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## A novel nondrug SFRP1 antagonist inhibits catagen development in human hair follicles *ex vivo*

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DEAR EDITOR, Wnt signalling is one of the most fundamental molecular pathways for modulating the human hair cycle and is essential for maintaining hair growth.<sup>1</sup> We recently identified that the Wnt antagonist, secreted frizzled related protein 1 (SFRP1), prematurely induces catagen in microdissected human scalp hair follicles (HFs) *ex vivo*.<sup>1</sup> SFRP1 is inhibited by the potent hypertrichosis-inducing immunosuppressant ciclosporin A (CsA), and the specific SFRP1 antagonist WAY-316606, both of which prevent spontaneous catagen development.<sup>1</sup> Collectively, this establishes SFRP1 antagonists as attractive candidate therapeutics for management of hair loss disorders.

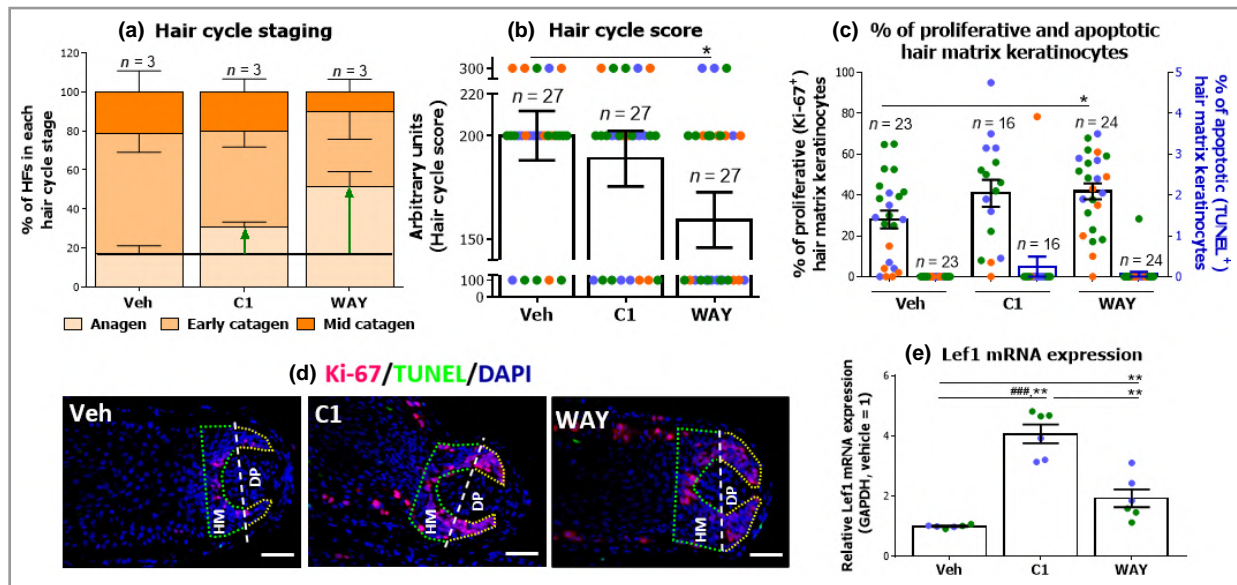
However, the immunosuppressive potency and overall toxicity of CsA<sup>2</sup> prohibit its long-term application for hair loss indications, while the hair-growth-promoting candidate WAY-316606<sup>1</sup> awaits licensing for clinical use. Therefore, it would be optimal to develop topically applicable, nondrug SFRP1 antagonists, the long-term application of which in cosmetic formulations is expected to limit agent toxicity. Therefore, we have explored a group of potentially cosmetically applicable imino-oxothiazolidine derivatives that are known to inhibit SFRP1 activity *in vitro*<sup>3</sup> as candidate human hair growth promoters.

First we evaluated the toxicological profile of selected imino-oxothiazolidine derivatives<sup>3</sup> using VEGA, a quantitative *in silico* platform that predicts a range of compound toxicity parameters. This revealed compound C1 – methyl 2-[[2-(2-imino-4-oxo-thiazolidin-5-yl)acetyl]amino]-4,5-dimethylthiophene-3-carboxylate<sup>3</sup> – to be the most favourable derivate due to the lack of toxicological alerts. C1 was next assessed for its safety in dermatological applications. Specifically, *in vitro* assays determined that C1 is not a skin irritant (OECD test method 439), ocular irritant (OECD 492) or sensitizer (RHE IL-18).<sup>4</sup>

Next, genotoxic evaluation *in silico* (VegaNIC version 1.0.8; www.vegahub.eu) and in three different *in vitro* tests (Ames test, micronuclei assay and comet assay)<sup>4</sup> demonstrated that C1 is not predicted to be genotoxic. Frog embryo teratogenesis assay-Xenopus (FETAX)<sup>4</sup> was also performed to exclude effects on embryogenesis, and gave a no observable adverse effect level (NOAEL) of 50 µmol L<sup>-1</sup>. Finally, we assessed whether C1 is immunosuppressive, in terms of inhibiting T-cell proliferation and interleukin-2 production, two well-established key downstream indicators of CsA-associated immunosuppression. This showed that C1 had a NOAEL of 45 µmol L<sup>-1</sup>. Furthermore, with a molecular weight of 341.4 Da and logP = 0.6, C1 is expected to be minimally absorbed systemically and not accumulating.<sup>5</sup> Collectively, these data suggest that C1 is suitable for cosmetic applications. All toxicology and dermatological safety data are available upon request.

