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Selenium disulfide: a key ingredient to rebalance the scalp microbiome and sebum quality in the management of dandruff

Background: Dandruff is a chronic and relapsing scalp condition characterized by flaky scalp. Environmental and host factors (exposome) may alter the sebaceous gland activity, sebum composition, epidermal barrier function, and scalp microbiome balance, resulting in dandruff. Selenium disulfide (SeS_2) improves the clinical signs of dandruff. *Objectives*: To investigate the mode of action of SeS_2 shampoo during treatment and relapse phases. *Materials & Methods*: Two single-center studies assessed dandruff severity, subjective efficacy perception, microbial balance, microbiota diversity and sebum lipids. *Results*: SeS_2 significantly ($p \leq 0.01$) reduced scaling and led to a significant decrease of *Malassezia* and *Staphylococcus* spp. counts in both lesional and non-lesional areas, compared to the vehicle at D28 returning to baseline levels at D56. *Cutibacterium* spp. levels were not different between the SeS_2 and the vehicle treatment groups but had significantly increased with SeS_2 ($p<0.001$) in the lesional zone at D56. The ratio *Malassezia* spp./*Cutibacterium* spp. decreased significantly in lesional zones compared to baseline levels, at both D28 and D35 ($p<0.001$). The total squalene content significantly increased ($p<0.05$), whereas peroxided squalene had significantly decreased by almost 50% at D31. The ratio triglycerides/free fatty acids significantly ($p<0.0001$) increased, almost 5-fold, between D0 and D31. SeS_2 shampoo was very well tolerated. *Conclusion*: SeS_2 is beneficial in scalp dandruff, even after treatment interruption. It is well tolerated, rebalances the equilibrium between the main bacterial and fungal populations, and improves sebum quality.

Key words: selenium disulfide, scalp microbiota, *Malassezia*, *Staphylococcus, Cutibacterium*, scalp squalene

Dandruff is a mild form of seborrheic dermatitis (SD), a chronic and relapsing inflammatory condition of the scalp, face, and upper chest. Dandruff mainly goes along with flakes and no visible signs of inflammation [1]. The prevalence of dandruff has been estimated to up to 50% in the general population [2]. The activity of the sebaceous gland and sebum composition, epidermal barrier function, host immune function, colonization by fungi such as *Malassezia (M.)* yeasts, and the host-inhabitant interplay have been suggested to trigger dandruff. At a species level, *M. restricta* and *M. globosa* are the most frequently observed species on healthy scalp, while *M. globosa and M. sympodialis* accounted for less than 1% of all sequences retrieved [3, 4]. *M. restricta* has been associated to dandruff/SD [5]. Moreover, several *Malassezia* spp., especially *M. restricta*, induce cytotoxicity to skin cells *in vitro,* suggesting an active role in accelerated scale formation [6, 7]. The ability of *Malassezia* spp. to metabolize and oxidize sebum-derived lipids such as triglycerides, squalene and fatty acids into inflammatory compounds and to produce indole derivatives including malassezin and indolocarbazole with an activity against aryl hydrocarbon receptors, may activate skin inflammation [8, 9].

Next generation sequencing-based studies showed that, in addition to *Malassezia* spp., bacterial scalp microbiota changes are associated with the pathogenesis of dandruff/SD [10, 11, 12]. *Cutibacterium* spp. and *Staphylococcus* spp. are the two major bacterial genera found in dandruff and non-dandruff scalps, accounting respectively for about 90% of all sequences retrieved [13, 14].

In dandruff/SD, bacterial changes correspond to a higher diversity, with disequilibrium between the two dominant bacteria, consisting in a reverse correlation between the abundance of *Staphylococcus* spp. (higher) and *Cutibacterium* spp. (lower) [13, 15, 16, 17, 18]. Thus, dandruff is not only associated with a higher incidence of one specific *Malassezia* species, but also with a disequilibrium between the fungal and bacterial scalp populations [14, 16, 17, 18]. These modifications may be related to the scaling severity and may extend to the forehead [16].

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Current dandruff/SD treatments involve topical application of antifungals; for SD, addition of anti-inflammatory agents is also required [19]. Selenium disulfide (SeS_2) has been shown to be effective in managing both dandruff and SD after an initial treatment with ketoconazole [20]. SeS_2 reduces scales, itching, irritation and redness of the scalp. Moreover, it rebalances the skin microbiome [21, 22].
We hereafter present results from 2 studies showing the clinical benefits of a shampoo containing SeS_2 (Dercos® Anti-Dandruff Shampoo, Laboratoires Vichy, France) on dandruff scalp and its impact on the fungal and bacterial scalp microbiota and surface squalene.

Materials and Methods

Both single-center studies were conducted at two study sites in Paris and Lyon, France, in compliance with the World Medical Association Declaration of Helsinki, and national and EU regulations. This non-interventional study did not require regulatory or ethics committee approval. All volunteers provided written informed consent.

