

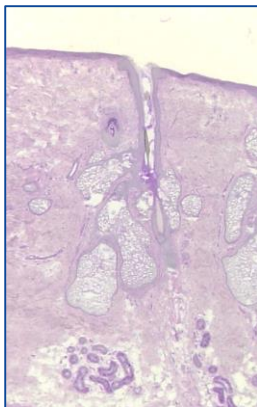
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Male and Female Pattern Hair Loss

male pattern hair loss



female pattern
hair loss



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our clients' success in
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changing skin and hair
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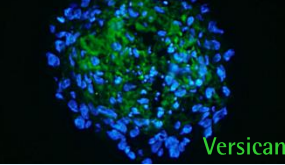
In vitro models to investigate potential beneficial effects of therapeutics on pathologic features in male and female pattern hair loss

Analysis of dermal papilla function *in vitro*

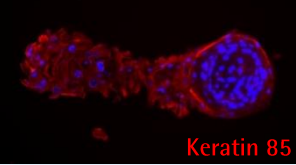
- 1) reduced dermal papilla inductivity
- 2) decreased secretion of morphogens by dermal papilla fibroblasts



Dermal papilla spheroids



Hair organoid



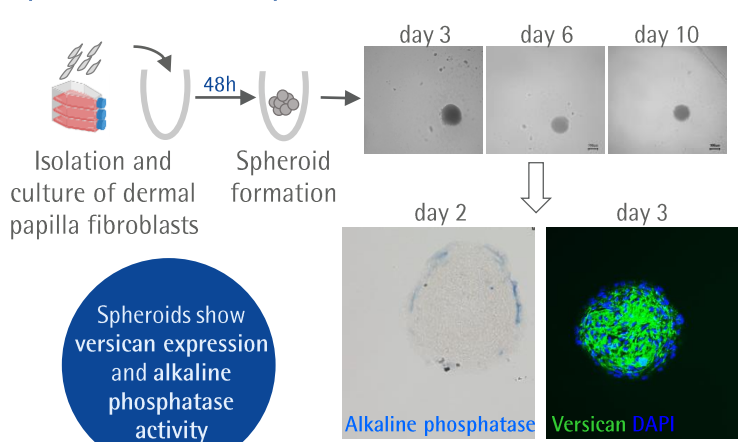
Our methods: 3D *in vitro* culture of primary dermal papilla fibroblasts spheroids (middle) or hair organoids (right)

Read-Out Parameters: Alkaline phosphatase activity (*in situ* enzymatic activity); Versican-, Noggin-, HGF expression; activation of signaling pathways involved in hair growth (e.g. Wnt/beta-catenin-, BMP-, Shh signaling);

Study Examples

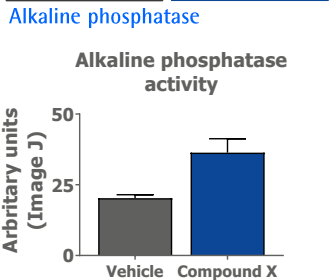
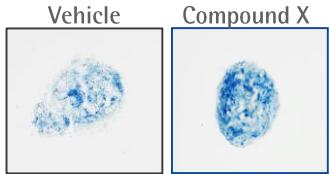
Compound X increases dermal papilla fibroblast inductivity in spheroids *in vitro*

