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# Topical odorant application of the specific olfactory receptor OR2AT4 agonist, Sandalore®, improves telogen effluvium-associated parameters

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## Abstract

**Background:** Human hair follicles (HFs) express the olfactory receptor (OR)2AT4, which is selectively stimulated by the synthetic sandalwood-like odorant, Sandalore®. In organ-cultured, human scalp HFs, Sandalore® prolongs anagen and suppresses apoptosis by up-regulating intrafollicular IGF-1 mediated signaling.

**Aims:** The objective of this study is to demonstrate whether effects of Sandalore® observed ex vivo translate into a clinically relevant effect in patients with telogen effluvium.

**Patients/Methods:** In a randomized, double-blinded, placebo-controlled, clinical trial, 60 female volunteers (18-65 years) affected by telogen effluvium received over a period of 24 weeks treatment with either 1% Sandalore® solution (n = 30) or placebo (identically smelling, but non-OR2AT4 activating sandalwood oil n = 30). The study read-out parameters were the degree of hair shedding, hair volume, terminal/vellus hair ratio, anagen/catagen-telogen ratio, and patient self-assessment.

**Results:** Sandalore® 1% ameliorated clinical signs of telogen effluvium, namely it reduced hair shedding, and increased hair volume and the percentage of anagen HFs, the latter two parameters significantly more than placebo when changes were calculated to baseline. Sandalore® also increased the ratio of terminal/vellus hairs at week 8. Most of the anti-hair shedding effects were seen after 8 weeks and maintained at week 24. Patient questionnaire showed that verum group patients were more satisfied than the placebo group in regard to the overall results.

**Conclusion:** This clinical trial supports previous findings of anagen-prolonging effects of Sandalore® ex vivo with similar results now reproduced in clinical practice. It also provides proof-of-principle that a topically applied cosmetic odorant acting through HF olfactory receptors can be a therapeutic alternative to treat hair loss disorders characterized by excessive hair shedding such as telogen effluvium.

Francisco Jimenez and Esmeralda López equally contributed.

This study has been presented as a poster at the following meetings: Society of Investigative Dermatology in Chicago (May 8th, 2019); 11th World Congress for Hair Research in Barcelona (April 24th, 2019); ESDR meeting in Bordeaux (Sept 10th, 2019).

  

KEYWORDS

claim substantiation, hair growth, olfactory receptor 2AT4, Sandalore®, telogen effluvium

## 1 | INTRODUCTION

Olfactory receptors (ORs) represent a very large and evolutionarily ancient family of G protein-coupled chemosensory receptors that specialize in detecting odorants and thus are a key element in the sense of smell.[1,2] However, given that the appearance of ORs during evolution far predates the existence of brain structures, ORs exert many additional, older activities than only the complex central nervous system function of olfaction.[3] Therefore, it is not surprising that many of these ancestral functions unrelated to the sense of smell, such as cell proliferation/apoptosis, differentiation, and migration, have been conserved in mammals, including human skin.[3,4]

It has been shown that human epidermal keratinocytes (KC), melanocytes, and hair follicle outer root sheath cells are among the many nonclassical cells outside of the mammalian olfactory system that express functional ORs.[5-7] Recently, Chéret et al[6] showed that Sandalore®, a synthetic sandalwood odorant that selectively stimulates olfactory receptors (OR)2AT4, stimulates human hair growth ex vivo by prolonging anagen and inhibiting apoptosis by up-regulation of the intrafollicular IGF-1-mediated signaling pathway. These ex vivo findings provided the first evidence in support of the novel concept of using selected odorants to stimulate human hair growth, and encouraged the development and commercial introduction of a topical Sandalore® formulation ("Bioscalin Signal Revolution®"), which contains 1% Sandalore® in an alcoholic vehicle. However, it remains to be shown whether this topical formulation exerts any significant hair growth effects at all in clinical practice, ie, after topical application to human scalp skin.

For this reason, we designed a randomized, double-blinded, placebo-controlled, prospective study with 60 volunteer patients diagnosed with telogen effluvium, with a treatment period of 24 weeks. Given the anagen-prolonging effect of Sandalore® ex vivo,[6] we chose to perform this study on patients with telogen effluvium as this is a disorder characterized by diffuse hair loss resulting from HFs prematurely entering into catagen phase and subsequently telogen.[8-10] Hair shedding, hair volume, hair miniaturization, and the anagen/catagen-telogen ratio were the main parameters assessed. This was complemented with a patient self-assessment questionnaire and clinical observations regarding safety and tolerability.