Next we determined whether C1 (15 µmol L<sup>-1</sup>)<sup>3</sup> prolongs anagen in microdissected, organ-cultured human occipital/temporal scalp HFs cultured over 5–6 days<sup>1,2</sup> compared with the vehicle control (0.1% dimethylsulfoxide) and using WAY-316606 (2 µmol L<sup>-1</sup>) as a positive control.<sup>1</sup> Standardized quantitative (immuno)histomorphometry<sup>1</sup> revealed that a higher percentage of HFs remained in anagen VI after treatment with either C1 or WAY-316606, compared with the vehicle control (Figure 1a). Despite the substantial inhibition of catagen development exerted by C1, only treatment with WAY-316606 significantly reduced the hair cycle score (Figure 1b).

To validate this effect, we quantified the proliferation and apoptosis of hair matrix keratinocytes (HMKs) using quantitative Ki-67/TUNEL immunohistomorphometry.<sup>1,2</sup> The percentage of Ki-67<sup>+</sup> germinative HMKs tended to increase after C1 treatment compared with vehicle control, and to a similar extent to that of WAY-316606 (Figure 1c). Conversely, there



**Figure 1** Compound C1 stimulates Wnt signalling and prolongs anagen in the human hair follicle (HF). Microscopic quantification of hair cycle staging (a) and hair cycle score (b) (arbitrary assignment of a score to HFs in anagen = 100, early catagen = 200, mid catagen = 300) using Masson–Fontana (images not shown) and Ki-67/TUNEL. Mean  $\pm$  SEM, 27 HFs per experimental group from three independent experiments (donors). (c) Quantification of Ki-67<sup>+</sup> and TUNEL<sup>+</sup> cells in the hair matrix. Mean  $\pm$  SEM, 16–24 HFs per experimental group from three independent experiments (donors). (d) Representative images of Ki-67/TUNEL immunofluorescence showing reference areas for evaluation of Ki-67 (yellow line) and TUNEL (yellow and green lines) and Auber's line (white line). DAPI, 4',6-diamidino-2-phenylindole. Scale bar = 50  $\mu$  m. (e) Quantitative reverse-transcription polymerase chain reaction analysis of LEF1 mRNA. Mean  $\pm$  SEM, three technical replicates per donor from two independent experiments (donors). RNA was isolated from eight HFs per experimental group per donor. Kruskal–Wallis test and Dunn's multiple comparison test or one-way ANOVA and Tukey's multiple comparison test, \*\*\* $P$  < 0.001, and Mann–Whitney test or Student's  $t$ -test, \* $P$  < 0.05, \*\* $P$  < 0.01. DP, dermal papilla; HM, hair matrix; Veh, vehicle; WAY, WAY-316606.

was no significant change in the percentage of TUNEL<sup>+</sup> cells within the hair matrix of treated HFs (Figure 1d), validating our previous report that SFRP1 inhibition does not affect apoptosis in human HFs.<sup>1</sup> This observation also corroborates our C1 toxicology data, as we did not detect indicators of HF cytotoxicity, such as enhanced apoptosis and reduced proliferation of HMKs (Figure 1c), or disruption of the HF pigmentary unit (data not shown), which are typically seen with cytotoxic compounds.<sup>6</sup>

As SFRP1 is a prominent inhibitor of Wnt activity in the human hair matrix,<sup>1</sup> we finally assessed whether C1 increases Wnt activity. Six-hour treatment with C1 significantly increased the transcription of the Wnt target gene, LEF1,<sup>1</sup> *ex vivo*, and to an even greater extent than WAY-316606 (Figure 1e), indicating that C1 indeed stimulates Wnt activity in the human HF.

The fact that C1 is not as beneficial as WAY-316606 in positively regulating the human hair cycle *ex vivo*, despite the higher stimulation of Wnt signalling, may be related to the short *ex vivo* treatment, a suboptimal concentration, or the importance of fine-tuning Wnt signalling during regeneration processes.<sup>7</sup>

Collectively, we unveil a new cosmeceutically applicable SFRP1 antagonist, the Wnt-activating imino-oxothiazolidine derivative, C1,<sup>3</sup> which prolongs anagen and stimulates HMK

proliferation *ex vivo*. Given the favourable toxicological profile of C1, its topical application therefore deserves to be clinically explored as an adjuvant cosmetic therapy for hair loss disorders associated with premature catagen development leading to telogen effluvium, such as androgenetic alopecia.<sup>8</sup>

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