Experimental model: Spheroid formation



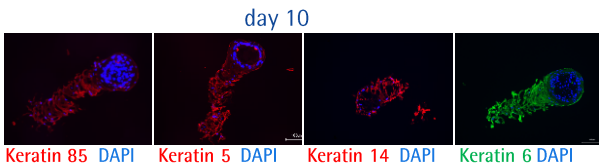
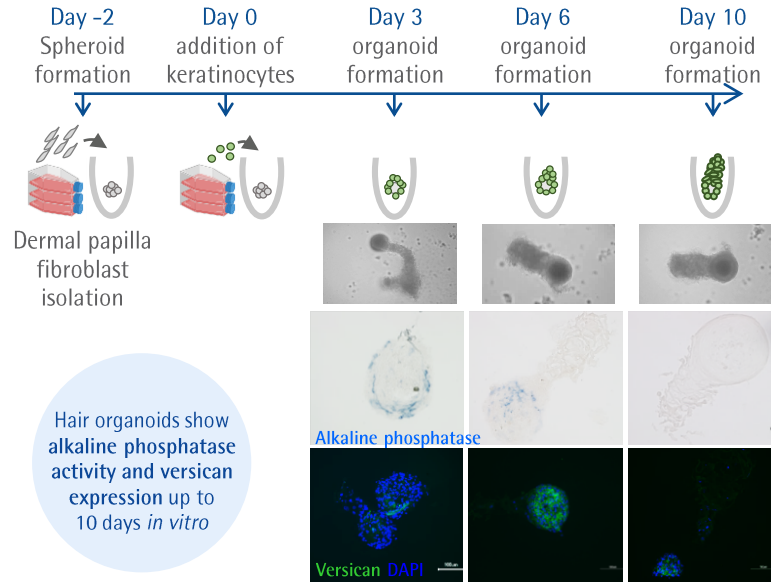
Keeping dermal papilla spheroids in culture (up to 10 days).

Alkaline Phosphatase and Versican expression: Marker for dermal papilla inductivity



Mean±SEM, n=2-4 dermal papilla spheroids/group from 1 donor

Hair organoids maintain dermal papilla characteristics *in vitro*



Spheroids and organoids allow for comprehensive compound testing *in vitro* ...

... and provide a valid model to investigate complex 3D cell-cell interactions

Organoids express keratins after 10 days *in vitro*

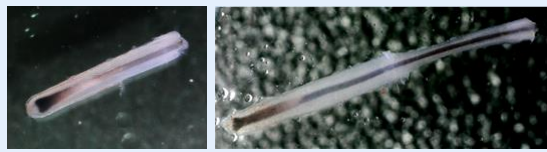
Bertolini et al. *Stem Cell Biol and Reg Med* (2022); Piccini et al. *J. Invest. Dermatol. Suppl.* (2019); Hawkshaw et al., *PloS Biol* (2018); Ahmed et al., *J Cell Biol.* (2014); Samuelov et al., *J Invest Dermatol* (2012)

Ex vivo models to investigate potential beneficial effects of therapeutics on pathologic features in male and female pattern hair loss

Analysis of healthy human hair follicle functions ex vivo

- 1) Hair shaft production
- 2) Hair cycle analysis
- 3) Dermal papilla fibroblast emigration
- 4) Reduced dermal papilla fibroblast inductivity
- 5) Reduced stem cell activity and progeny