## 2 | MATERIAL AND METHODS

### 2.1 | Study design

This was a single-center, parallel, double-blind, simple randomized (with balanced randomization [1:1]) study to assess the efficacy and safety of a 24 weeks treatment with the topical cosmetic formulation Bioscalin S-R® (verum), which contained 1% Sandalore®, in comparison to a formulation containing the Bioscalin S-R® vehicle plus 0.05% sandalwood oil (placebo), in patients affected by telogen effluvium. Each patient was randomly allocated to either of two groups (n = 30 in each group): a treatment group receiving verum or a placebo group. The study investigator or designated member of staff dispensed either verum or placebo according to a computer-generated randomization list provided by the sponsor. The trial was conducted at a private dermatology clinic (Mediteknia/ Monasterium Clinical Trial Unit, Las Palmas de Gran Canaria, Spain). The study was performed in accordance with the current version of the Declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland, October 2000) and in agreement with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practise (GCP). Ethics approval was obtained for the study (CEIm-CHUIMI-2017/961, Complejo Hospitalario Insular de Gran Canaria, Spain), and patients received and signed appropriate consent forms.

### 2.2 | Patients

60 healthy female volunteers from 18 to 65 years complaining of excessive hair shedding of more than 4 weeks were recruited. Only patients having a shedding degree of 4 or higher according to the recently validated Hair Shedding Visual Scale[11] were included in the trial. Diagnosis of telogen effluvium was performed by two dermatologists (FJ and EL) after ruling out other causes of noncicatricial alopecia (ie, alopecia areata, chemotherapy induced alopecia, or androgenetic alopecia). Inclusion criteria included no administration of any topical or systemic therapies for hair loss for two months (including oral nutritional supplements) prior to the initiation of the study.

### 2.3 | Investigational products

The verum product was the commercially available preparation Bioscalin S-R® (Giuliani S.p.A., Milan, Italy), containing 1% Sandalore® as active ingredient. As a placebo, instead of using only the vehicle of Bioscalin S-R®, we chose to add natural sandalwood oil (0.05%), which smells identical to Sandalore® but does not stimulate OR2AT4-mediated signaling of human epidermal KCs in vitro.[7] We thus opted for the most rigorous placebo control that smelled identical to the verum. Both the verum and placebo products were given in identical-looking containers numbered from 1 to 60. Neither examiners nor patients knew which containers were verum or placebo (double-blinded). Patients were asked to apply 2 mL of product topically (verum or placebo) on dry hair and scalp once a day by massaging softly the scalp, preferably before sleeping, for the entire trial period of 24 weeks.

## 2.4 | Technical procedures

Patients visited the clinic at weeks 0, 8, and 24 for the following evaluations: hair shedding visual scale, hair volume (cross-section trichometry), terminal/vellus hair ratio, anagen/catagen-telogen ratio, patient self-assessment questionnaire, and clinical evaluation for adverse effects (ie, redness and irritation of scalp).

### 2.4.1 | Hair shedding visual scale

After two nonwash days, patients were asked to wash, comb, and collect all hairs into a plastic bag before visitation to the clinic. The collected hairs were subsequently evaluated using an arbitrary unit from 1 to 9 by following the Hair Shedding Visual Scale, described by Sinclair and recently validated.[11]

### 2.4.2 | Hair volume (cross-section trichometer: hair check®, Divi International Co, Miami, FL)

It measures the cross-sectional area of a bundle of hair selected from a specific 2cm x 2cm area located in the mid-parietal area of the scalp, using a measuring template (numbered at 10 every time), which corresponded to approximately 12.5 cm from the glabella. The quantitative result, known as the hair mass index, is an indirect measurement of hair volume.[12] Hair mass index increases either when the number of hairs or the thickness of the hairs increase, or both.[13] It is a reliable parameter that has proven to have high reproducibility.[12,14,15]

