ACTIVE BEAUTY

Evernityl™

Porphyr'ageing™ control for timeless Beauty

Well-ageing / Prevention

Givaudan

Human by nature

Focus on the product

Porphyr'ageing™, when postbiotics influence ageing

Givaudan Active Beauty has unveiled a ground-breaking discovery regarding the impact of porphyrins, metabolites produced by the skin microbiota, on skin ageing. This significant advancement highlights that porphyrins, which are prevalent molecules in various organisms, play a crucial role in various parts of the ageing process.

Porphyrins actively contribute to inflammation by promoting cytokine release and increasing oxidative stress, as already described in the literature, both of which can accelerate premature skin ageing. They also stimulate pigmentation by enhancing melanogenesis, resulting in dark spot precursors that can later lead to visible pigmentation issues. Furthermore, fibroblasts exposed to porphyrins lose their capacity to produce collagen, which accelerates cellular ageing and emphasises the harmful effects of porphyrins on skin. These findings, made by Givaudan Active Beauty experts, underscore the critical role of porphyrins in the ageing process, giving rise to the concept of Porphyr'ageing™, and highlight the urgent need for strategies to regulate their levels to promote healthier, more youthful skin.

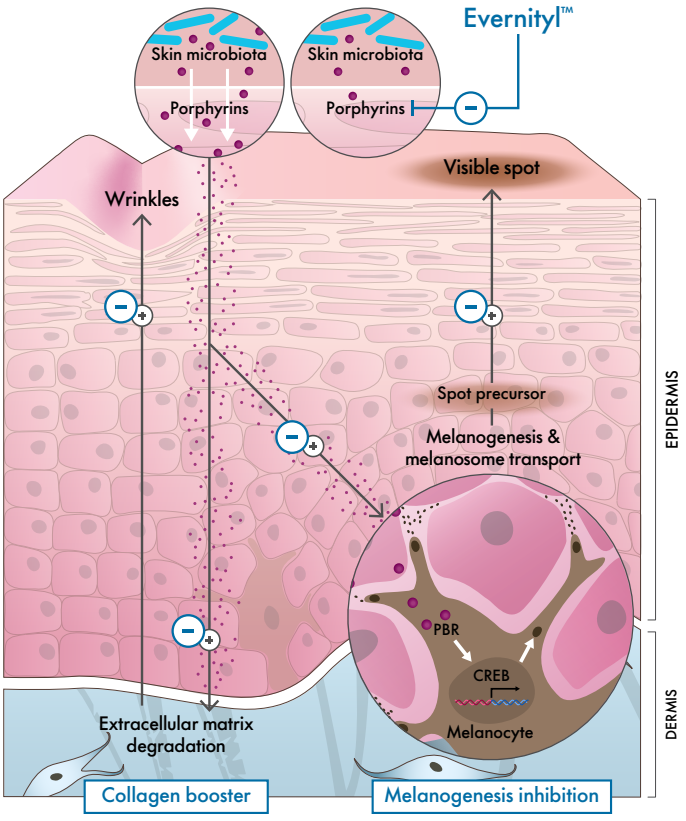
Harnessing the ocean's power for ageless skin

Through a rigorous biological screening, Active Beauty scientists have identified among numerous candidates: Evernityl™, a unique and powerful active ingredient derived from the depths of the ocean in Brittany, for its potent action against Porphyr'ageing™.

Evernityl ™ is extracted from *Laminaria hyperborea*, a macroalgae found in rocky coastal areas of the North Atlantic. Harvested in the Iroise Sea from September to November; at this time, the algae are richest in laminaran, a specific glucan-derivative.

The harvesting process is highly regulated, ensuring ecological balance by allowing sites to remain fallow for 3 to 4 years post-harvest.

Evernityl™ is produced through an innovative upcycling process, transforming fresh algae into a powder extract using a sustainable, solvent-free method. Its uniqueness lies in a substituted carbon on the active molecule laminaran, which boosts its anti-ageing properties and efficacy, making Evernityl™ a powerful ally for achieving youthful skin.



Evernityl™ decelerates ageing and corrects visible signs of time

Evernityl™ operates through a multifaceted mode of action to combat the signs of ageing linked to Porphyr'ageing™, a post-biotic induced ageing. It effectively reduces porphyrin production while facilitating their elimination via autophagy.