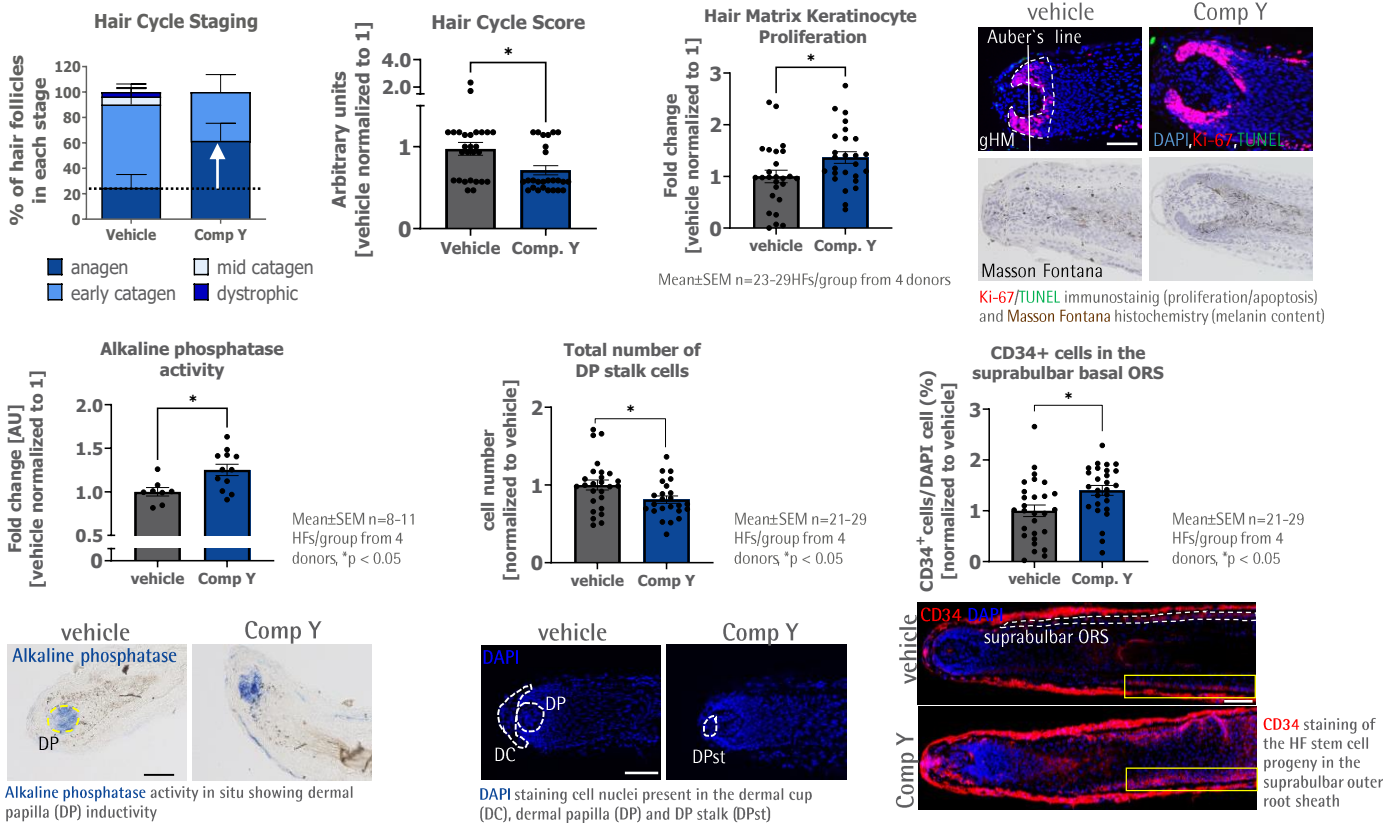
Piccini et al., *Nutrients* (2022); Campiche et al., *Int J Cosmet Sci.* (2022); Mardaryev et al., *J Invest Dermatol* (2021); Bertolini et al., *Br J Dermatol* (2021); Lisztes et al., *J Invest Dermatol* (2020); Alam et al., *Br J Dermatol* (2020); Chéret et al., *Nat Commun* (2018)



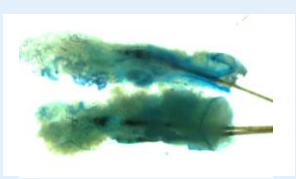
Our methods: Isolation and culture of healthy hair follicles ex vivo. Amputated microdissected hair follicle at day 0, after isolation (left). Amputated microdissected hair follicle at day 6 of organ culture with newly formed hair shaft and outer root sheath (right)

Study Examples

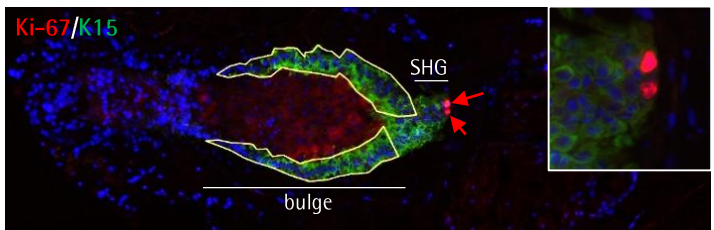
Compound Y prolongs anagen phase by enhancing DP inductivity, reducing DP fibroblast emigration and increasing HF stem cell progeny in female hair follicles ex vivo



Isolation of telogen hair follicles from healthy donors



Our methods: isolation of telogen hair follicles from healthy donors (methylene blue staining).



Bulge area of telogen hair follicle. **Ki-67**: proliferation marker **K15**: stem cell marker **SHG**: secondary hair germ

- 1) Hair follicle stem cell proliferation
- 2) Expression of Wnt ligands
- 3) Analysis of signalling pathways associated with anagen induction

Ex vivo models to investigate potential beneficial effects of therapeutics on pathologic features in in male and female pattern hair loss

Analysis of healthy human scalp skin functions ex vivo

- 1) Hair cycle analysis
- 2) Hair follicle miniaturization
- 3) Dermal papilla fibroblast emigration
- 4) Reduced dermal papilla fibroblast inductivity
- 5) Reduced stem cell activity and progeny



Our methods: human scalp skin ex vivo culture (left); different drug delivering modes are possible, including topical (middle), systemic (right) or intradermal (not shown) application.