**TABLE 1** Self-assessment questionnaire

| |
|---|
| Q1. Visible decrease in hair shedding |
| Q2. I find less hairs in the comb/brush |
| Q3. It seems that my hairs are growing |
| Q4. My hairs are stronger |
| Q5. My hairs are denser |
| Q6. My hairs are shiny |
| Q7. My hairs are more resistant after stress |
| Q8. The quality of my hairs is better |
| Q9. My hairs are thicker |
| Q10. My hairs are soft |
| Q11. My hairs have increased volume |
| Q12. I am satisfied with the results |
| Q13. Smell impression |
| Q14. Tolerability to product |
| Possible answers for questions Q1-Q12, 0: totally disagree, 1: slightly agree, 2: agree, 3: strongly agree; for question Q13, 0: negative impression, 1: positive impression; for question Q14, 0: not good, 1: ok, 2: good, 3: very good. |

### 2.4.3 | Terminal/vellus hair ratio

The hair diameter of 15 hairs per volunteer was measured with the Folliscope® device (Lead M Corp, Seoul, South Korea), at the same area of the scalp as the hair check, and the mean hair diameter was calculated. Based on these measurements, the ratio of terminal (defined as hairs > 40 microns) and vellus or miniaturized hairs (defined as hairs < 40 microns) was determined.

### 2.4.4 | Phototrichogram (anagen/catagen-telogen ratio)

The anagen/catagen-telogen ratio was determined using a digital video microscopy device (TrichoScan®, Tricholog GmbH). The number of growing (anagen) hairs was counted 48 hours after dying the area and shaving. Hairs that did not grow after 48 hours were classified as catagen/telogen.

### 2.4.5 | Patient self-assessment questionnaire

A self-assessment questionnaire was given to all subjects at week 8 and week 24 (questions outlined in Table 1).

## 2.5 | Statistical analysis

D'Agostino & Pearson omnibus normality test was used to test whether the data were consistent with a Gaussian distribution. A two-way repeated measures ANOVA and Sidak's multiple comparisons test were used to analyze significant differences between the means of the repeated measures between placebo and verum at W0, W8, and W24 using data from all patients attending both visits (W8 and W24) and terminating the study (not significant). A parametric unpaired Student's *t* test or Mann-Whitney test was used to analyze significance between placebo and verum for each time point analyzed using data from all patients after elimination of outliers according to the Grubb´s test (*$P < .05$ only between W0 placebo and verum for Figure 1E). A parametric repeated measures ANOVA, and Dunnett's multiple comparisons test or nonparametric repeated measures Friedman test and/or Dunn's multiple comparisons test with fixed comparison to W0 were used to analyze significance between repeated measures (W0, W8, and W24) within each experimental group (placebo or verum) using data from all patients attending both visits (W8 and W24) and terminating the study, #$P < .05$, ##$P < .01$, and ####$P < .0001$ (Figure 1A-F). A parametric paired Student's *t* test or Wilcoxon matched-pairs signed rank test was used to analyze significance between one collecting time point and W0 within each experimental group (placebo or verum) using data from all patients after elimination of outliers according to the Grubb's test, *$P < .05$, **$P < .01$, and ****$P < .0001$ (Figure 1A-F). A parametric unpaired Student's *t* test or Mann-Whitney test was used to analyze significance between placebo and verum for each time