By lowering the levels of porphyrins that trigger melanogenesis and extracellular matrix (ECM) degradation:

- It inhibits porphyrin-induced melanogenesis, reducing the formation of dark spot precursors and thus visible hyper-pigmentation, further contributing to a more even skin tone.
- It enhances skin elasticity and firmness by stimulating ECM synthesis, vital for maintaining skin integrity, helping prevent the formation of wrinkles.

Overall, Evernityl™ addresses existing signs of ageing while pro-actively preventing future skin damages, making it a comprehensive solution for healthier, more youthful-looking skin.

Biological activity

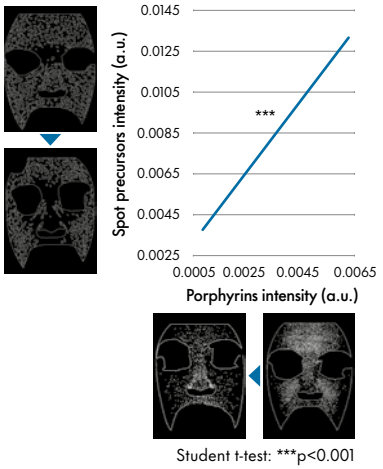
Porphyr'ageing™: the impact of porphyrins on skin ageing

1. Significant correlation between porphyrins and ageing signs (*in vivo*)

Givaudan Active Beauty gathered clinical data from 100 volunteers over a five-year period to analyse skin parameters across various body and face areas. This study aimed at enhancing the global understanding of skin health and possible correlation between various parameters.

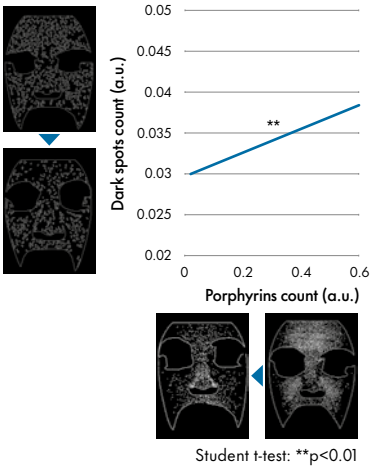
Porphyrins, spot precursors, visible spots and wrinkles were measured by VISIA CR 2.3® methodology.

Porphyrins and spot precursors



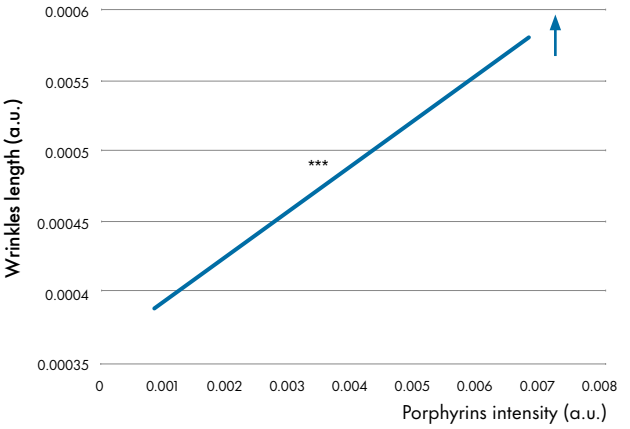
Student t-test: ***p<0.001

Porphyrins and dark spots



Student t-test: **p<0.01

Porphyrins and wrinkles



Student t-test: ***p<0.001

Results: The severity of **spot precursors and visible spots** is significantly correlated **with a higher quantity of porphyrins**, regardless of age. More spot precursors lead to an increase in dark spots.

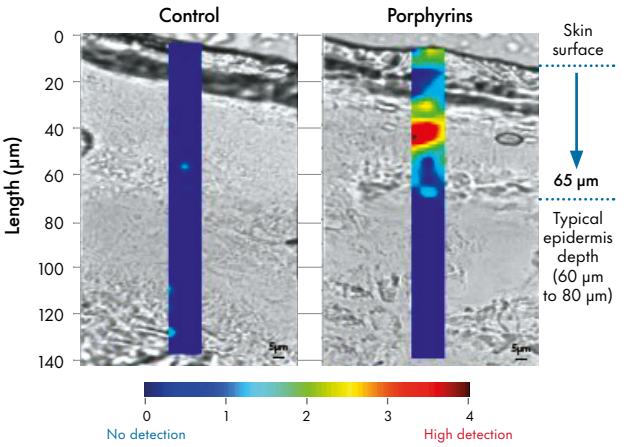
Results: The severity of wrinkles is significantly correlated with high porphyrin levels across all ages.