Study 1: single blind vehicle-controlled design

Study population

Adult subjects with an adherent dandruff score of more than 2.5 and total dandruff score (adherent + non-adherent) ≥4.5 (ranging from 0 = none to 10 = very severe) were included in this study. Adherent dandruff was scored from 0 (no dandruff) to 5 (very severe dandruff) using a visual dandruff severity scale based on a modified Van Abbe scale [23]. The subjects were asked to wash their hair 3 times per week with the vehicle shampoo during a 3-week run-in period prior to baseline visit; the last wash was made 3 days prior to study onset. At the baseline visit, the subjects were randomly assigned for 4 weeks to either the SeS_2 or the vehicle shampoo, to be used 3 times per week for 28 days (treatment period). After 28 days, the subjects reverted to the vehicle shampoo for a further 4-week period. Samples were collected at baseline, 28, 35 and 56 days.

Clinical assessments

Adherent and non-adherent dandruff scores were assessed at Day (D) -21, D0 baseline and at D21, D28, D35 and D56 (adherent dandruff and non-adherent score ranging from 0 to 5).

Microbiota diversity

With regards to the scalp microbiota pattern described above in the introduction, a focus on 16S for bacterial sequencing of the conserved ribosomal unit regions was made to assess the change on alpha diversity using the Shannon index [24]. The Bray-Curtis index was used to quantify the OTU composition dissimilarity between two different groups or sites [25]. A detailed methodology to assess the microbiota diversity is provided in Clavaud *et al.* [26].

Genomic DNA was extracted from skin swabs using the PowerSoil® DNA Isolation kit (MO BIO Laboratories Inc., CA, USA) following the manufacturer's instructions at the University of Colorado (Boulder, CO, USA). PCR amplification of the V1-V2 region of *16S* rRNA gene was performed using the primer set (27F/338R), PCR mixture conditions, and thermal cycling. PCR amplicons from triplicate reactions for each sample were pooled at approximately equal amounts and sequenced on a 454 Life Sciences Genome Sequencer FLX Titanium® instrument (Roche, Basel, Switzerland) [27]. All sequences were processed, barcoded and clustered following the standard QIIME pipeline. High-quality sequences were trimmed to 300bp and clustered into operational taxonomic units (OTUs) using an open reference-based approach that implements reference-based clustering followed by *de novo* clustering using the UCLUST algorithm. Clustering was conducted at a 97% similarity level using the GreenGenes database (https://greengenes.secondgenome.com/). Sequences were assigned to taxonomic groups using the RDP classifier. Readable data were obtained from 440 of the 448 samples. Samples were rarefied to 7790 sequences each.

Quantification of microbial scalp species

qPCR was used to quantify *Staphylococcus* spp., *Cutibacterium* spp. and *Malassezia* spp. and to determine potential changes in the abundance of specific microbial species at both lesional and non-lesional zones in both groups. Microbial abundance was assessed at baseline (D0), at D28, D35 and D56. The method described by Clavaud et al. was used to quantify microbial scalp species [18]. As for the assessment of the global bacterial diversity, the sampling procedure was repeated on the same scalp area at D28 (end of treatment), and D35 and D56 (respectively after 1 and 4 post-treatment weeks).
In total, 2 mL of the swab scalp suspension were pelleted by centrifugation for 30 min at 10,000 *g*. Supernatants were carefully removed to obtain dry pellets and frozen at -20°C. Samples were processed within 14 days after collection. Two pellets were generated from each sample, one was used for fungal DNA isolation, the other for bacterial DNA isolation. For fungal DNA, pellets were resuspended in 600µL sorbitol buffer (1 M sorbitol, 10 mM disodium EDTA, 14 mM ß-mercaptoethanol) with 200 U of Zymoliase T20® (MP Biomedicals, Illkirch, France) and incubated at 30°C for 30 min. For bacterial DNA, pellets were resuspended in 180µL of Tris-EDTA buffer (20 mM Tris-Cl pH 8.0, 2 mM disodium EDTA, 1.2% Triton® X-100) with 20 mg/mL lysozyme and incubated for 30 min at 37 C. After centrifugation for 10 min at 300 *g*, 180 µL ATL buffer (Qiagen, Hilden, Germany) and 20 µL proteinase K (Qiagen, Hilden, Germany) were added to the pellet, mixed thoroughly by vortexing, and incubated at 56°C for 15 minutes for fungal DNA or 30 min for bacterial DNA. After incubation, 200 µL AL buffer (Qiagen) and 200 µL 96-100% ethanol were added to the spheroplasts and genomic DNA was purified using the DNeasy® blood & tissue kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. Resulting DNA samples were resuspended in 100 µL ultra-pure DNAse-RNAse free water and stored at -80 °C until processing.