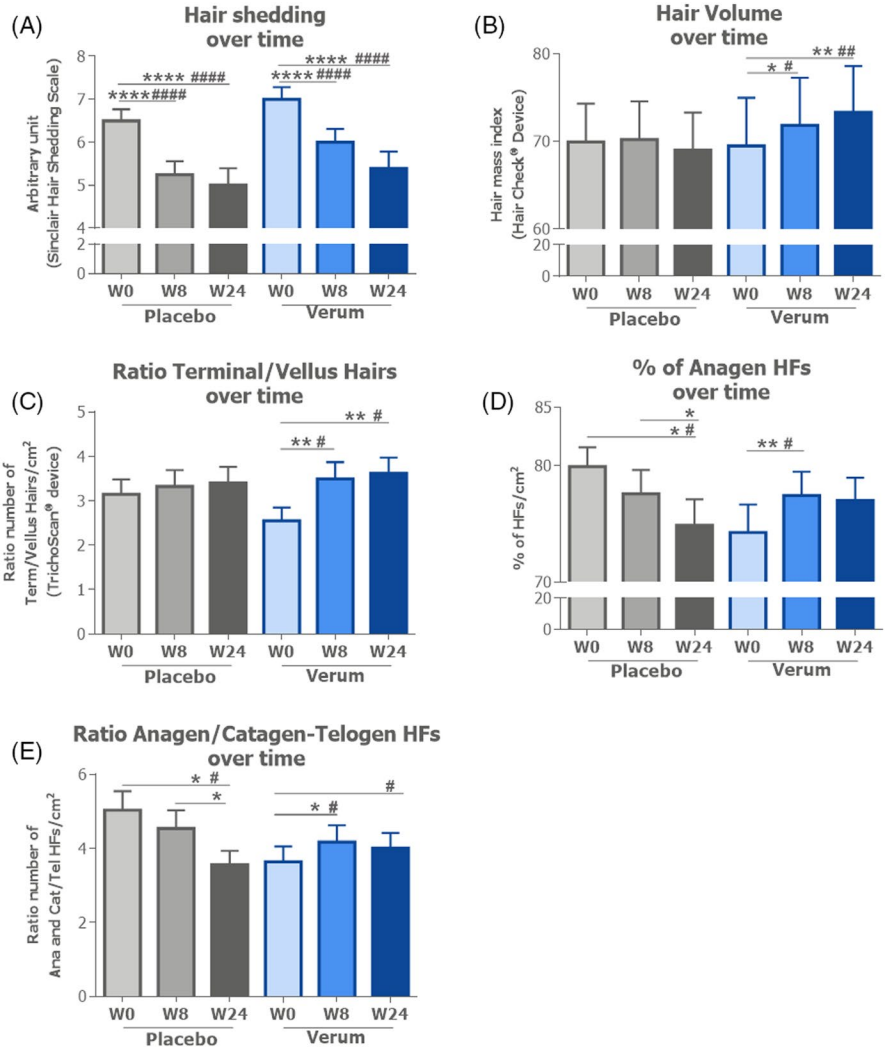


**FIGURE 1** Analysis of the response distribution to verum and placebo over time. Measurements of hair shedding (A), volume (B), ratio terminal/vellus hairs (C), % of anagen HFs (D), ratio anagen/catagen-telogen HFs (E). Mean ± SEM, N = 26-28 patients/group after exclusion of outliers according to Grubb´s test, D'Agostino & Pearson omnibus normality test. Paired Student's *t* test, or Wilcoxon matched-pairs signed rank test between different time points within each experimental group using all data after elimination outliers according to Grubb´s test, $^{*}P < .05$, $^{**}P < .01$, $^{****}P < .0001$. Parametric repeated measures ANOVA, and Dunnett's multiple comparisons test, or nonparametric repeated measures Friedman test and or Dunn's multiple comparisons test with fixed comparison to W0 was used to analyze significance between repeated measures (W0, W8, W24) within each experimental group (placebo or verum) using data from all patients attending both visits (W8, and W24) and terminating the study, $\#P < .05$, $\#\#P < .01$, $\#\#\#\#P < .0001$

point after calculation of changes at week 8 and 24 compared to baseline analyzed using data from all patients after elimination of outliers according to the Grubb´s test, §$P < .05$ and §§$P < .01$ (Figure 2A-F). Paired Student's *t* test or Wilcoxon matched-pairs signed rank test was used for the analysis of the patient self-assessment questionnaire results between different time points within each experimental group, $\#P < .05$ and $\#\#P < .01$; unpaired Student's *t* test, or Mann-Whitney test between Placebo and Verum, not significant (Figure 4A-N).

## 3 | RESULTS

During the study, a total of five patients dropped out for reasons unrelated to product administration. Two patients receiving placebo and two patients receiving verum dropped out of the study and did not participate in the visits at weeks 8 and 24. One patient who received placebo dropped out after the first day of collection (week 8), and only hair shedding and hair volume were evaluated at week 24 for another patient receiving placebo. One patient receiving verum declined scalp shaving for the phototrichogram analysis with the Trichoscan®.

### 3.1 | Hair shedding

Patients receiving placebo and verum both showed decreased hair shedding over the time of the study (Figure 1A), but verum was tendentially more efficient in reducing hair shedding at week 24 than placebo (Figure 2A). However, the change in the shedding score between the collecting points and baseline did not reveal statistically significant differences between the experimental arms (Figure 2A).