2. Porphyrins penetrate the skin (*ex vivo*)

Skin explants (57 years old) were treated with porphyrins (Coproporphyrin III at 100µM) for 8h.

Specific signal of porphyrins was defined by spectrofluorometry and used for tracking porphyrin skin penetration by micro-imagery Raman methodology.

Results: Porphyrins reach the basal layer of the epidermis and **act at the interface between the epidermis and dermis**.

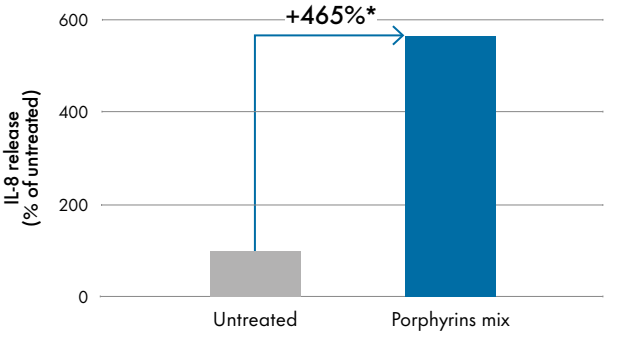


3. Porphyrins trigger inflammation (*in vitro*)

A co-culture of normal human melanocytes (NHM) and keratinocytes (NHEK) was treated with a mixture of Coproporphyrin III (10µM): Protoporphyrin IX (0.1µM) (100:1) (representing the natural porphyrins ratio on the skin) for 3 days. Pro-inflammatory cytokine (IL-8) release in the supernatant was then quantified by ELISA.

Results: **Porphyrins induce inflammation** by significantly increasing the pro-inflammatory cytokine (IL-8) release compared to the untreated condition.

Pro-inflammatory effects of porphyrins



Mann Whitney test: *p<0.05

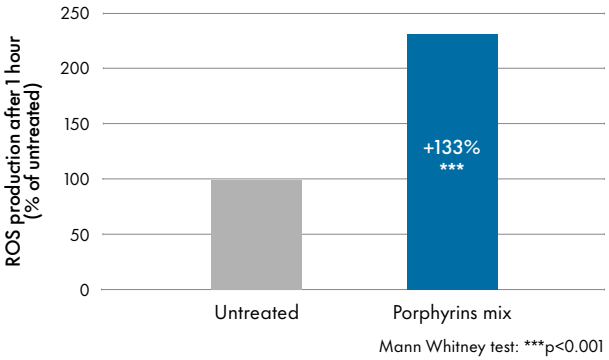
Biological activity

4. Porphyrins promote oxidation (*in vitro*)

Intracellular reactive oxygen species (ROS) production was measured in NHEK after treatment for 1 hour with a mixture of Coproporphyrin III (10 µM): Protoporphyrin IX (0.1 µM) (100:1). The measurement was performed using a fluorescent probe method.

Results: Porphyrins induce oxidative stress by **increasing the release of intracellular ROS by +133%** compared to the untreated condition.

Increased oxidation



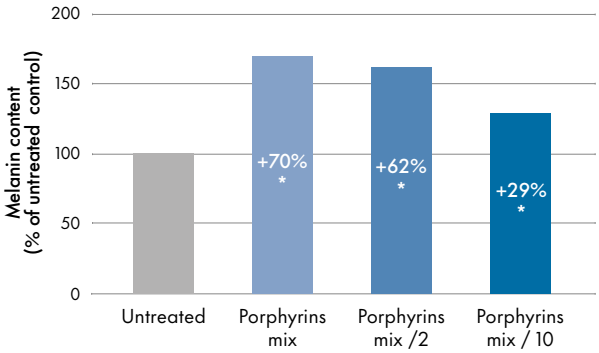
Mann Whitney test: ***p<0.001

5. Porphyrins foster skin pigmentation (*in vitro*)

Skin pigmentation was evaluated on a a co-culture of NHM and NHEK treated with a mixture of Coproporphyrin III (10µM) : Protoporphyrin IX (0.1µM) (100:1) at different doses (without or with dilution by 2 or 10) for 3 days. Melanin content was then extracted from the cell lysates and quantified using a spectrophotometer.

