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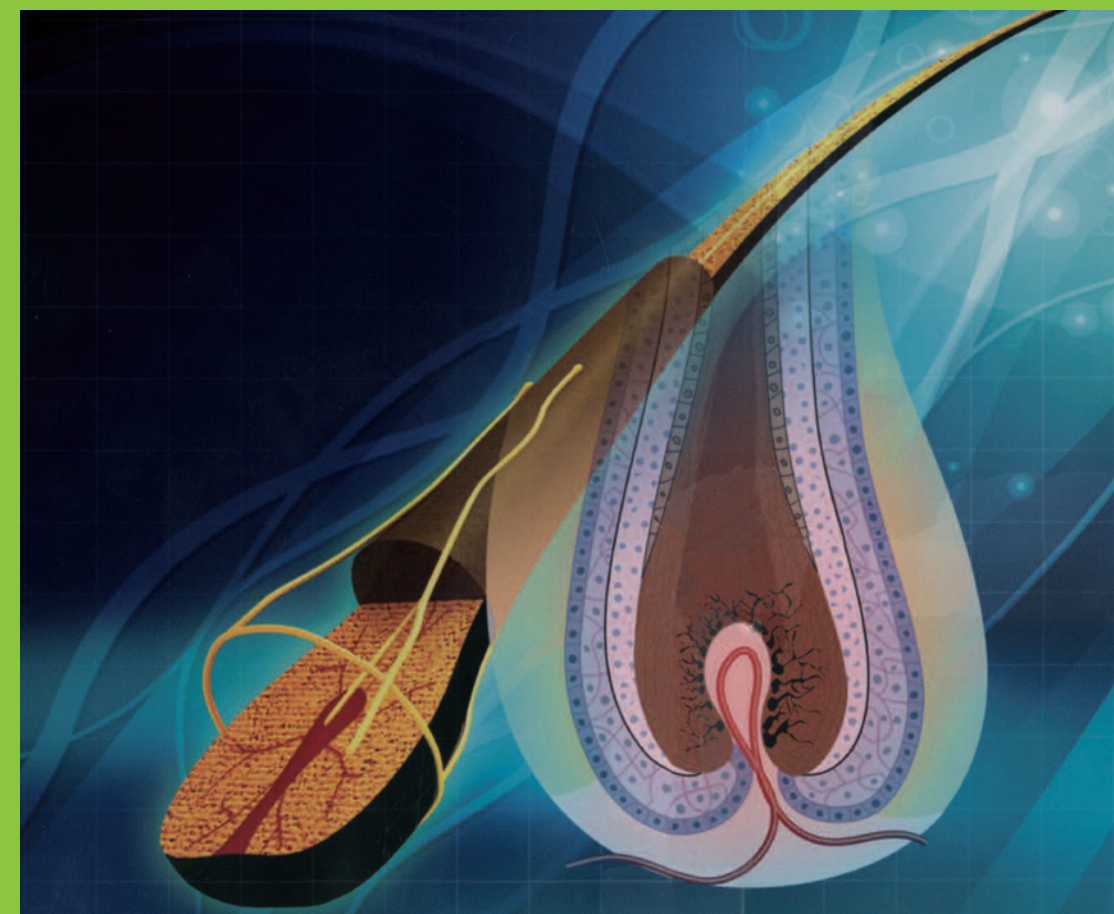
DIETARY SUPPLEMENTS

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Progressive hair loss

Benefits of TRIPHASIC^{VHT} ATP INTENSIF

M. Lévêque, A. Jérôme, C. Merial-Kieny





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PARIS

Treat hair loss
and extend the life of your hair

PROGRESSIVE HAIR LOSS

TRIPHASIC^{VHT}

ATP INTENSIF

René FURTERER reveals the full anti-hair loss effectiveness of ATP, an essential source of energy for the follicular papilla, to treat hair loss and extend the life of your hair. TRIPHASIC's formula with 3 active phases fights against the 3 factors responsible for progressive hair loss. Existing hair is preserved, hair grows back stronger and thicker. Easy-to-use. No-rinse.

+ 4 583 hairs in the growth phase*
No.1 on the anti-hair loss market **



ESSENTIAL HAIR CARE



Pierre Fabre

CONCLUSION

The new formula of TRIPHASIC^{VHT} ATP INTENSIF, incorporating the new active ingredient ATP, therefore acts on all the factors involved in the aggravation of progressive hair loss (hormonal, vascular and tissular factors).

All of the *ex vivo* studies described above made it possible to demonstrate the significant benefits of ATP against hair loss through its triple action: stimulation of growth factors (KGF), improvement of hair follicle anchoring (fibronectin) and improvement of survival in the anagen phase of the hair bulbs.

This efficacy was confirmed in the finished product TRIPHASIC^{VHT} ATP INTENSIF by a program of dermatologically controlled clinical studies based on AGA reference assessment criterion: phototrichogram, change in the number of hairs in the anagen phase and A/T ratio.