### 3.2 | Hair volume

Verum-treated patients showed a statistically significant improvement in hair volume at week 8 and week 24 of treatment compared to baseline (week 0) (Figure 2B). In contrast, no change was detected for patients receiving placebo over the time of the study in terms of hair mass index (Figure 1B). This beneficial response to verum treatment was also confirmed by the significant differences observed when the change of hair mass index at weeks 8 and 24 from week 0 was calculated in the placebo and verum groups (Figure 2B).

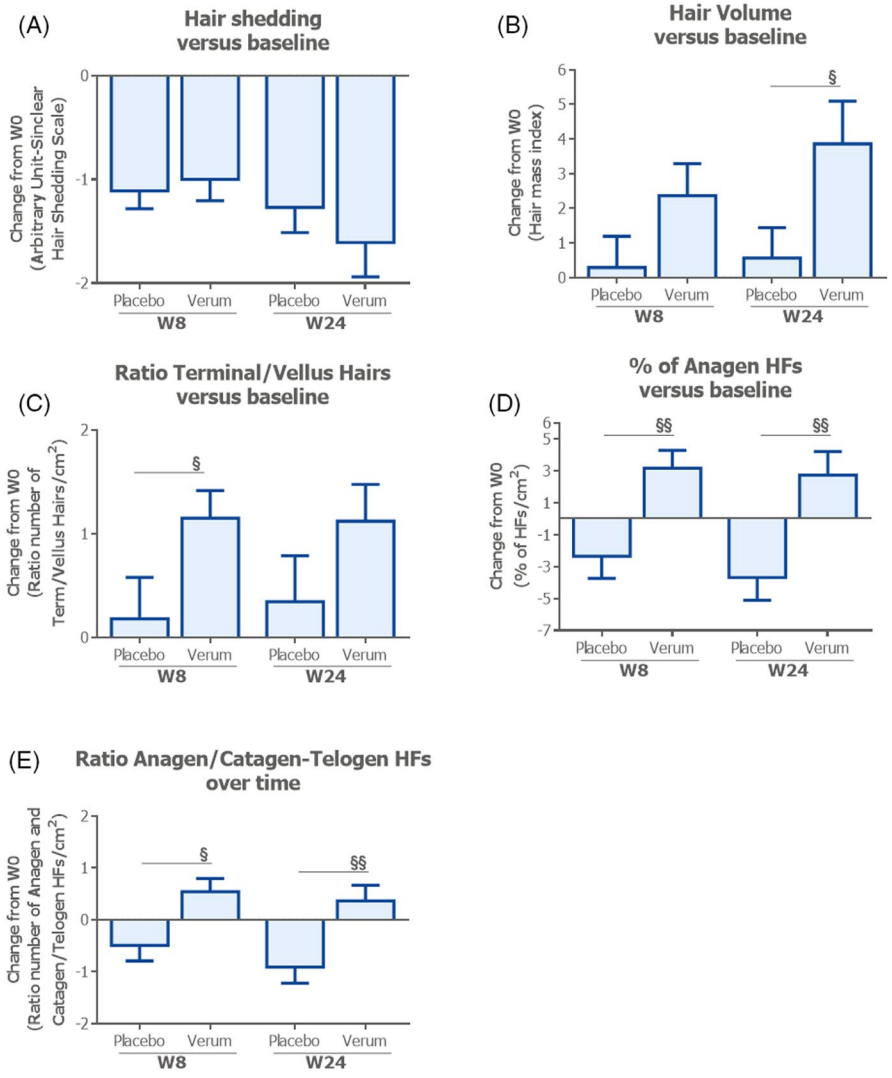


**FIGURE 2** Analysis of the changes at week 8 and 24 compared to baseline. The change between the collection points and baseline was calculated for each patient and each parameter, that is, hair shedding (A), volume (B), ratio terminal/vellus hairs (C), % of anagen HFs (D), ratio anagen/catagen-telogen HFs (E). Mean ± SEM, N = 26-28 patients/group after exclusion of outliers according to Grubb's test, D'Agostino & Pearson omnibus normality test, Unpaired Student's *t* test, or Mann-Whitney test between Placebo and Verum at each time point, §$P < .05$, §§$P < .01$