Results: Porphyrins induce **melanin synthesis** in a dose-dependent manner. Reducing the concentration of porphyrins by a factor of 10 results in a 41%# decrease in melanogenesis, making porphyrins' level reduction a promising strategy to combat hyperpigmentation.

Induction of melanin synthesis



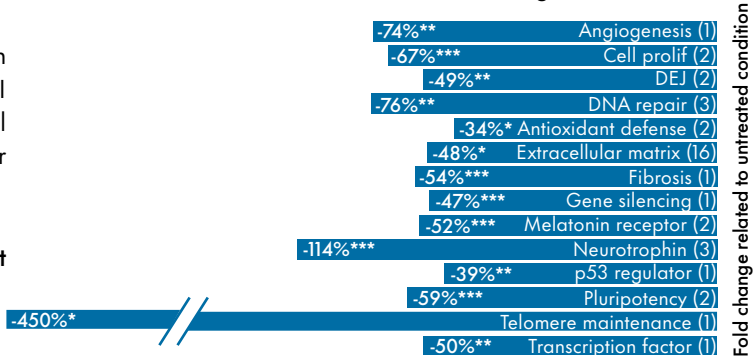
Mann Whitney test: #p<0.1; *p<0.05

6. Porphyrins target several ageing pathways (*in vitro*)

A transcriptomic analysis was performed on normal human dermal fibroblasts (NHDF) treated with a mix of Coproporphyrin III (10µM): Protoporphyrin IX (0.1µM) (100:1) for 6 hours. Total RNA were extracted and retrotranscripted in cDNA for further transcriptomic analysis using targeted RT-qPCR methodology.

Results: Porphyrins downregulate 38 genes **modulating fibroblast functions** (cell proliferation, DNA repair, extracellular matrix).

Genes downregulation



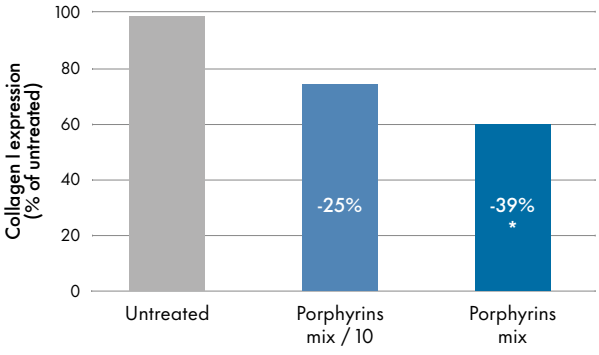
Student t-test: *p<0.05; **p<0.01; ***p<0.001

7. Porphyrins decrease collagen synthesis (*in vitro*)

NHDF were treated with a mix of Coproporphyrin III (10 µM or 1 µM): Protoporphyrin IX (0.1 µM or 0.01 µM) at a ratio of 100:1 for 48h. Supernatant was collected and Collagen I expression was then analysed by ELISA.

Results: Fibroblasts **lose their capacity to synthesise collagen** when they are stressed with porphyrins down to -39%. Reducing the concentration of porphyrins by a factor of 10 results in a protection of the collagen synthesis.

Decrease of collagen synthesis



Mann Whitney test: *p<0.05

Biological activity

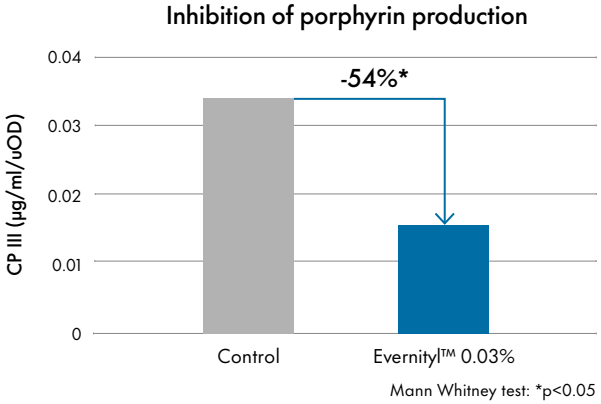
Evernityl™ a powerful preventive and corrective action

A. Tackling the roots of Porphyr'ageing™

1. Reduction of porphyrin production (*in vitro*)

The production of Coproporphyrin III by Gram+ bacteria was quantified in the supernatant after 48h of culture in the presence of Evernityl™.