Quantitative PCR of the major bacterial and fungal species was performed at Bertin Pharma Campus CEA (Fontenay aux Roses, France) using published methods [18, 28]. *Cutibacterium* spp., *Staphylococcus* spp. and *Malassezia* spp. were quantified via qPCR using specific primers and TaqMan® MGB probes targeting a specific region of bacterial 16S rDNA, or fungal ITS-28S rDNA. Reaction mixes consisted of 20 µL TaqMan® Universal Master Mix II without UNG (Applied Biosystems, Thermo Fisher Scientific, MA, USA), 200 nM each primer, 250 nM TaqMan® probes (Applied Biosystems) and 0.5-5 ng DNA. Amplification and detection were performed with the iCycler iQ® (BIO-RAD, CA, USA) with the following cycle parameters: 55 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 30 seconds and 55 °C for 30 seconds, for *Malassezia* spp. or 55°C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 30 sec and 55 °C for 45 sec for bacterial species. Each sample was run in triplicate. Direct linear correlations were confirmed between the density of *M. restricta*, *C. acnes* and *S. epidermidis* cells (between 10^2 to 10^7 cells) and the cycle threshold (Ct) values.

Study 2: open label design

Study population

Adult subjects with mild to severe scalp desquamation and mild to severe pruritus were suitable for inclusion. The subjects included in the study were asked to wash their hair 3 times per week during the 2 weeks preceding the study onset with a provided shampoo devoid of any antidandruff ingredient.

During the first 28 days, SeS_2 was to be used 3 times per week (treatment phase), followed by a 42-day period during which a bland shampoo was to be used (follow-up phase) 3 times per week. The subjects attended the study facilities at baseline (D-3 and D0), D10, D17, D24, D31, D45, D59 and D73.

Clinical and instrumental assessments

Adherent and non-adherent dandruff scores were assessed with adherent dandruff and non-adherent score ranging from 0 to 5. The subjects self-assessed the severity of scaling, pruritus and scalp greasiness on a 10-point scale from 0 (none) to 9 (very severe).

To evaluate the skin barrier function, Trans Epidermal Water Loss (TEWL) was recorded at D-3, D31, D73, using a Vapometer© (Delfin Technologies, Crendon House, UK) on a shaved area of the scalp vertex.

Lipid analysis

Scalp sebum lipids were sampled at D0 and D31 through successive contacts between silica rods (Synelvia™, Labège, France) and the vertex site of the scalp. Prior to analysis, rods were stored at –80°C. The analysis focused on the balance between triglycerides (TG) and free fatty acids (FFA), reflecting the lipolytic activity, and the ratio of squalene monohydroperoxide (SQOOH) to squalene (SQ), showing the lipoperoxidation activity of *Malassezia* spp. The analytical methods used have been described elsewhere [8, 11, 12].

Local tolerance

Local tolerance was assessed for all subjects during both studies.

Statistical analyses

Statistical analyses for both studies were performed using the SAS® software version 9.2 (or higher) (SAS Institute Inc., Cary, North Carolina, USA). All statistical tests were two-sided and type I error (alpha) set to 5%.

Quantitative variables were summarized for the number of non-missing observations (n), the mean and standard deviation (SD) and 95% CI of mean, the median, the minimum and maximum, and quartiles.

Qualitative variables were summarized for the number of non-missing observations (n), frequency and percentage.

Results

Study 1 vehicle-controlled

A total of 53 subjects with adherent dandruff scores of at least 2.5 and 6 subjects with a score between 2.0 and 2.5 all having total dandruff scores of at least 4.5 were included in the study; 56 completed the study (25 in the SeS_2 and 31 in the vehicle group). Mean age was 42.4 years; 58% were women.

Clinical efficacy

Both groups had similar adherent dandruff scores at baseline. SeS_2 significantly reduced adherent dandruff scores at all post-baseline visits, with reductions ranging from 40.8% to 58.3% compared to baseline (all $p<0.001$, *figure 1*) with a maintenance of the clinical benefit until D56. Compared to baseline, no significant change was observed with the vehicle throughout the study.

Bacterial diversity of the scalp

At baseline, both lesional and non-lesional zones showed broad bacterial diversity with *Staphylococci*, *Corynebacterium*, *Cutibacterium*, *Acinetobacter*, and *Micrococcus* species representing more than 90% of the global number of bacterial sequences (data not shown). Lesional zones presented higher abundance of *Staphylococci* spp., whereas the presence of *Corynebacteria* spp. and *Acinetobacteria* spp. was higher in non-lesional zones (data not shown). The Shannon index showed that SeS_2 and vehicle formula did not impact the bacterial richness of the scalp neither immediately after the end of treatment nor a month after. SeS_2 and vehicle formula maintained the microbiota diversity (*figure 2*).