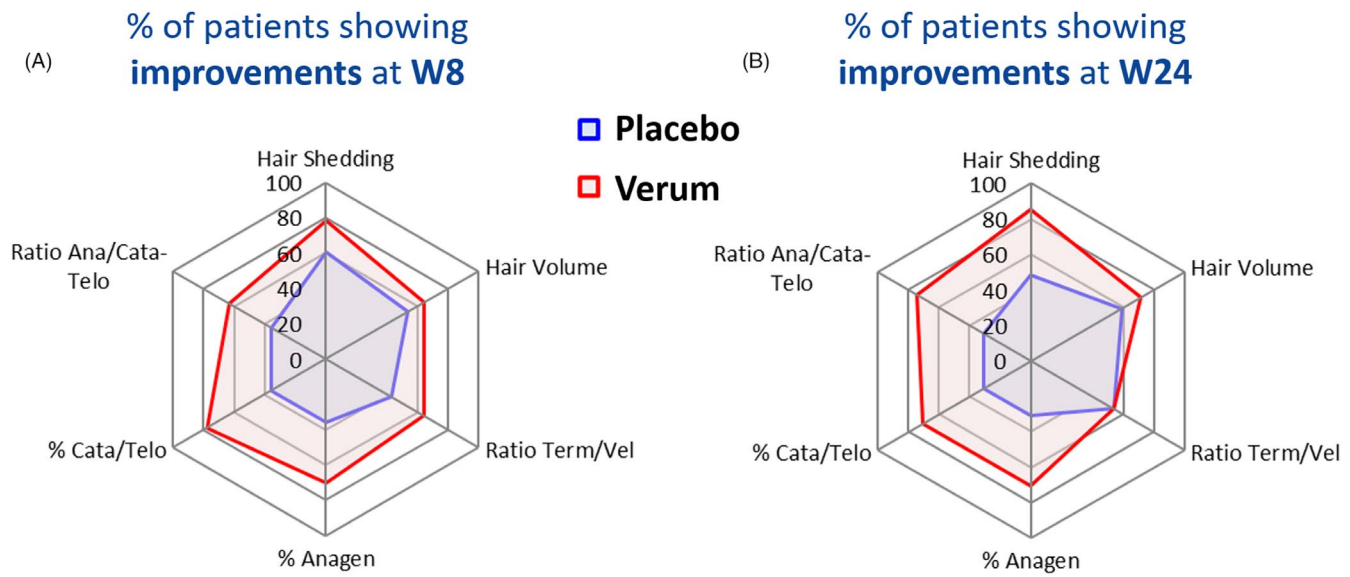


**FIGURE 3** Overall Response. Radar chart showing the % of patients in the placebo and verum group showing improvements at week 8 (A) and week 24 (B)