This proven efficacy combined with simple dosing (2 applications/week the 1st month and 1 application/week the 2nd and 3rd months of treatment) guarantee good treatment compliance.

Affiliations: the authors declare the following affiliations: A. Jérôme: clinical trial coordinator (Pierre Fabre Dermo-Cosmetics). C. Merial-Kieny: employed by Pierre Fabre Laboratories. M. Lévêque: participation as principal investigator in the fundamental PFDC study.

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Progressive hair loss:

Benefits of TRIPHASIC^{VHT} ATP INTENSIF

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PROGRESSIVE HAIR LOSS

The hair follicle

The hair follicle is a mini-organ anchored in the skin down to the hypodermis, and whose primary function is the production of a hair shaft (body or head hair). The human body has around 5 million hair follicles of which 100,000 to 150,000 are located on the scalp. Their distribution is established during *in utero* growth and their number is genetically determined [1].

The hair follicle is composed of a connective tissue sheath (CTS), an outer root sheath (ORS) and an inner root sheath (IRS) which form a wall serving as a guide to the hair shaft during its growth (*Figure 1*).

The **connective tissue sheath** is composed of fibroblasts and an extracellular matrix formed by collagens, proteoglycans and a major glycoprotein: fibronectin [2]. Involved in cellular migration and adhesion, fibronectin is described as being

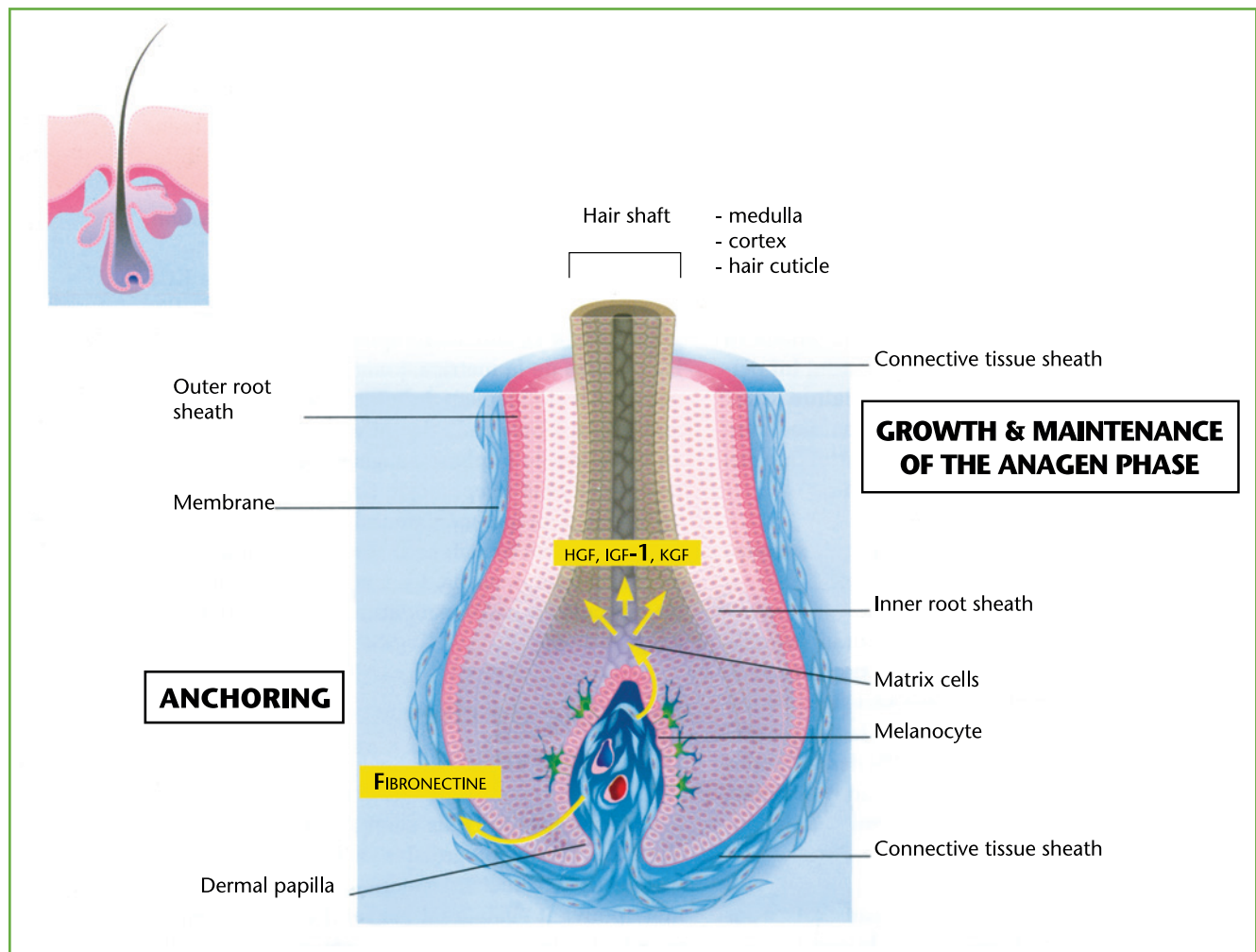


Figure 1: Hair cycle and anatomy of the hair follicle (G. Cotsarelis, Journal of Investigative Dermatology, 2006)

indispensable to anchoring the follicle. The inner and outer root sheaths are composed of follicular keratinocytes which express panels of specific keratins, which are markers of their differentiation.