SeS_2 induced a global change in the distribution of the abundance relatives of the operational taxonomic units (OTUs) on lesional zones, as measured by the Bray-Curtis Index (*figure 3*). At baseline, the OTU distribution was decreased on lesional zones, while no distribution change was observed in non-lesional zones. Over time, no change of the bacterial diversity on

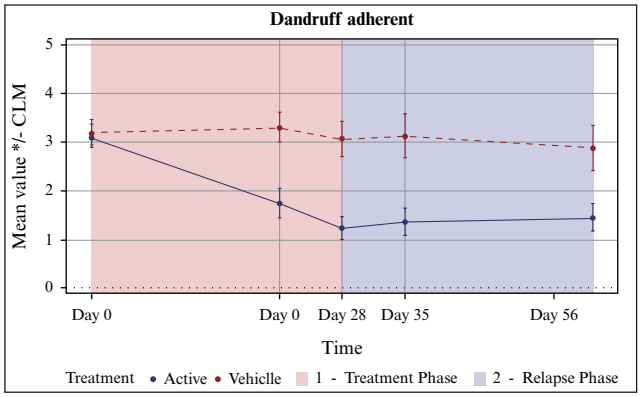

Figure 1. Study 1: Evolution over time of mean scores for adherent dandruff.
Differences between SeS_2 and the vehicle were significantly (*p<0.001) at all post-baseline (D0) visits for mean adherent dandruff scores.

lesional zones was found (*figure 3B*) with the vehicle, while changes were observed at D28 and D35 with SeS_2 (*figure 3A*), with a notable increase in the OTU distribution returning to initial diversity levels at D56.

Malassezia quantification

No significant difference between both groups was observed in the *Malassezia* spp. load between lesional and non-lesional zones at baseline (*figure 4*). After 4 weeks of treatment, the *Malassezia* spp. load was significantly reduced by ~-2Δlog (p<0.001) at both zones following the use of SeS_2, while no such effect was observed with the vehicle. A maintenance effect with SeS_2 was observed at D35, which was no longer present at D56.

Bacterial species quantification

SeS_2 significantly (p<0.001) reduced the *Staphylococcus* spp. load by ~-1Δlog at both the lesional and non-lesional zones at D28, returning to baseline levels at D56. This effect on *Staphylococcus* spp. loads was not observed with the vehicle. *Cutibacterium* spp. loads were not different between both treatment groups, except at D56 at the lesional zone, where loads were increased by ~0.4Δlog with SeS_2 (p<0.001).

Between-species ratios

The ratio *Malassezia*/*Cutibacterium* spp. was highly increased in lesional zones compared to non-lesional zones. At the lesional zones receiving SeS_2, this ratio was significantly decreased compared to baseline level after 4 weeks of treatment and at D35 as well (p<0.001), whereas no effect was observed with the vehicle (*figure 5*).

Study 2 open label

32 healthy Caucasian subjects (24 women, 8 men) aged between 29 and 42 years and with mild to severe dandruff participated in this study.

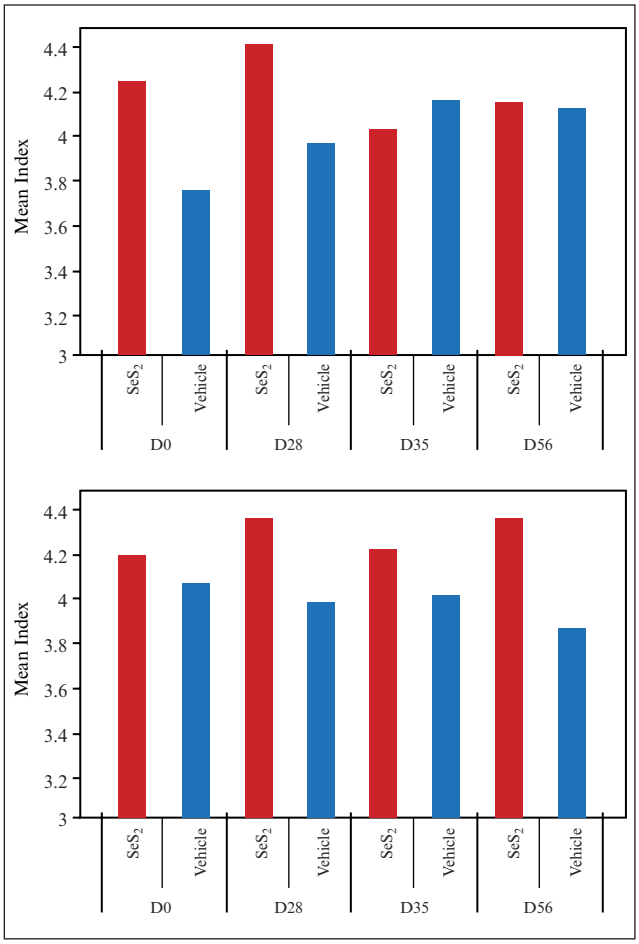

Figure 2. Study 1: Scalp bacterial diversity using the Shannon Index for lesional and non-lesional zones.
A) Lesional zone
B) Non-lesional zone
Shannon indices measuring microbial diversity and richness were used to compare both lesional and non-lesional zones from both treatment and vehicle groups over time.
Bacterial diversity was not modified by SeS_2 compared to the vehicle, either during the active phase or the follow-up period, at both lesional and non-lesional zones.