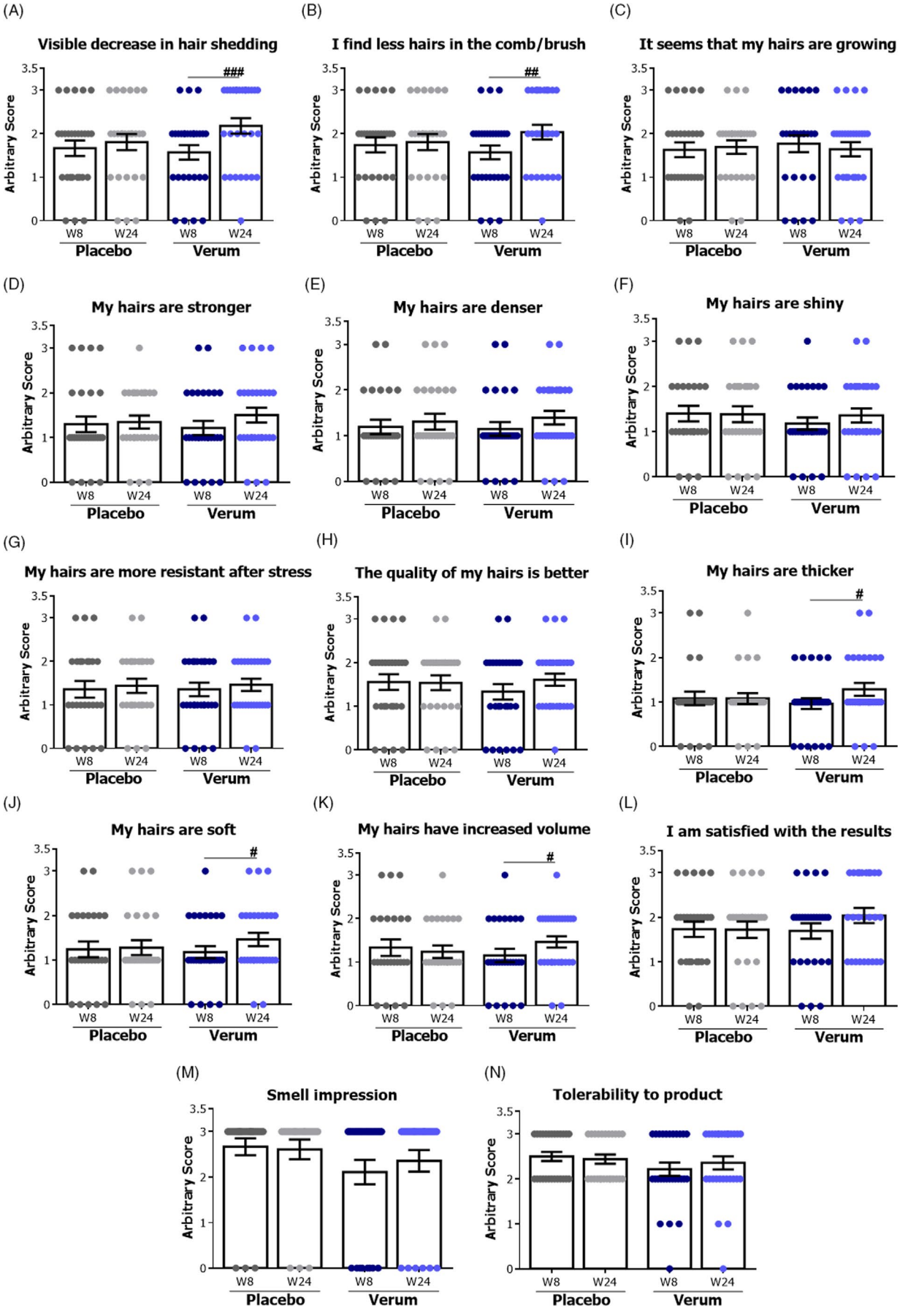
(A)
Visible decrease in hair shedding
###
(B)
I find less hairs in the comb/brush
##
(C)
It seems that my hairs are growing
(D)
My hairs are stronger
(E)
My hairs are denser
(F)
My hairs are shiny
(G)
My hairs are more resistant after stress
(H)
The quality of my hairs is better
(I)
My hairs are thicker
#
(J)
My hairs are soft
#
(K)
My hairs have increased volume
#
(L)
I am satisfied with the results
(M)
Smell impression
(N)
Tolerability to product
Arbitrary Score
0
1
2
3
3.5
W8
W24
Placebo
Verum

**FIGURE 4** Analysis of the Patient Self-Assessment Questionnaire. Questionnaire results for visible decrease in hair shedding (A), I find less hairs in the comb/brush (B), it seems that my hairs are growing (C), my hairs are stronger (D), my hairs are denser (E), my hairs are shiny (F), my hairs are more resistant to stress (G), the quality of my hairs is better (H), my hairs are thicker (I), my hairs are soft (J), and my hairs have increased volume (K), I am satisfied with the results (L), smell impression (M), and tolerability to product (N). Bar chart showing mean ± SEM with data points overlap, n = 25-27 patients/group, Graph Pad Prism 6, D'Agostino & Pearson omnibus normality test, paired Student's *t* test or Wilcoxon matched-pairs signed rank test between different time points within each experimental group, #$P < .05$, ##$P < .01$; unpaired Student's *t* test, or Mann-Whitney test between Placebo and Verum, not significant

### 3.3 | Ratio terminal/vellus hairs

Our data show that verum treatment significantly increases the ratio terminal/vellus hairs over time (Figure 1C). In contrast, the ratio did not change over time in patients receiving placebo (Figure 1C). A significant increase in the change of the terminal/vellus ratio over the treatment days and baseline was detected in verum compared to placebo at week 8 (Figure 2C).