The **hair bulb** is located at the proximal extremity of the follicle. It is composed of the dermal papilla, melanocytes and matrix cells, which are cells which generate follicular keratinocytes of the inner root sheath and the hair shaft.

The **dermal papilla** is composed of specialized fibroblasts called dermal papilla cells (DPC), which control the proliferation and differentiation of the matrix cells, the diameter of the hair shaft, as well as the hair cycle (namely the duration of the anagen phase) via the secretion of growth factors [3]. *In vivo*, the dermal papilla is a highly vascularized connective tissue which supplies the hair follicle with oxygen and nutrients.

The hair follicle also holds a reserve of epithelial and melanocyte **stem cells** under the sebaceous gland located in the outer root sheath: the **bulge**. These cells are characterized by a slow cellular cycle and the ability to differentiate into different cell types, including the cells of the outer root sheath and the matrix cells [4].

The hair cycle

Over the course of its post-natal life, the hair follicle undergoes a succession of cycles during which it will generate 20 to 30 hair shafts. In humans, these phases are not synchronized among the follicles; there is therefore a constant loss of hair with a constant replacement.

Each **hair cycle** is made up of three major characteristic phases [5-6]:

- a growth phase (**anagen**), during which the hair shaft is produced. During this period, the proliferation and differentiation of the follicular keratinocytes allow the hair shaft to grow.

In a healthy scalp, 85 to 90% of the hair follicles are in this phase of which the duration, which can range from two to six years, will determine the length of the resulting hair shaft.

- a regression phase (**catagen**), characterized by a decrease in the exchanges between the dermal papilla and the matrix cells. During this very transient phase lasting from 15 to 20 days, two-thirds of the hair follicle are destroyed by apoptosis. The cells of the dermal papilla, which express the anti-apoptotic

factor BCL2 (B-cell CLL/lymphoma 2), are not eliminated and lodge themselves under the bulge.

- a resting phase (**telogen**), during which the exchanges between the various cells of the follicle are significantly reduced. It generally lasts two to three months before the follicle enters a new anagen phase and produces a new hair shaft.

Regulation of the hair cycle

The hair cycle is a highly regulated mechanism.

For this reason, the various compartments of the follicle closely interact through numerous molecular exchanges which take the follicle from one phase to another in the cycle.

Among these exchanges, the signals from the dermal papilla to the bulge and matrix cells play a central role, in particular during the initiation and maintenance of the anagen phase [5]. These signals are mediated by factors secreted by the DPC acting on the epithelial shaft cells of the bulge (initiation of the anagen phase) or on the matrix cells of the bulb (maintenance of the anagen phase) and inducing their proliferation and their differentiation.

Among these signals, the keratinocyte growth factors (KGF) and insulin-like growth factor 1 (IGF1), of which the receptors are expressed by the matrix cells, play an important role in the development of the hair follicle by stimulating the growth the hair shaft and participating in the maintenance of the anagen phase [3]. Hepatocyte growth factor (HGF), described as the primary mediator of the interactions between mesenchymatous and epithelial cells, is also involved in controlling follicle growth by participating in the maintenance of the anagen phase [7-8].

Progressive hair loss

One of the most common and best known forms of progressive hair loss is androgenetic alopecia (AGA), mainly affecting men, but which can also occur in women, under effect of androgenic hormones in genetically disposed individuals. In the Caucasian population, it affects 50% of men from 50 years of age. The onset of AGA also has a significant psychological impact in affected patients [9]. We differentiate between two types of AGA: the male type, characterized by a symmetrical recession of the temporal and frontal hairline (up to hair loss at the crown of the head), and the female type, in which the temporal

and frontal hairline is preserved, and which manifests as diffuse rarefaction of the hair on the vertex.

A multifactorial etiology has been observed as has the role of other aggravating factors.