Clinical assessment

Figure 6 summarizes the changes over time of the total dandruff severity. At each time point during the treatment phase (D0-D31), the mean total dandruff scores significantly (p<0.01) improved compared to baseline. During the follow-up phase (D31-D73), an increase in dandruff severity was observed, with a maintenance of a significant benefit compared to baseline at D73 (p<0.01). *Figure 7* illustrates mean severity scores for self-perceived scaling, itch and scalp greasiness integrating an additional self-assessment at D3. At each time point of self-assessment starting from D3, the subjects significantly (p<0.05) self-perceived the decrease of scores for the 3 signs (itch, scaling, and scalp greasiness) as compared to baseline. The mean values of perceived symptoms remained stable during the follow-up phase with no significant variation between D31 and D73.

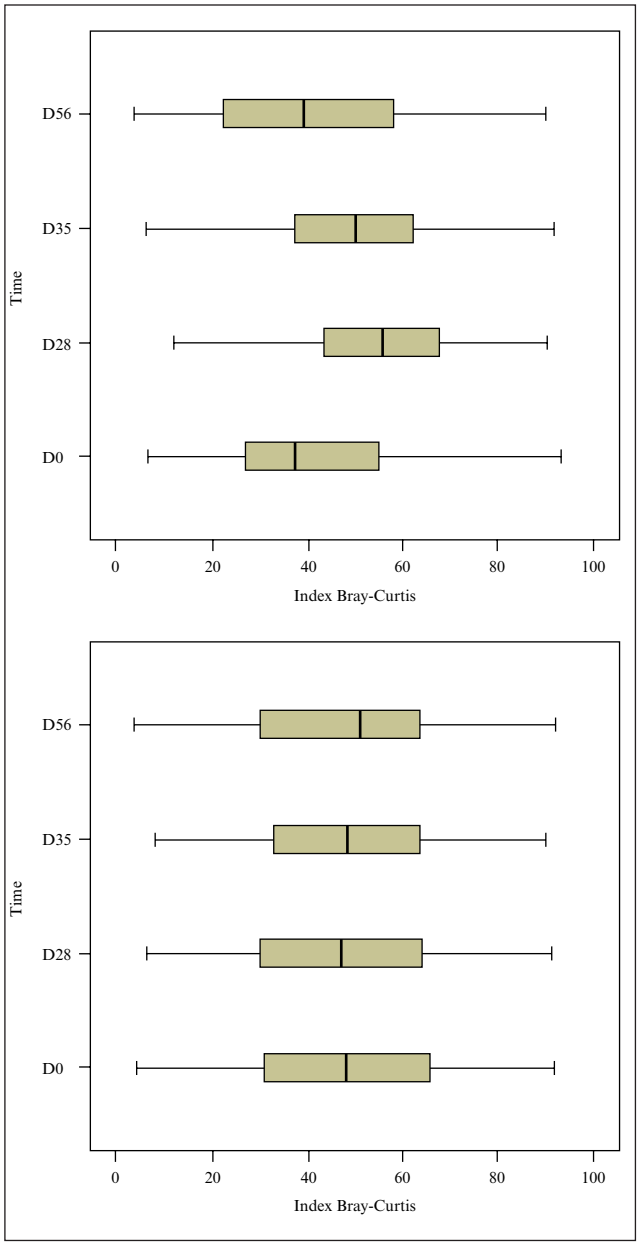

Figure 3. Study 1: bacterial OTU composition dissimilarity as measured using Bray-Curtis , with SeS_2 and the vehicle Bray-Curtis Indices were used to compare changes in the spread of bacterial OTUs at lesional zones in both treatment and vehicle groups over time.
A) SeS_2 caused changes in the relative distribution of bacterial OTUs towards greater diversity, at D28 and D35. This variation returned to baseline levels after 56 days.
B) Vehicle-treated group showing no change over time in the relative OTU distribution

Lipid Analysis
The regular use of SeS_2 shampoo resulted in significant changes of several sebum compounds. The total squalene content significantly increased ($p<0.05$) in parallel with a decrease by almost 50% of squalene peroxide at D31. Between D0 and D31, the ratio TG/FFA had significantly increased (+89.60±76.05, $p<0.0001$) corresponding to the restoration of the stock of TG through the decrease of the lipasic activity of *Malassezia* spp. after 4 weeks of use of SeS_2.
The ratio of SQOOH/SQ significantly decreased (-46.05±57.62, $p<0.0001$) after 4 weeks, correlating with the decrease of squalene peroxide with SeS_2.
Table 1 provides complete results for lipid ratios at baseline and D31.