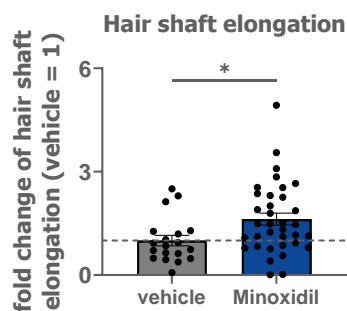
Read-Out Parameters: Hair follicle growth, hair shaft elongation, anagen (growth phase) maintenance, catagen (regression phase) induction, proliferation and apoptosis of hair matrix keratinocytes, dermal papilla fibroblast emigration, activation of signaling pathways involved in hair growth; production of hair follicle morphogens, ...

Edelkamp et al., *Skin Pharmacol Physiol* (2023); Alam et al., *Br J Dermatol* (2020) Hawkshaw et al., *PLoS Biol* (2018); Ahmed et al., *J Cell Biol* (2014); Samuelov et al., *J Invest Dermatol* (2012)

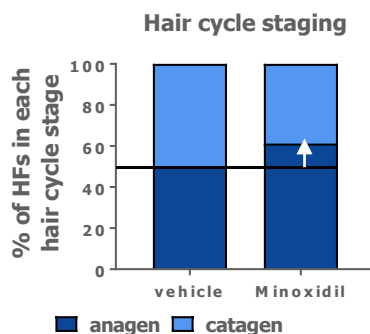
Study Examples

Topical application of Minoxidil promotes hair shaft elongation, prolongs anagen phase and induces hair matrix keratinocyte proliferation ex vivo

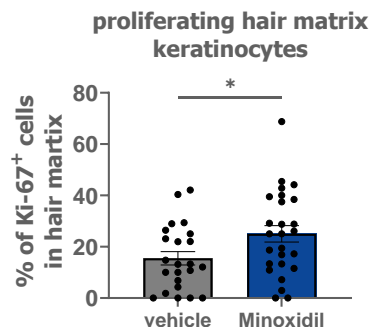
Minoxidil:
FDA approved
drug



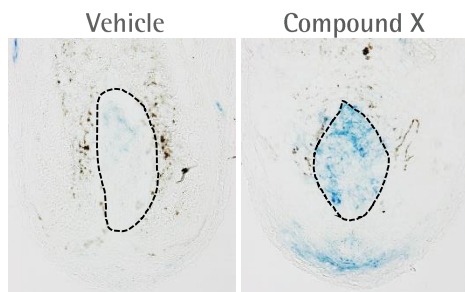
Mean±SEM, n=19-25 hair follicles/group from 2 donors, Mann Whitney test, *p<0.05.



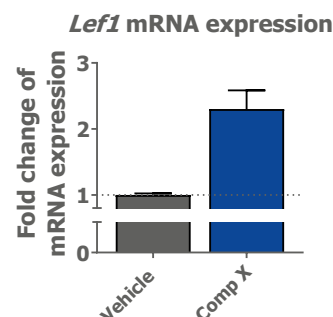
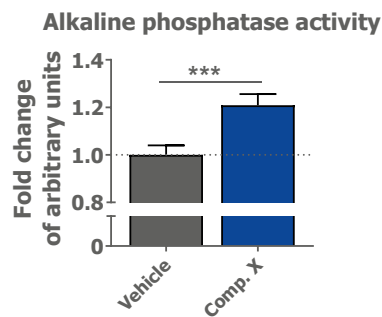
Mean±SEM, n=24-26 hair follicles/group from 2 donors, Mann Whitney test, *p<0.05.