Genetic and hormonal factors

Manifesting between puberty and 40 years of age, AGA is mainly due to **genetic and hormonal factors**. In the dermal papilla of hair follicles affected by AGA, greater expression of the androgen receptor is observed, as well as more intense activity of the enzyme 5-alpha-reductase than in healthy follicles [10-11]. This enzyme reduces a steroidal hormone, testosterone, to dihydrotestosterone (DHT), which has a much greater affinity for the androgen receptor. Once formed, the DHT-androgen receptor complex is translocated to the cell nucleus where it induces the transcription of genes coding inhibitors of the hair cycle (IL-6, DKK1, TGF- β 1) [12-14]. The latter precipitate the passage from the anagen to the catagen phase and bring about a failure to activate by the stem cells of the bulge, thus delaying the initiation of a new anagen phase.

At the vascular level

During AGA, a decrease in superficial cutaneous blood flow has been observed as well as modifications in the vascularization of the dermal papilla of the hair follicle during the hair cycle.

The vascular network, which is highly developed during the active hair growth phase almost completely disappears during the hair loss phase. There is a potent angiogenic factor, vascular endothelial growth factor, VEGF, among the growth factors involved in regulating the hair cycle that controls the active growth phase.

At the tissular level

On the micro-environment of the hair bulb:

The hardening of the collagen fibers in the perifollicular connective tissue can also accompany androgenetic hair loss. This tissue undergoes free-radical attacks and the phenomenon of glycation.

Moreover, it has been shown that perifollicular micro-inflammation also plays an essential role in the cause of the onset of androgenetic hair loss and sometimes ends in the complete destruction of the affected follicles in advanced cases.

Inside the follicular papilla:

All of the aforementioned phenomena give rise to a decrease in the duration of the anagen phase and an acceleration of the hair cycles. This acceleration of the cycles leads, firstly, to the appearance of miniaturized hairs called «vellus» hairs, and finally to the premature exhaustion of the capacity of the hair follicles to produce new hair shafts [15]. It is also essential to act on the growth factors involved in regulating the hair cycle, such as keratinocyte growth factor (KGF).

With the genetically programmed number of hair cycles being 25-30 over a person's life, the assets of cycles run out and the prematurely aged follicle becomes unproductive. The effect aimed for to treat this type of hair loss is therefore to act on the 3 aggravating factors in AGA and to keep the hair follicles in the anagen phase as long as possible in order to slow down the frequency of the cycles.

Benefits of TRIPHASIC^{VHT} ATP INTENSIF against progressive hair loss

Since 1989, René Furterer has marketed the product TRIPHASIC, aimed at individuals with progressive hair loss. Over time, this product has continually benefitted from the advances of Pierre Fabre Research to optimize its effectiveness. It combines essential oils and plant-based active ingredients with complementary action and acting on the associated factors which can induce progressive hair loss:

- the vascular factor, through a *Pfaffia Paniculata* extract and essential orange and lavender oils to promote the microcirculation of the scalp.
- the hormonal factor, through a *Curbicia* extract, regulating the secretion of sebum (associated with the activity of 5-alpha-reductase).
- the tissular factor, through Stearyl Glycyrhettinate (*Licorice* extract), to reabsorb the micro-irritation of the hair bulb, and Hesperidin Methyl Chalcone (extract of *Citrus Aurantium*) to combat glycation.

Recently, TRIPHASIC was reformulated with the introduction of ATP (nucleoside adenosine 5' triphosphate) to give rise to the product **TRIPHASIC^{VHT} ATP INTENSIF**.

ATP is the primary source of energy within a cell, the precursor of cofactors NAD⁺ and co-enzyme A [16]. Moreover, ATP is an intermediary in cell communication. It plays an essential role in numerous tissues via its action on the purinergic P2Y receptors (7 transmembrane helices, coupled with heterotrimeric G proteins) and P2X receptors (transmembrane protein forming ion channels). In the human hair follicle, the expression of the P2Y and P2X receptors has been shown in distinct compartments during the anagen phase [17, 18], suggesting a role of ATP in the physiology of the hair follicle.

The aim of this publication is to present, firstly, the *ex vivo* studies demonstrating the interest of ATP in an anti-hair loss product and, secondly, the efficacy and tolerability of the product TRIPHASIC^{VHT} ATP INTENSIF in androgenetic alopecia.

Effect of ATP on *ex vivo* hair follicles

Methodology

A first experiment involved a primary culture of dermal papilla cells from 3 different donors. The proteins **KGF**, **HGF**, **IGF1** and **fibronectin** were measured in the culture supernatants after 24 hours of incubation of the cells with ATP (0.0018%). Prior to this a cytotoxicity test (reduction in MTT and morphological observations with a microscope) was performed on dermal papilla cells in order to confirm the viability of the cells under culture conditions.

In a second series of experiments, isolated hair bulbs were obtained, by micro-dissection of fragments of human skin at the level of the hypodermis, and cultured. They were then incubated with or without ATP (0.03%) for 6 hours (monitoring the level of expression of genes of interest) or for 28 days (monitoring the survival of cultured hair follicles).