Skin barrier markers
The use of SeS_2 shampoo led to a significant ($p<0.02$) decrease of 4.91 units ($g/m^2/h$) for the TEWL during the active phase, returning to baseline values during the follow-up phase.

Local tolerance

No local tolerance issues were reported for SeS_2 in any of the 2 studies.

Discussion

SeS_2 is a well-known active ingredient to manage dandruff [22]; however, only limited clinical work has been carried out to confirm its efficacy and mode of action [21, 29, 30]. We herewith report results from 2 studies that, in addition to the assessment of clinical parameters, also assessed the impact of SeS_2 on the scalp full microbiota and sebum in subjects with dandruff.
The studies confirm that SeS_2 significantly reduces adherent and non-adherent dandruff from baseline, correlating well with changes of the scalp microbiome. During the follow up phase, the clinical benefit was maintained up to 6 weeks with a trend to relapse correlating with a dysbiosis, confirming the chronicity of dandruff and the importance of a maintenance care.
At baseline, in lesional zones, *Staphylococci genus* abundance was more important while *Corynebacteria* and *Acinetobacteria* genus abundance was more frequently observed in non-lesional zones, confirming results presented by Clavaud *et al*. in 2013 [18]. The SeS_2 shampoo did not significantly impact the bacterial alpha diversity of the scalp, neither during the active nor during the follow-up period.
SeS_2 changed the global bacterial distribution with a notable decrease in *Staphylococci* and an increase in *Cutibacterium* load, confirming that SeS_2 acts on the bacteriome.
After 28 days of SeS_2 use, *Malassezia* spp. and *Staphylococcus* spp. loads were significantly reduced ($p<0.001$) in both zones. However, after 56 days of follow-up, the loads returned to baseline levels with *Cutibacterium* spp. levels not differing between the SeS_2 and the vehicle treatment group. Clinical signs and symptoms remained unchanged This may be due to the fact that dandruff is a multifactorial condition, and that SeS_2 shampoo is not an antifungal, but a fungistatic, agent resulting in an increase of *Malassezia* stains once treatment has stopped.
The ratio *Malassezia*/*Cutibacterium* and *Cutibacterium*/*Staphylococcus* decreased to reach that of non-dandruff