Results: Evernityl™ **reduces porphyrin production.** The inhibition of porphyrin production is very **specific to the extract from Laminaria hyperborea,** with its specific substitution versus a standard laminaran (data not shown).

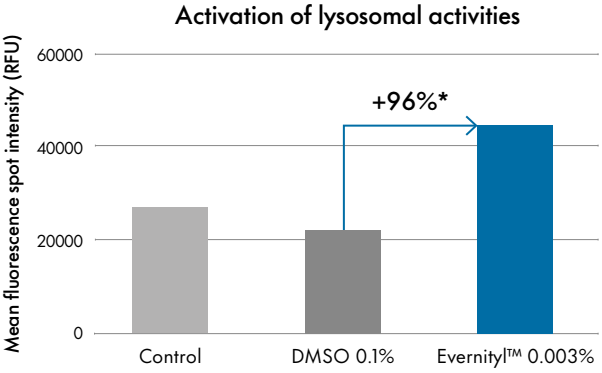


Mann Whitney test: *p<0.05

2. Porphyrin elimination by autophagy (*in vitro*)

NHEK were treated for 30 hours with Evernityl™ at 30 µg/mL (0.003%). Then fluorescent lysosome detection probe was added for 1 hour and lysosomes were observed and quantified by automated fluorescence microscopy. Results are expressed in Mean fluorescence spot intensity (RFU).

Results: Evernityl™ enhances **lysosomal activities**, triggering the clearance of porphyrins via autophagy.



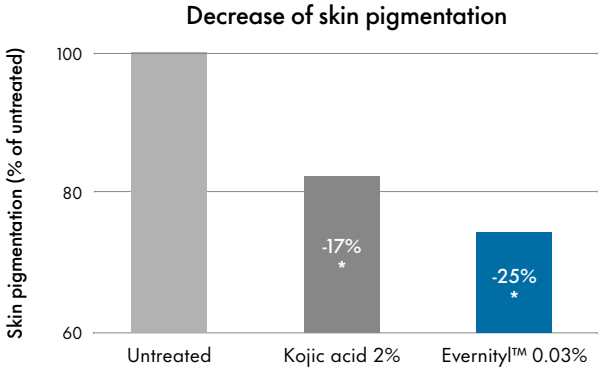
Mann Whitney test: *p<0.05

B. Addressing visible signs of ageing

1. Strong inhibition of melanin synthesis (*ex vivo*)

Skin explants from a phototype V donor (36 years old) were treated for 5 days with Evernityl™ at 0.03% versus Kojic acid at 2%, as positive control. Melanin content was analysed after Fontana Masson staining and image analysis (data not shown).

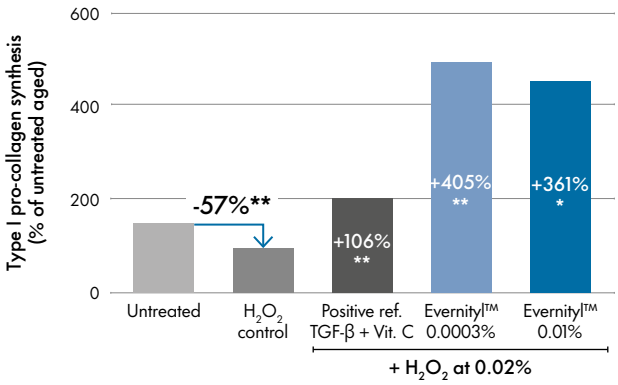
Results: Evernityl™ significantly **reduces the melanin content** in skin explants.



Mann Whitney test: *p<0.05

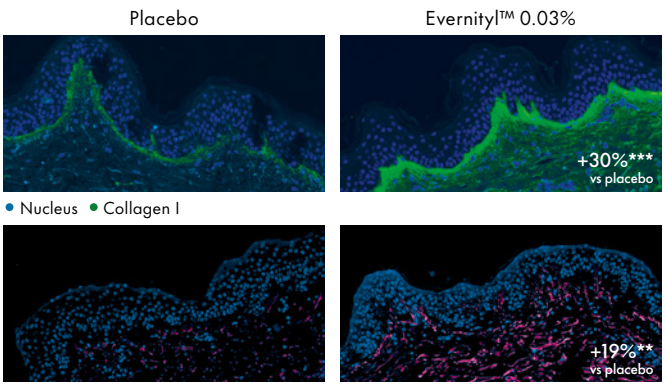
2. Boost of collagen and elastin content (*in vitro* and *ex vivo*)

Premature ageing was induced by hydrogen peroxide (H_2O_2) for two hours on NHDF. After incubation, NHDF were treated with Evernityl™ at two doses or with reference (TGF-β at 10 ng/ml + ascorbic acid at 20 µg/ml) or kept untreated (negative control). After 48h of incubation Collagen I was quantified in the supernatant by using ELISA.