In order to determine the effect of ATP on the expression of certain target genes expressed in the hair follicle, the RNA of the hair follicles was extracted and measured after 6 hours of incubation with ATP. Reverse transcription of RNA was performed and the cDNA obtained were then used to perform a quantitative PCR in real time.

In order to determine the effect of ATP on the survival of cultured hair follicles, the degeneration of the hair follicles in culture was assessed by observing the morphological attacks of the hair bulbs over time. The follicles were kept in culture until the degeneration of all of the control condition follicles, or for 28 days.

Results

The choice of study model on the hair follicles makes it possible to observe the effects of treatment with ATP on the whole tissue.

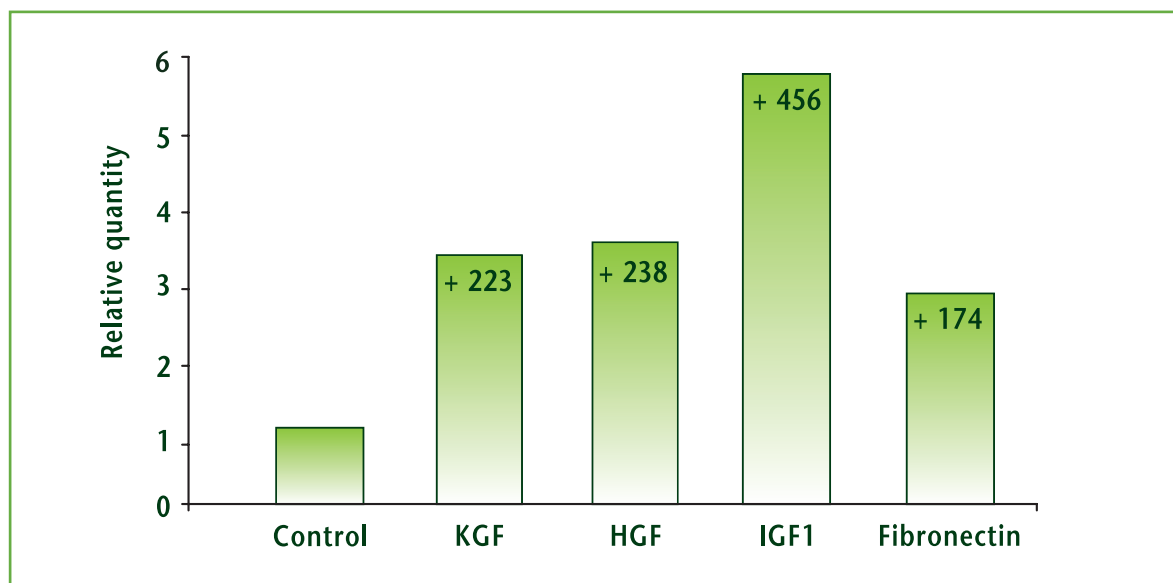


Figure 2: Study of the transcription of genes involved in the regulation of the hair cycle after 6 hours of incubation with ATP.

Impact of ATP on the expression of key hair follicle growth factors: KGF

Several genes coding key hair cycle regulator mediators (growth factors, signal proteins, matrix proteins) and expressed by dermal papilla cells were modulated after 6 hours of treatment with ATP.

Our results show that ATP stimulates the transcription of 3 growth factors (**KGF**, HGF, IGF1), major regulators of the anagen phase (Figure 2).

In order to confirm these gene expression induction results, the KGF content was measured in the dermal papilla cell culture supernatants.

After 24 hours of incubation, ATP induces the production of the protein KGF in the three donors studied, with an average of 37% activation (Figure 3).

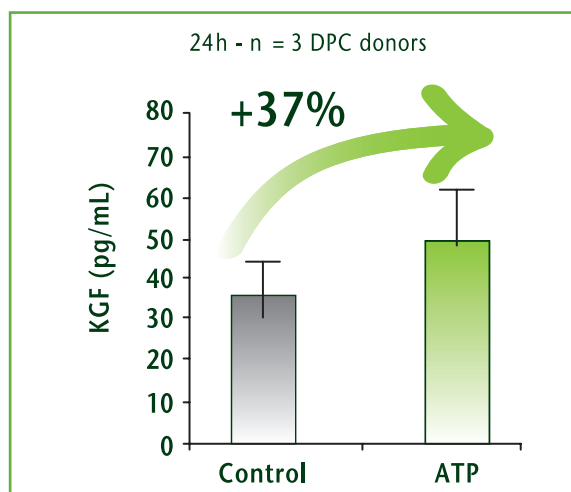
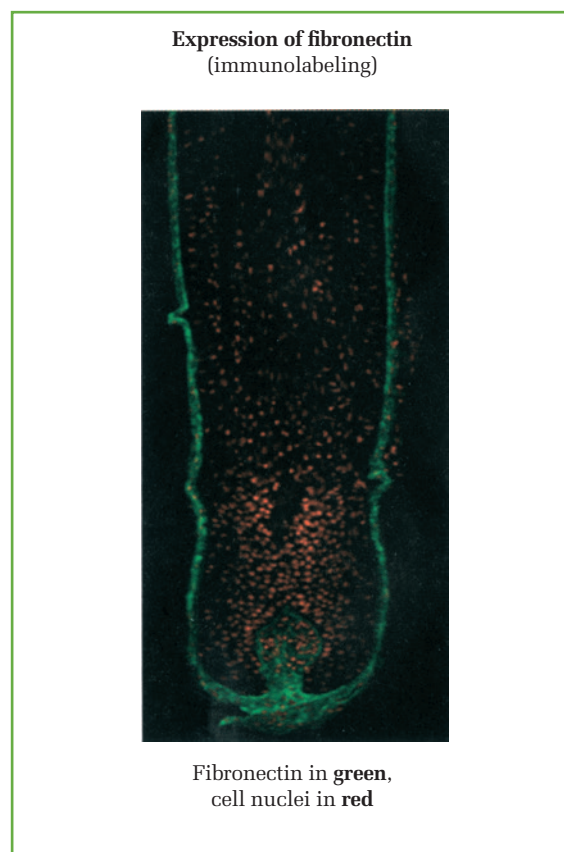