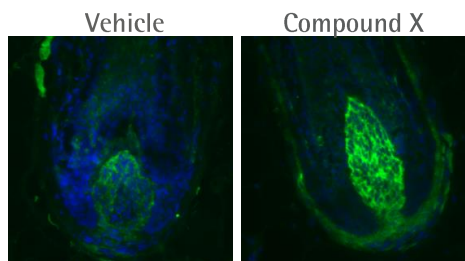
Compound X increases dermal papilla fibroblasts inductivity ex vivo and stimulates Wnt signaling



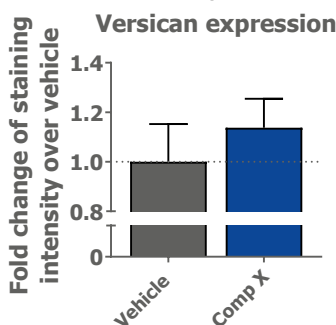
Alkaline phosphatase



Mean±SEM, n= 3-12 HF/group from 3 donors.



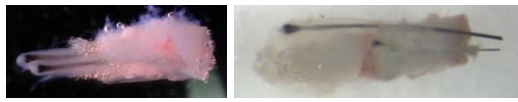
Versican DAPI



Mean±SEM, n=16-22 hair follicles/group from 1 or 3 donors, Mann Whitney test, ***p<0.001.

Investigating and characterizing affected and non-affected hair follicles from male pattern hair loss patients

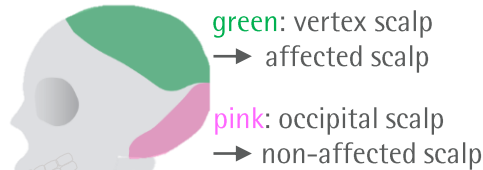
Analysis of lesional scalp skin samples or follicular units from AGA patients *in situ*



Our methods: isolation of hair follicular units from healthy donors (left) or isolation of follicular unit from balding area of AGA patients (right)
→ induction of hair follicle dysfunction (+/- testing compound)

Read-Out Parameters:

Hair follicle miniaturization characterized by the number of fibroblasts in the dermal papilla, the dermal cup and dermal stalk; size of the dermal papilla; size of the hair bulb; activation of signaling pathways involved in telogen-to-anagen conversion, transcriptome and proteome analysis, cytokine release, ...

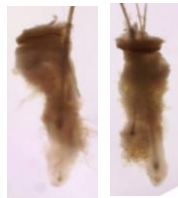


Characterization of hair follicle miniaturization

Occipital scalp (non-affected)

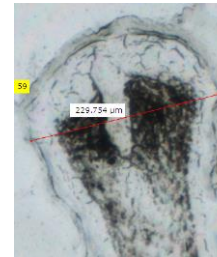


Vertex scalp (affected)

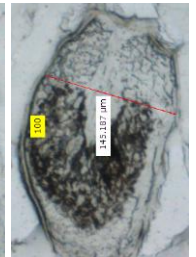


Organ-culture (up to 7 days)

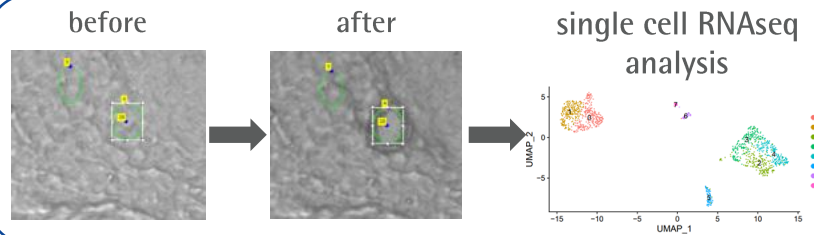
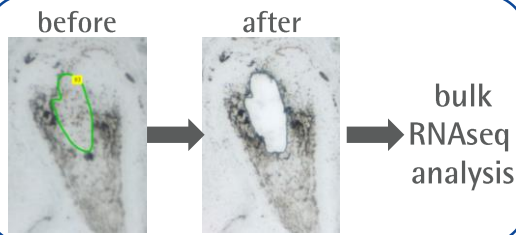
terminal HF (non-affected)



intermediate HF (affected)