Mann Whitney test:*p<0.05; **p<0.01

Skin explants from a 56-year-old woman were subjected to topical application of Evernityl™ at 0.03% and 0.1% in emulsion, compared to a placebo, for 4 days. Immunofluorescence targeted Collagen I and Elastin IHC, and quantification was performed by analysing fluorescence intensity.



Student t-test: **p<0.01; ***p<0.001

Results: Evernityl™ significantly improves skin **collagen and elastin** content in skin.

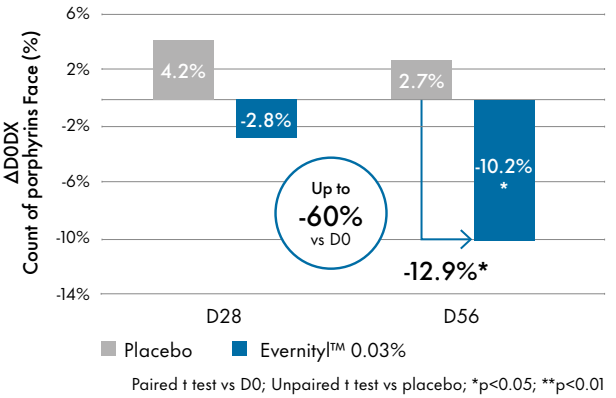
Clinical efficacy

A. Effective prevention

A panel of 37 women aged between 45 and 73 years old (65.3 years old ± 4.5 years), with wrinkles, spot precursors, and pigmented spots on the face and upper chest, was divided into two groups. One group applied a cream containing Evernityl™ at 0.03% twice a day for 56 days, the other group applied a placebo (the same formula without the active ingredient). Skin parameters were measured at D0, D28 and D56.

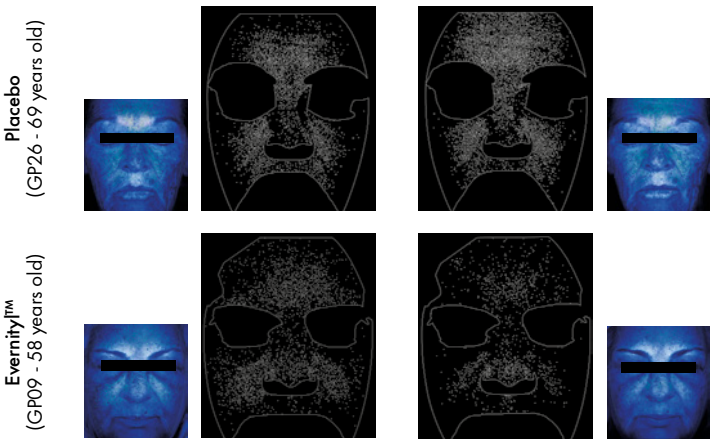
Reduction of porphyrins and spot precursors

Invisible spots, dark spots, and porphyrins on the face were observed using Visia CR2.3®.