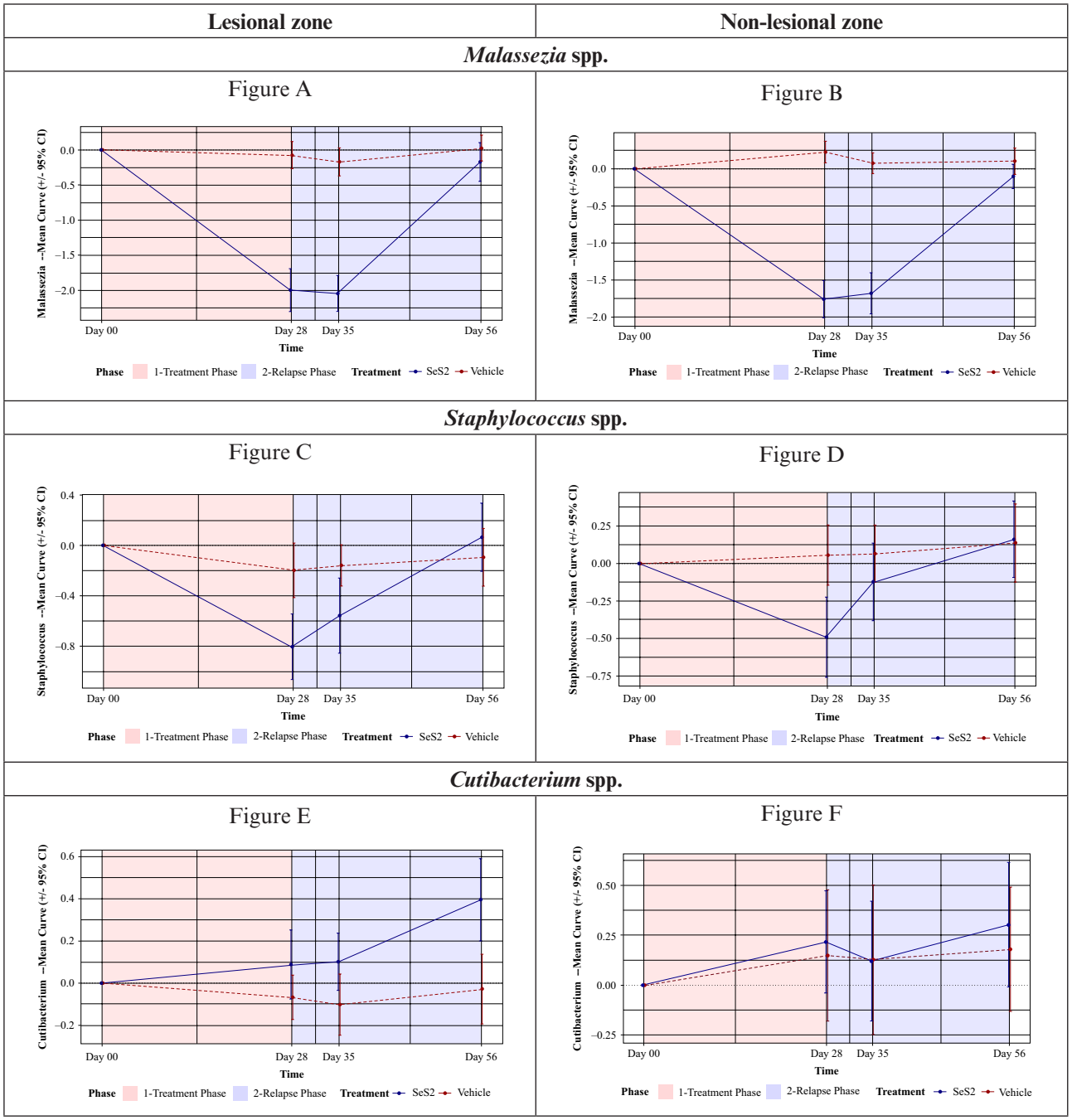

Figure 4. Study 1: SeS_2 reduces numbers of *Malassezia* spp. and *Staphylococcus* spp.
Subjects having received SeS2 showed reduced numbers of *Malassezia* spp. and *Staphylococcus* spp. by about -2Δlog to -1Δlog respectively, at D28 and 35 at both lesional and non-lesional zones ($p<0.001$) when compared to D0 and the vehicle group.
By D56, the number of these 2 microorganisms had returned to near-D0 levels. *Cutibacterium* spp. numbers did not vary at D35 and between the SeS_2 and the vehicle group for both lesional and non-lesional zones. *Cutibacterium* spp. numbers were significantly increased by ~+04Δlog with SeS_2 treatment ($p<0.001$), but only at the lesional zone at D56.

volunteers, indicating that the SeS_2 shampoo restores the microbial scalp equilibrium after 28 days of treatment.
Results from the scalp lipid analysis revealed that the regular use of SeS_2 shampoo resulted in significant changes of several sebum compounds. SeS_2 significantly ($p<0.05$) increased the total squalene content and decreased the quantity of SQOOH by almost 50%. Furthermore, the ratio glycerides/free fatty acids significantly ($p<0.0001$) increased after 28 days, corresponding to a restoration of the lipid stock of triglycerides through the decrease of the lipasic activity of *Malassezia* spp. after 28 days, paralleling observations made by

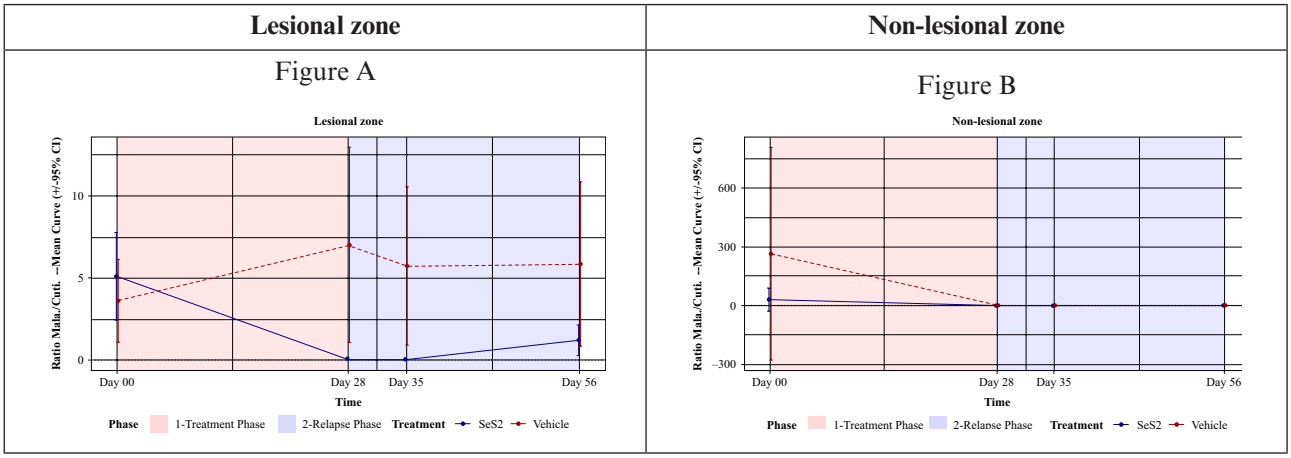

Figure 5. Study 1: Ratio *Malassezia* spp. vs *Cutibacterium* spp.
A significant ($p<0.001$) difference at D28 and D35 compared to baseline were observed between SeS_2 and the vehicle for the ratio of number of *Malassezia* spp. *Cutibacterium* spp.