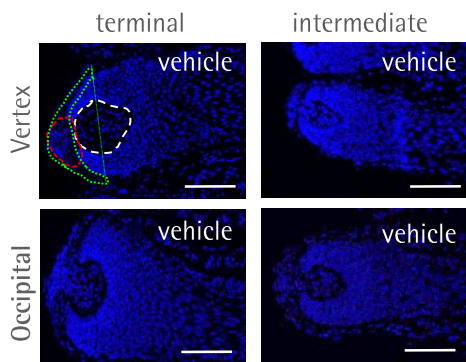


Laser Capture Microdissection (LCM) to select hair follicle compartments or single cells

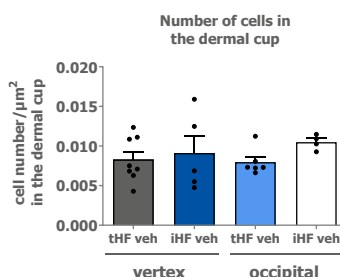
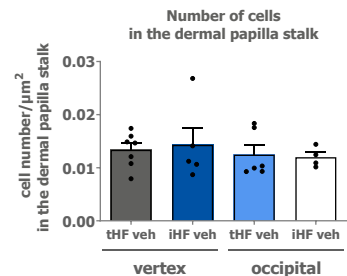
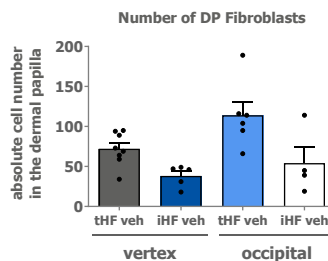


Study Example

Ex vivo organ culture of affected hair follicles to examine emigration of dermal papilla fibroblasts



White dotted line: dermal papilla
Red line: dermal papilla stalk
Green dotted line: dermal papilla cup



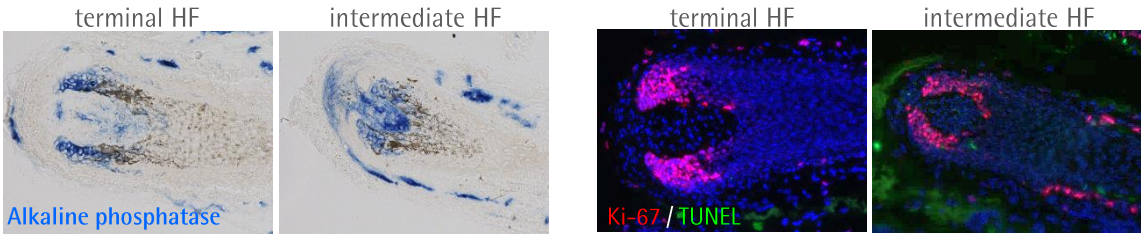
Mean±SEM n=4-8 HF's analysed/group.

Investigation and quantification of hair follicle miniaturization allows for comprehensive testing of your compound

Investigating the effects of therapeutics in affected and non-affected hair follicles from male pattern hair loss patients

Study Example

Effect of testosterone treatment on affected terminal vs. intermediate hair follicles



→ Investigate the effect of testosterone on affected HFs by analysis of dermal papilla and dermal cup cell number, changes in the hair cycle, effect on dermal papilla inductivity, changes in gene expression (bulk RNAseq, *in situ* hybridization), and changes in secretory profiles (ELISA)

Investigating and characterizing the metabolomic phenotype of affected and non-affected hair follicles from female pattern hair loss patients

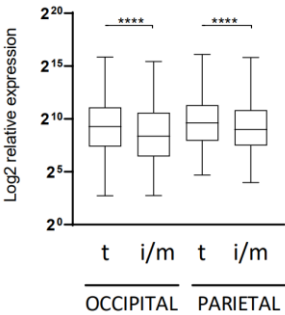
Study Example

Intermediate Hair Follicles from Patients with Female Pattern Hair Loss Are Associated with Nutrient Insufficiency and a Quiescent Metabolic Phenotype