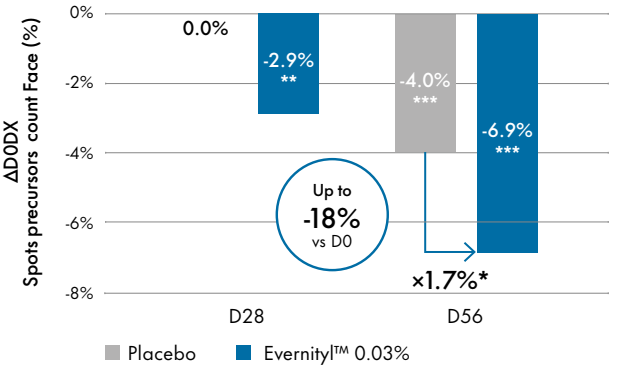


Paired t test vs D0; Unpaired t test vs placebo; *p<0.05; **p<0.01

Porphyrins count

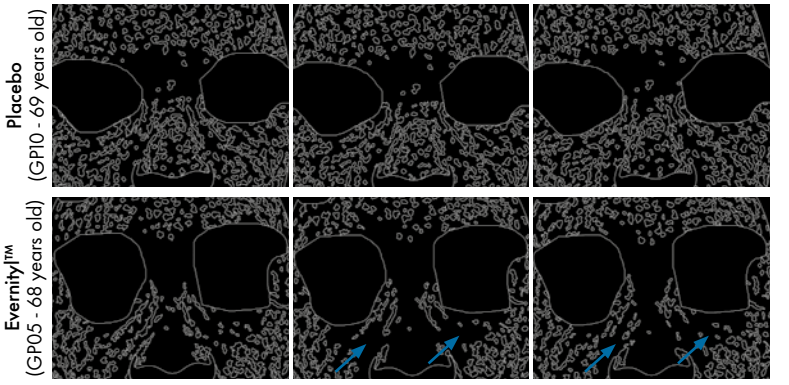


Results: Evernityl™ significantly **reduces the count of porphyrins** after 56 days on the face, by respectively -10.2% versus D0, and -12.9% versus placebo.



Paired t test vs D0; Unpaired t test vs placebo; *p<0.05; **p<0.01; ***p<0.001

Spot precursors

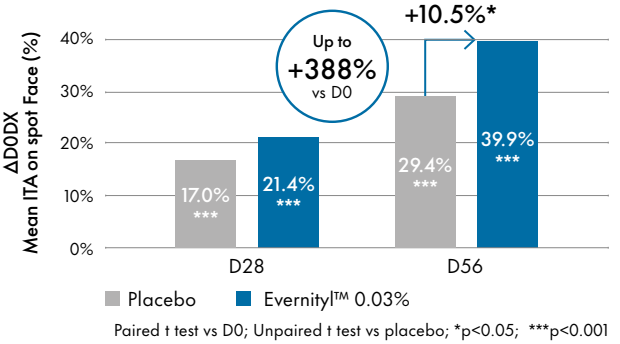


Results: Evernityl™ significantly **decreases the count of spot precursors** after 56 days on the face, by -6.9% versus D0, and up to 1.7 times better than the placebo.

B. Corrective actions

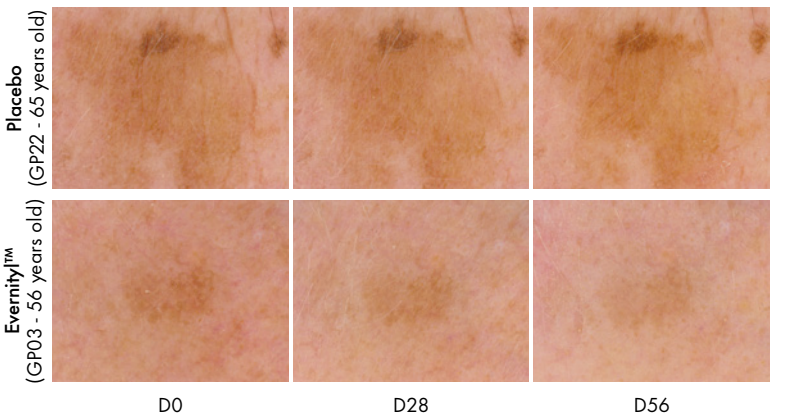
1. Brightening effect

C-cube® measurements were performed on the face to measure the melanin level in the spots.



Paired t test vs D0; Unpaired t test vs placebo; *p<0.05; ***p<0.001

Melanin level in spots

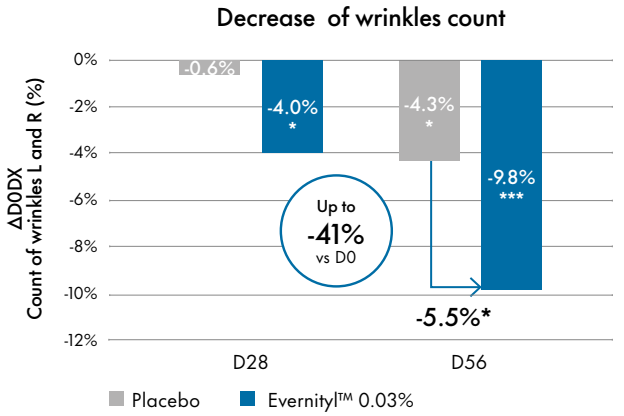


Results: Evernityl™ significantly increases the mean ITA on spots after 56 days on the face by 10.5% versus the placebo. Evernityl™ decreases the melanin level **inducing a brightening effect** of the spots.