### 3.4 | Anagen vs catagen/telogen ratio

There was a significant increase in the percentage of anagen HFs in patients treated with verum after 8 weeks of treatment, which was maintained until 24 weeks (Figure 1D). In contrast, placebo-treated patients showed a significant decrease in the percentage of anagen HFs at the end of the study (Figure 1D). The ratio of anagen/catagen-telogen HFs was significantly decreased in placebo-treated and significantly increased in verum-treated patients, mainly after 8 weeks of treatment (Figure 1E). The change to baseline of the percentage of anagen HFs and the ratio of anagen/catagen-telogen HFs at week 8 and 24 between placebo and verum resulted also to be significant (Figure 2D,E).

### 3.5 | Overall response

A clear separation of responder and nonresponder was not possible to achieve due to the high inter-individual differences in the response of patients receiving verum. As depicted in the radar charts (Figure 3A,B), more than the 50% of patients treated with the verum product showed improvement for all analyzed parameters already after 8 weeks of treatment (Figure 3A). After 24 weeks of treatment, the percentage of patients showing improvements in the verum arm was either increased or not changed (Figure 3B). Instead, only 20%-40% of the patients receiving placebo revealed an improvement in selected parameters (Figure 3A,B).

### 3.6 | Patient self-assessment questionnaire results

The majority of the patients, regardless of the treatment received, perceived reduced hair shedding, and found fewer hairs in the comb/brush. However, only the difference between the arbitrary scores given by patients receiving verum at week 8 and week 24 reached statistical significance (Figure 4A,B). The majority of the patients also agreed that they experienced "hair regrowth," and slightly agreed that their hairs were "stronger, denser, shinier, and more resistant to stress," with "increased quality," irrespective of the treatment received (placebo vs. verum) (Figure 4A-H). Although the majority of the patients included in the study slightly agreed that they experienced "thicker and softer hairs," and "increased volume," only the difference between the arbitrary scores given by patients receiving verum at week 8 and week 24 reached statistical significance (Figure 4I-K). Overall, patients treated with verum were satisfied with the results (Figure 4L).

### 3.7 | Clinical screening for adverse effects and tolerability to product

Throughout the study, observations were made for any adverse effects/reactions as a result of either control or verum application and recorded if present. However, clinical observation did not reveal the presence of any adverse effects, as in the appearance of redness or scaly skin, in scalp from patients treated with either placebo or verum. Overall, patients were also not disturbed by the smell of sandalwood (Figure 4M), and they perceived a good tolerability to the product (Figure 4N).

## 4 | DISCUSSION

This study shows that Sandalore®-treated patients had significantly improved selected hair growth parameters (anagen vs catagen-telogen ratio, terminal/vellus hair ratio, and hair volume) over time compared to placebo-treated patients. Thus, this stringently controlled pilot clinical trial in a small but rigorously defined and homogenous cohort of otherwise healthy women with substantial telogen effluvium provides the first evidence that a topically applied synthetic odorant preparation containing the selective OR2AT4 agonist, Sandalore®, as active ingredient prolongs anagen in vivo, resulting in an increased anagen vs catagen-telogen ratio and increased overall hair volume. This is perfectly in line with our previous ex vivo study which showed that OR2AT4 stimulation significantly prolongs anagen duration.[6]

It is interesting to note that patients treated with verum showed an increase in the terminal/vellus hair ratio, which reached statistical significance at week 8 of the study. If this effect can be reproduced in follow-up studies, this would obviously make Sandalore® a promising active ingredient for the treatment of female pattern hair loss, where telogen effluvium is combined with HF miniaturization.[9]

Given that the OR2AT4 receptor is most strongly expressed in the lower portion of the HFs [6] and that the solution used in our study was topically applied, it would also be interesting to design further studies that consider supplementing the formulation with additional epidermal penetration enhancers as well as testing Sandalore® at higher concentrations.

In conclusion, despite the limitations of our study, these results indicate that topically-applied Sandalore® 1% solution is an attractive novel, safe, well-tolerated, adjuvant cosmetic treatment option for hair loss disorders characterized by premature anagen termination, as is the case in patients with telogen effluvium.

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