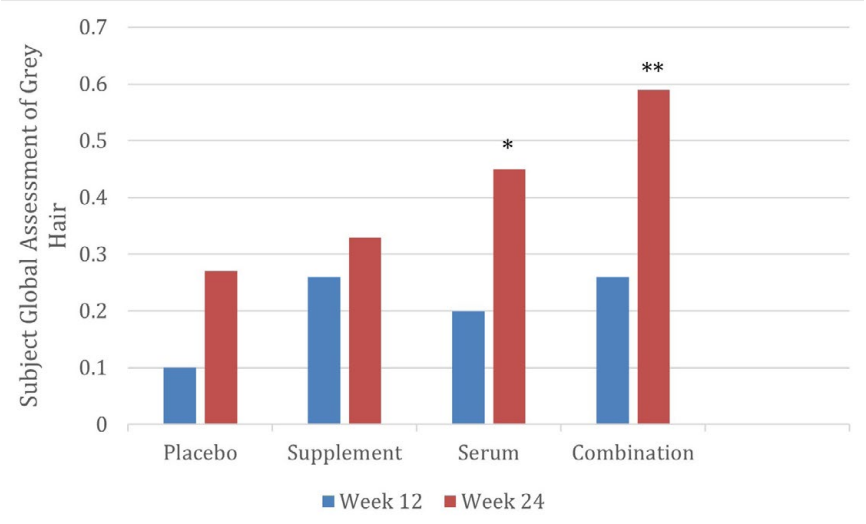
(D)



Combination N=31 at week 12 and week 24, supplement N=27 at week 12 and N=28 week 24, serum N=30 at week 12 and week 24, placebo N=44 at week 12 and week 24.

*P<0.05, **P<0.01, ***P≤0.001 vs baseline.

FIGURE 5. Subject Global Assessment – improvement of grey hair vs baseline.



Combination N=34 at week 12 and N=39 at week 24, supplement N=38 at week 12 and N=39 at week 24, Serum N=41 at week 12 and N=42 at week 24, placebo N=48 at week 12 and N=49 at week 24. *P=0.032; **P=0.002 vs baseline.

Photo Grading

Photo grading results of grey hair were similar to the results obtained by counting grey hair. Significant reduction was visible in the combination group at week 24 (the mean difference vs baseline was -0.32, P=0.021 (Figure 2). In the placebo group, photographs showed worsening at week 12 and week 24 (+0.25 and +0.23). At week 24, a comparison of the combination group vs those who received placebo showed a significant difference (-0.55, P=0.002). Figure 3 shows three representative cases to show improvement in grey hair and other hair quality attributes in the combination group vs placebo group. Photographic grading showed that only 5/31 (16.1%) of subjects in the combination group had more grey hair at week 24 vs 19/44 (43.2%) of those in the placebo group.

Other Hair Quality Attributes Assessed by Photographic Grading

Subjects in the combination group showed progressive and statistically significant improvement at week 12 and week 24 in hair density, total amount of hair, volume, shine, and overall health (Figure 4). Subjects in the supplement group and serum group also showed improvement in hair density and total amount of hair at week 12 and 24. For hair density and total amount of hair, changes in the combination group, supplement group, and serum group were all better than changes in the placebo group. The combination group and supplement group showed improvement in hair volume and shine compared with placebo. Overall hair quality at week 24 was better in the serum, supplement, and combination groups than in the placebo group, and the change in the combination group was highly statistically significant at that time (P<0.001 vs baseline and P=0.001 vs placebo).

Subject Global Assessment

Results showed that, at week 24, subjects in the combination group and in the serum group reported noticing improvements in the appearance of grey hair (combination P=0.002, serum P=0.032); those who received supplement alone also observed improvement, but the results did not achieve statistical significance (P=0.053) (Figure 5). Responses from subjects in the placebo group did not report a statistically significant improvement in grey hair.

Subjects in all non-placebo groups observed improvement in the total number of hairs at week 24 (combination P=0.017, supplement P=0.003, serum P=0.011). Subjects in the placebo group also observed improvement in hair numbers, but at a lower level of statistical significance (P=0.041).

DISCUSSION

The results of this study show clear evidence that over the course of 24 weeks, the combination of Not Today, Grey oral supplement and To The Root® topical serum resulted in progressive (12 weeks and 24 weeks) and statistically significant changes from baseline in grey hair and other hair quality attributes (hair density, count, shine, volume, and overall quality). In subjects who received the combination, there was a 9.2% reduction in grey hair (scaled) at week 12 (P=0.034 vs baseline) and a 13.0% reduction in grey hair at week 24 (P=0.016 vs baseline). In contrast, the placebo group showed worsening of grey hair, with a 10% increase in grey hair count at 24 weeks. Compared to placebo, the combination

group showed significant reduction in grey hair at week 24 (-22.7% vs placebo, *P*=0.004). Among the individual treatments, the supplement was more effective than serum in reducing grey hair; but adding the serum to the supplement resulted in a more robust effect in reducing grey hair. These results were confirmed by photo grading of grey hair, which showed the combination was clinically and statistically superior to placebo at week 24 (*P*=0.002). The results were further confirmed by the subjects' observations, showing an improvement in appearance of grey hair at week 24 in those who received the combination (*P*=0.002) and in those who received the serum (*P*=0.032).

Similarly, there was a progressive and statistically significant improvement observed for other hair quality attributes (hair density, count, volume, shine, and overall health) determined by photo grading in subjects in the combination group. Photographic grading showed that for most hair attributes, both supplement and serum improved results, but the combination was superior to each ingredient separately. The objective data was supported by subject self-assessment.

In summary, study met its primary efficacy endpoint, showing that a combination of supplement (Not Today, Grey) and serum (To The Root®) resulted in a clinically and statistically significant reduction in the appearance of grey hair after 12 and 24 weeks of treatment. Results from hair counting were confirmed by photo grading and subject assessments of grey hair. The combination was also effective in improving other hair attributes. The synergistic and compounded effects of the combination of supplement and serum contributed to the clinically and statistically significant improvements observed in this study.

DISCLOSURES

Dr. Kenneth Conrad is a medical advisor for Two Lines Inc. Allison Conrad is an employee of Two Lines Inc. Other authors have no conflicts of interest to disclose.

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Institutional Review Board Statement: The study was approved by the Advarra Institutional Review Board, located in Columbia, Maryland, USA.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study before they participated in any study-related activities.

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