Figure 3: Measurement of the growth factor KGF in dermal papilla cell culture supernatants.

Impact of ATP on the anchoring of the hair follicle

The rate of transcription of a glycoprotein of the extracellular matrix, **fibronectin**, is also significantly increased after stimulation of the follicles by ATP (Figure 2). Fibronectin is necessary to anchor the hair follicle and promotes the aggregation of dermal papilla cells.



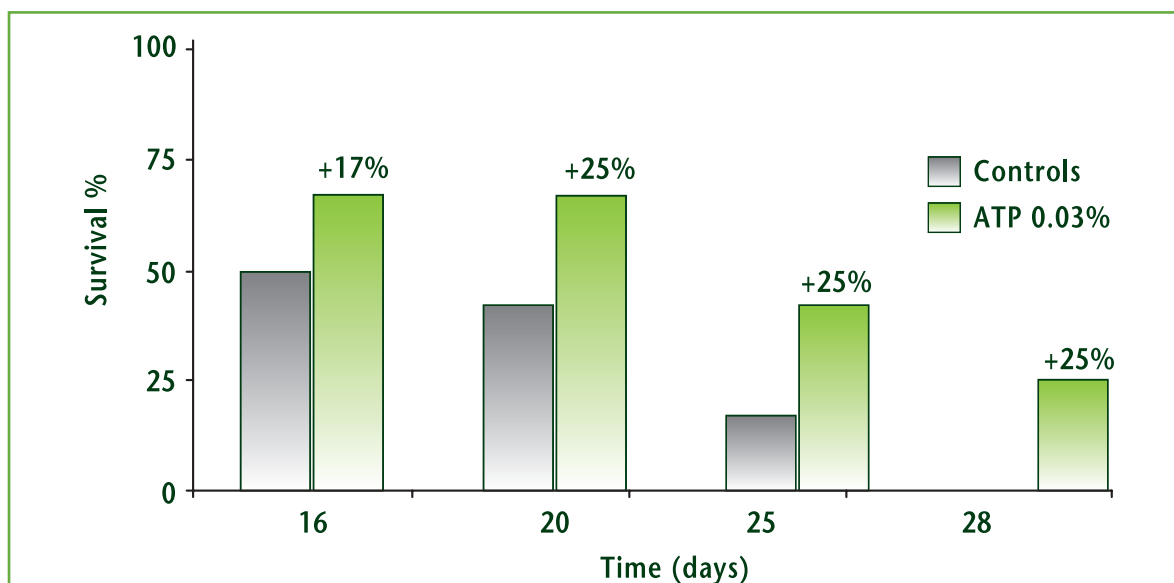
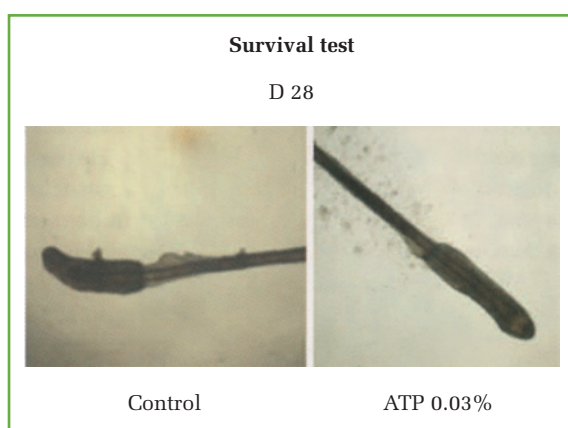


Figure 4: Survival of bulbs in culture as a function of time.

