Table 1 Sonographic features of cutaneous leishmaniasis

Sonographic features	
Diameter [mm]	9.5 ± 5.6 [1.96, 22.6]
Under skin thickness [mm]	7.88 ± 3.25 [2.77, 14.63]
Depth ulcer [mm]	0.65 ± 0.38 [0.2, 1.54]
Type of predominant involvement of the hypodermis	
Septal	55%
Lobular	45%
Echogenicity dermis	
Isoechoic	5%
Hypoechoic	90% [18 of 20]
Hyperechoic	5%
Type of alteration of the dermis	
Irregular	95%
Band-like	5%
Echogenicity hypodermis	
Isoechoic	–
Hypoechoic	10%
Hyperechoic	90%

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A.C. Saavedra,[1] B.M. Valencia,[2] P. Tueros,[3] X. Wortsman,[4,5,6] A. Llanos-Cuentas,[7,8] R.J. Lavarello[1,*]

[1]Laboratorio de Imágenes Médicas, Departamento de Ingeniería, Pontificia Universidad Católica del Perú, Lima, Peru, [2]Viral Immunology Systems Program, Kirby Institute, University of New South Wales, Sydney, NSW, Australia, [3]División de Diagnóstico por Imágenes, Hospital Nacional PNP Luis N. Saenz, Lima, Peru, [4]Institute for Diagnostic Imaging and Research of the Skin and Soft Tissues-IDIEP, Santiago, Chile, [5]Department of Dermatology, Universidad de Chile, Santiago, Chile, [6]Department of Dermatology, Pontificia Universidad Catolica de Chile, Santiago, Chile, [7]Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, [8]Facultad de salud Pública y Administración, Universidad Peruana Cayetano Heredia, Lima, Peru

*Correspondence: Roberto J. Lavarello. E-mail: lavarello.rj@pucp.edu.pe

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Preclinical evidence that the PPARγ modulator, *N*-Acetyl-GED-0507-34-Levo, may protect human hair follicle epithelial stem cells against lichen planopilaris-associated damage

Editor

Permanent hair loss in lichen planopilaris (LPP) results from the depletion of keratin 15 ($K15^+$) epithelial stem cells (ESCs) localized in the bulge of hair follicles (HFs) that have lost their physiological immune privilege (IP), were attacked by a cytotoxic $CD8^+$ T-cell-driven inflammatory infiltrate and have undergone apoptosis or pathological epithelial–mesenchymal transition (EMT).[1–4] Currently, only palliative off-label treatments exist that reduce symptoms and slow down hair loss progression, but do not reliably and effectively stop the latter without unacceptable adverse side-effects.[5] One example is pioglitazone (oral administration),[5,6,7] a peroxisome proliferator-activated receptor (PPAR)γ agonist.[6]

Previously, we have shown that a new PPARγ modulator[8] with agonistic activity developed by the sponsor of this study (Nogra Pharma Ltd., Dublin, Ireland), *N*-Acetyl-GED-0507-34-Levo (NAGED),[2,9,10] is of interest in LPP management, since it stimulates the expression of the stem cell-associated keratin, K15[9], and protects/partially rescues HFeSCs from experimentally-induced EMT[2], in 'clinically healthy' human scalp HFs *ex vivo*. Moreover, NAGED can partially reverse the EMT signature in the bulge of lesional LPP HFs *ex vivo*.[2] Therefore, we have investigated in the current pilot study whether NAGED interferes with other key events involved in LPP development,[3] by treating organ-cultured lesional scalp skin from two LPP patients showing lymphocytic inflammatory cell infiltrates in/around the isthmus (Fig. 1a) with vehicle or 0.1 mmol/L NAGED.[2,9]

This showed that the number of $K15^+$ HFeSC, and K15 protein expression, is increased in the bulge of lesional LPP HFs compared with vehicle HFs (Fig. 1b). This preliminary