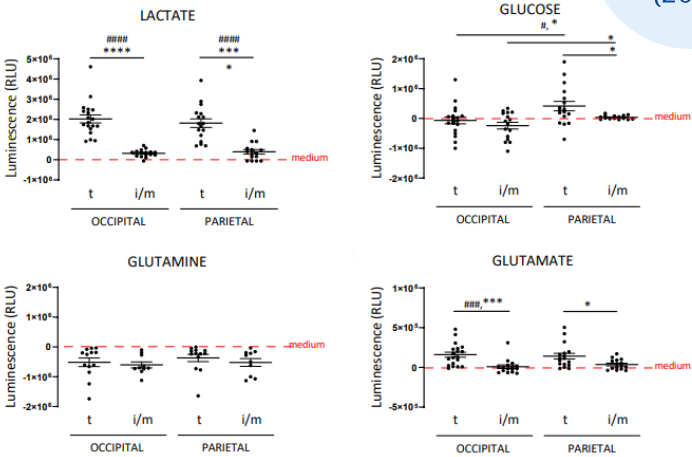
1) Intermediate HFs have a significantly lower abundance of metabolites, analyzed by UPLC-MS

2) Intermediate FPHL HFs have a lower metabolic activity *ex vivo*

Piccini et al.
Nutrients
(2022)

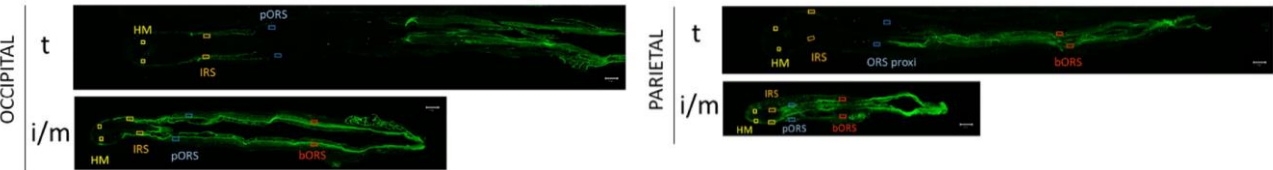


Log2 average relative expression of n=141 identified metabolites in at least 2 patients in terminal (t) and intermediate/miniaturized (i/m) hair follicles from occipital and parietal scalp skin samples from n=3 FPHL donors.



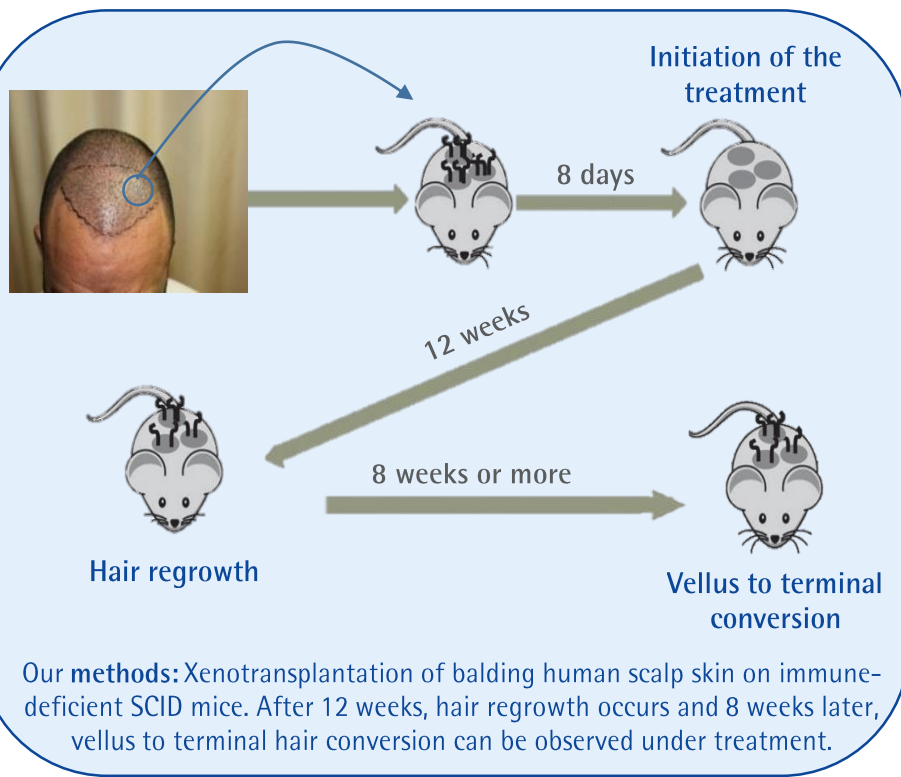
3) Intermediate FPHL HFs are able to absorb fluorescent labeled metabolites *ex vivo*

Pantothenic acid-FAM



Terminal hair follicles (t) from FPHL occipital and parietal scalp skin and of intermediate/miniaturized hair follicles (i/m) from FPHL parietal scalp skin absorb Pantothenic acid-FAM (green).