Impact of ATP on the survival of bulbs in culture

ATP inhibits the degeneration of hair bulbs throughout the study and shows an increase of 25% in survival on the last day of the study (Figure 4).

In conclusion: ATP induces the expression of genes such as KGF, IGF1, HGF or fibronectin on *ex vivo* hair follicles, stimulates the production of the protein KGF and increases the survival of the hair bulbs. This result suggests that it may slow down hair loss by prolonging the anagen phase (increase the survival and strengthen the anchoring of the follicle): an effect which should be clinically confirmed.



Efficacy and tolerability of the product TRIPHASIC^{VHT} ATP INTENSIF in androgenetic alopecia

In light of the results obtained *ex vivo*, ATP was added to René Furterer's new product TRIPHASIC^{VHT} ATP INTENSIF. Its efficacy and tolerability were assessed in an open-label, intra-individual clinical study, with each subject being his or her own control.

Study protocol

Inclusion criteria:

Included in this study were male Caucasian volunteers aged over 18, with phototypes I to IV according to the Fitzpatrick classification, with light or medium brown hair, with a hair density of 150 hairs/cm², and confirmed hair loss (stage III to IV on the Hamilton-Norwood scale), a proportion of telogen hairs greater than or equal to 20% and having signed a written consent form.

The subjects were supposed to use the René Furterer's TRIPHASIC^{VHT} ATP INTENSIF twice a week in the first month and then once a week for the next two months.

Non-inclusion criteria:

Excluded from the study were subjects with gray or blond hair in the area studied, with known allergies to one of the product ingredients, with skin lesions on the scalp (eczema, seborrheic dermatitis, psoriasis, etc.), subjects included in another study protocol, receiving topical or systemic treatment which might affect hair growth in the 6 months prior to inclusion.

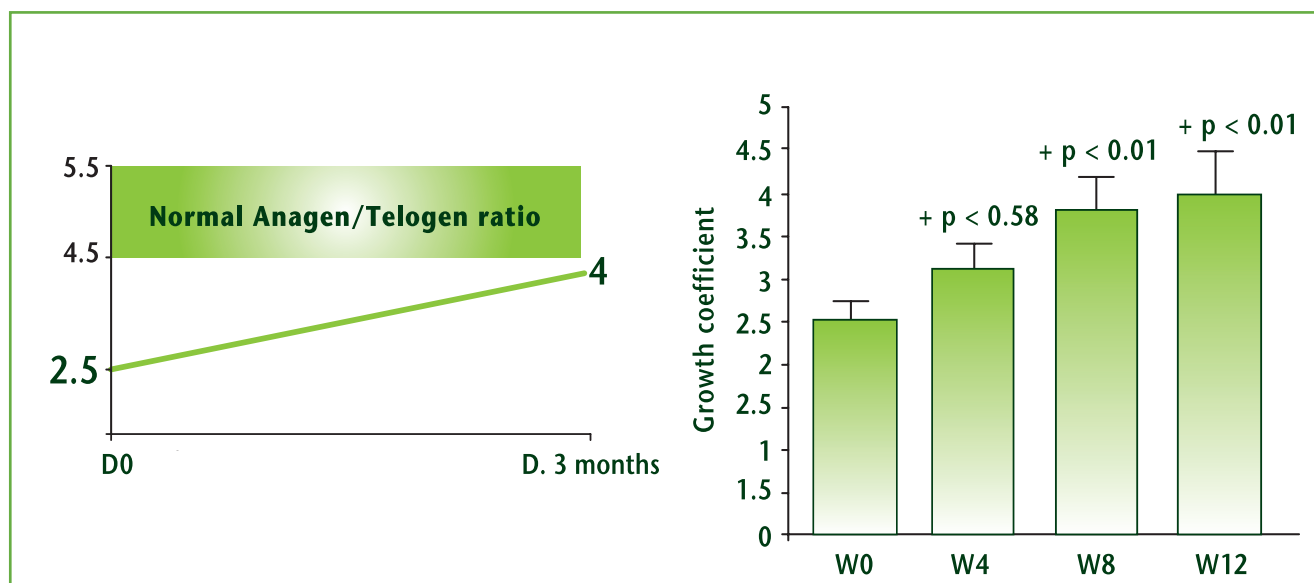


Figure 5: Hair growth coefficient.

Assessment criteria:

The primary **assessment criterion was the assessment of the anti-hair loss efficacy** of the product after 4, 8 and 12 weeks of use of the product. This assessment is based on the reference method, the **phototrichogram**, performed on D0 and D2 for each assessment period in order to differentiate hair in the growth phase (anagen) from hair in the shedding phase (telogen) on an area of shaved head measuring 0.7 cm². **Hair density** (number of hairs/cm²), the number and proportion of **hairs in the anagen phase** (A) and in the telogen phase (T) (number of hairs/cm² and percentage, respectively) were assessed and the hair growth coefficient (**A/T ratio**) calculated after 4, 8 and 12 weeks of use of the product.