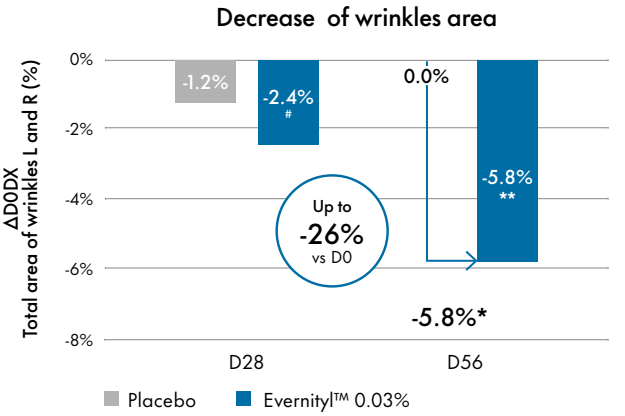
Clinical efficacy

2. Reduction of wrinkles

The analysis of wrinkles on the face was conducted using Visia CR2.3®.



Paired t-test vs D0; Unpaired t test vs placebo; #p<0.1; *p<0.05; ***p<0.001



Wilcoxon test vs D0, Mann Whitney test vs placebo: #p<0.1; *p<0.05; **p<0.01

Results: Evernityl™ significantly **decreases the count of wrinkles** after 56 days on the face, by -9.8%, and 2.3 times better than the placebo and significantly **decreases the total area of wrinkles** after 56 days of use on the face by -5.8%.

3. Extra clinical benefits: skin roughness improvement and body care applications

Evernityl™ also significantly decreases skin roughness in 1 month and even improves further after 2 months. An additional study shows that Evernityl™ has similar effects on the bust area, with a significant reduction in dark spots after 1 and 2 months (up to +117% vs D0, mean ITA on pigmented spots).
These studies illustrate the strong corrective efficacy of Evernityl™ on dark spots and wrinkles.

C. Visualising efficacy: the long-term benefits of Evernityl™ on skin appearance thanks to AI

A 3D virtual avatar was created to demonstrate the effects of Evernityl™ versus a placebo over time. This AI-generated video uses multiple parameters to predict efficacy based on our extensive biological and clinical evaluations and visually shows how the active ingredient effectively reduces the number and intensity of dark spots and wrinkles over a life-time, highlighting its significant skin benefits.

27 years old

Placebo | Evernityl™

45 years old

Placebo | Evernityl™

74 years old

Placebo | Evernityl™

Summary

Technical information

INCI:	Laminaria Hyperborea Extract (and) Citric Acid
Origin:	Blue biotechnology
Preservation:	Sorbic Acid
Appearance:	Beige powder
Solubility:	Water dispersible
Dosage:	0.03%
Processing:	Disperse in water under rotor-stator (ratio: 1:4). Add this premix at the end of the formulation process at a pH between 4-8 and, at temperature below 40°C.

Benefits

Claims*:	Preventive, corrective, antioxidant, anti-porphyr'ageing™, porphyrins reduction, reduction of porphyrin-induced matrix degradation, anti-wrinkles, collagen production booster, reduction of porphyrin-induced melanogenesis, dark spot precursors inhibition, dark spots correction, pigmentation correction, skin brightening, longevity, well-ageing, skin whitening.
Applications:	Preventive serum or cream, anti-ageing serum or cream, anti-spots preventive and corrective cream, anti-wrinkle product, global anti-ageing product.

*Disclaimer: Safety assessment of Evernityl™ for cosmetic application has been fully done in accordance with SCCS Notes of guidance for the testing of Cosmetic ingredients and their safety evaluation and CSAR. However Evernityl™ has not yet been fully assessed from a safety standpoint for a specific whitening/ freckle-removing function in China. It is the customer's responsibility to assess which claims are compliant with cosmetic regulation in the countries where the final product is launched.

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Givaudan

Human by nature

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