Pre-clinical Research: Humanized mouse model for male and female pattern hair loss



long-term
in vivo
observations
allow for ...

... unique
investigation of

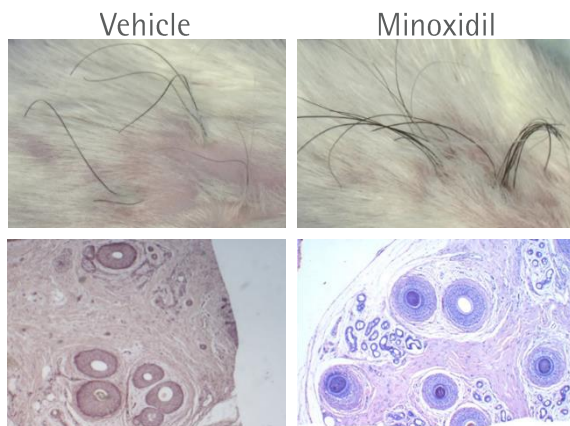
- 1) Hair regrowth
- 2) Vellus to terminal conversion

Analysis of
preventive or
therapeutic effects
of test agents on hair
physiology and
pathology
in vivo

Read-Out Parameters: Number of hairs per xenograft, terminal to vellus ratio, anagen to telogen ratio...

Study Example

Validation of the humanized mouse model for male pattern hair loss using Minoxidil to induce hair growth

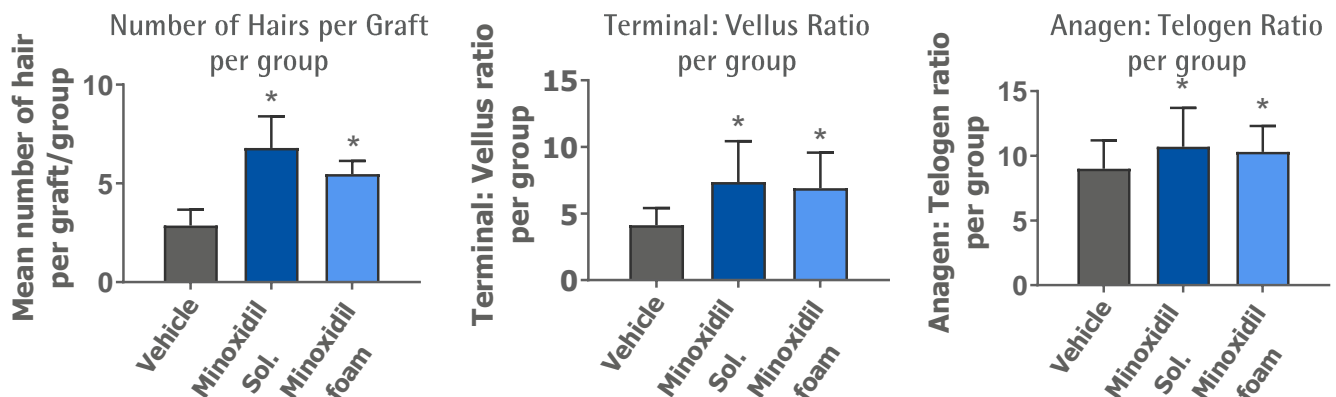


highly
clinically
relevant

quantifiable
read-out
parameters

Selection of our
publications:

Gilhar et al., *Acta Derm. Venerol* (2023);
Gilhar et al., *Exp. Dermatol* (2022);
Laufer Britva et al., *Br J Dermatol* (2021)



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Strong academic background & publication record

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- Establish novel cutting edge methodologies and techniques
- Design tailor-made & customized assays for all needs
- Identify, characterize, or validate novel targets and therapeutics for skin & hair disorders
- Discover mechanistic action stories, biomarkers & predictors of response
- Conduct investigator initiated skin & hair clinical trials
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