The secondary assessment criteria were:

- skin tolerance: the objective criteria assessed by a dermatologist (dandruff, seborrhea, erythema), and the subjective criteria perceived by the subjects (tightness, itching, tingling, burning sensation) were assessed according to a five point scale (none, very slight, slight, moderate, severe) after 4, 8 and 12 weeks of use of the product,
- the organoleptic characteristics and perceived effectiveness were assessed by the subject via an acceptability questionnaire.

Results

The subjects:

31 subjects (average age: 28 years) were included in this intra-individual open label study. The results were calculated in the 19 subjects who complied with the entire study protocol.

Primary assessment criterion: product effectiveness

A significant decrease was shown in the density of hair in the telogen phase: Decrease of 11% at W4 (p = 0.011), 24% at W8 (p = 0.003) and 23% at W12 (p = 0.006).

In parallel, a **significant increase was observed in the density of hair in the anagen phase:** increase of 10% at W4 (p < 0.001), 14% at W8 (p = 0.001) and 12% at W12 (p = 0.002).

Overall hair density increased significantly starting from 4 weeks of use of the product.

The hair growth coefficient, A/T ratio, **significantly changes over time**, as shown by Figure 5.

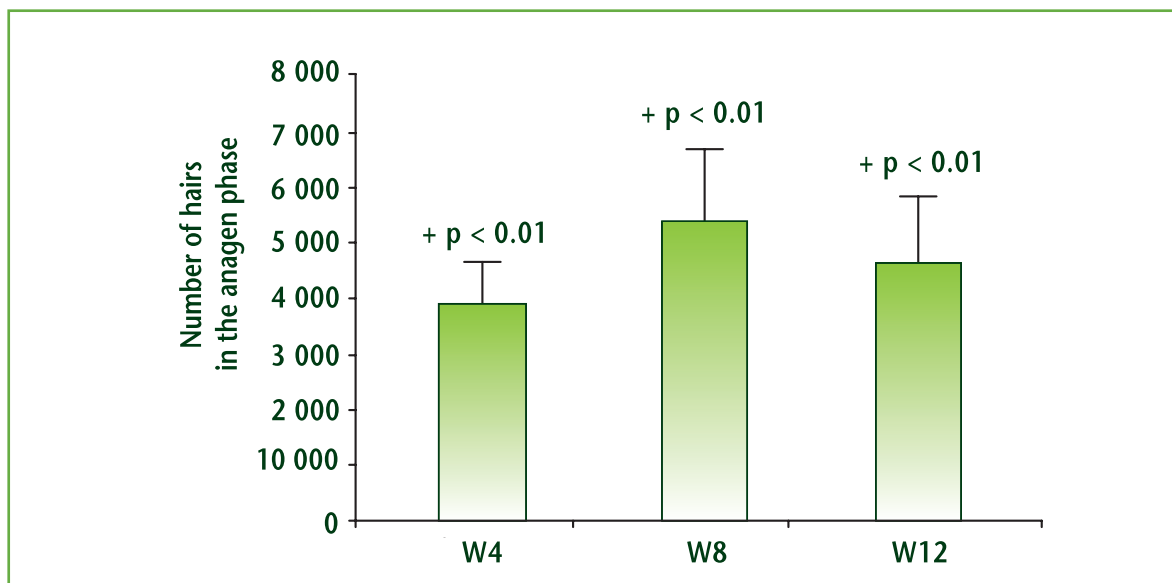


Figure 6: Number of hairs in the anagen phase.

The number of hairs in the anagen phase increases significantly starting from 4 weeks of use of the product (Figure 6): + 3,826 hairs in the anagen phase ($p < 0.001$). This number is even higher after a complete treatment, i.e. 12 weeks of use of the product:

**+ 4,583 hairs in the anagen phase
($p = 0.002$)**

Secondary assessment criteria:

Under the conditions of use of the product, tolerability was considered very good by the dermatologist.

The cosmetic appeal of the product was assessed after 4 weeks of use of the product. 80% liked the product. 86% of the subjects felt that the packaging allows the product to be dispensed well. They felt that the adaptation of the tip on the single-use containers is easy (86%), and applying the product strand by strand is easy (93%).

Item	%
Attractive color	93
Satisfying consistency	83
Light texture	93
Rapid penetration	93
Pleasant fragrance	93

The organoleptic characteristics of the products were touted:

Hair, after use of the product, was perceived as soft (83%), supple (79%), easy to style (90%), with hold (83%).

The subjects were satisfied with the condition of their hair (90%) and felt that the product respected the scalp balance (100%).

At the end of the study, after 12 weeks of use of the product:

92% of the subjects felt their hair was stronger, more robust.

81% of the subjects perceived an increase in the density of their hair.

And for 83% of them, the lotion stimulates hair growth.