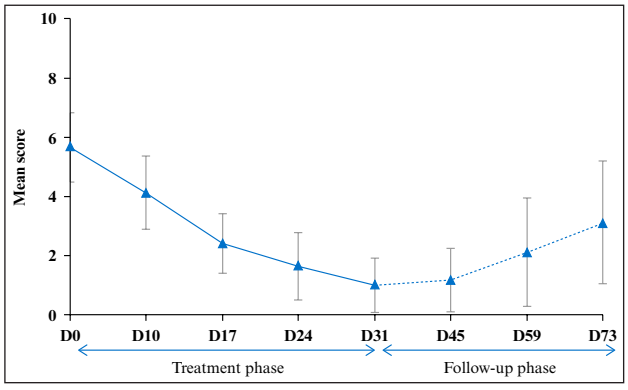

Figure 6. Study 2: Changes over time of mean total dandruff severity scores compared to baseline
At all-time points, mean total dandruff scores were significantly ($p<0.01$) improved from baseline. During the follow-up phase a slow increase to baseline values was observed, with a maintenance of a significant ($p<0.01$) clinical benefit.

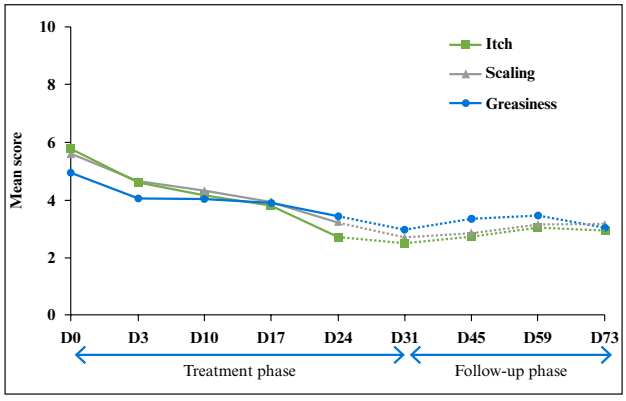

Figure 7. Study 2: Changes over time of the mean scores in the self-perception of the severity of scaling, itch and scalp greasiness
At all visits, the subjects significantly ($p<0.05$) self-perceived the decease of scores for the 3 signs as compared to baseline.

Table 1. Study 2: Scalp lipid analyses.

	Result (mean±SD)	
	Baseline	**Day 31**
Squalene (µg/mg lipidic extract)	97.41±29.23	109.2935.43
Ratio SQOOH/SQ ng/µg	85.96±69.58	39.90±37.68
Ratio Glyceride/FFA	28.52±23.53	118.12±88.05

SQ: squalene, SQOOH: squalene monohydroperoxide, FFA: free fatty acids

Jourdain *et al* [8]. The ratio SQOOH/SQ significantly ($p<0.0001$) decreased after 28 days correlating with a decreased peroxide level.
The tolerability to SeS_2 shampoo was excellent.
In conclusion, SeS_2 shampoo is effective and well-tolerated in dandruff due to its rebalancing effect of the equilibrium between the main bacterial and fungal populations and rebalancing of the SQOOH/SQ ratio, thus helping to maintain a healthy scalp. ■

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Conflict of interest: All authors are employees of L'Oréal Research & Innovation, France.

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Contribution. *Cécile Clavaud conducted the microbiome analysis, read the data and co-wrote the paper, Céline Michelin conducted the clinical study 2, Sayeh Pourhamidi conducted the study 1, read the data, Sarah Ziane conducted the clinical field activities, Charles El Rawadi designed the clinical study 1 and read the data, Benoit*

Muller coordinated, designed and read the data of study 1, Luc Souverain conducted the statistical analysis of both studies, Ségolène Panhard designed and read the data of study 2, Roland Jourdain analyzed the scalp lipids and read the data of study 2 and Philippe Massiot analyzed and wrote the paper, Richard Martin conducted the microbiome analysis, Olivia Isard conceived the formula, Fabien Cabirol analyzed the microbiome data, Damien Drillon contributed to data analysis, Lionel Breton read the data, Florence Pouradier read the data and wrote the paper, Luc Aguilar conceived the study and read the data, Geneviève Loussouarn coordinated and read the data of the study 2, Audrey Guéniche designed the trials, read the data, wrote the paper and coordinated the review.

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