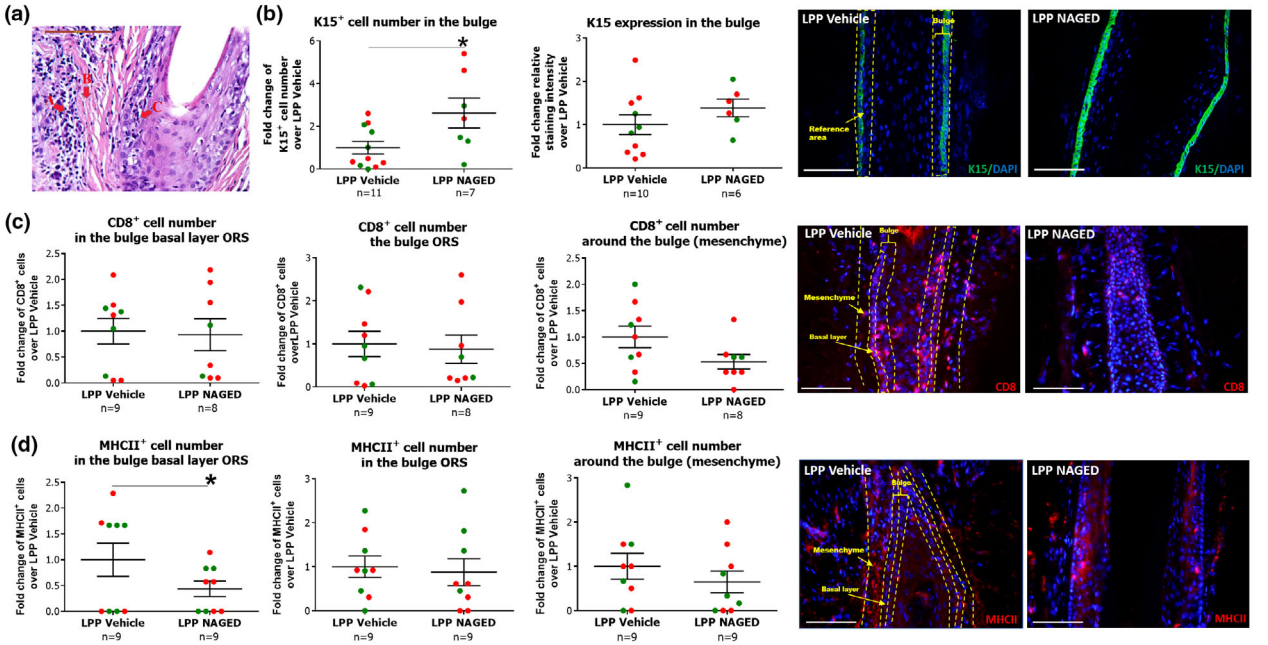

Figure 1 NAGED re-enforces K15$^+$ HFeSC pool, reduces intra- and perifollicular inflammation, and may restore immune privilege in the bulge of lesional LPP HFs *ex vivo*. (a) Representative image of Haematoxylin and Eosin histochemistry in lesional skin from one LPP patient showing inflammatory lymphocytic infiltrate (arrow labelled with A), perifollicular fibrosis (arrow labelled with B) and lichenoid reaction with apoptosis of ORS cells (arrow labelled with C). (b) Quantitative analysis of K15positive cell number and expression in the bulge region of lesional LPP HFs treated either with vehicle, or with 0.1 mmol/L NAGED, and representative images showing the reference area for the evaluation. (c, d) Quantitative analyses of CD8 (c) and MHC class (MHC) II (d) positive cells in the basal layer of the bulge, in the outer root sheath of the bulge and around the bulge (mesenchyme) of lesional LPP HFs treated either with vehicle or with 0.1 mmol/L NAGED, and representative images showing the reference areas for the evaluation. Scattered plots reveal also that cell infiltration is *almost* equally distributed within the LPP samples, as most of the vehicle HFs within the LPP samples showed CD8$^+$ T cells, and MHC class II$^+$ cells in and/or around the bulge. Data are expressed as fold change of mean ± SEM over LPP Vehicle; n = 6–11 HFs/group from two different patients. Graph Pad Prism 6, Student's t-test, $^*P < 0.05$. Scale bars: 100 μm. ORS, outer root sheath.

observation suggests that NAGED may not only prevent, but also partially reverse the depletion of the K15$^+$ HFeSC pool in LPP patients. Importantly, NAGED treatment also decreased the number of CD8$^+$ T cells, the key pathogenic T cells in LPP,[3,4] and of MHC class II$^+$ cells[4] in/around the bulge epithelium (Fig. 1c,d), which indicates that NAGED not only reduces the inflammatory infiltrate attack on the bulge, but may also partially restore bulge immune privilege.[4]

In addition, we compared the efficiency of NAGED with that of pioglitazone[2,5,6] (both at 0.01 mmol/L) in reversing experimentally induced bulge EMT in 'clinically healthy', full-length scalp HFs of three donors *ex vivo*.[2] Consistent with our previous results,[2] HFs treated with the EMT-promoting cocktail showed a significantly increased number of vimentin$^+$ or SLUG$^+$ cells, decreased E-cadherin expression and a reduced number of K15$^+$ HFeSCs within the bulge (Fig. 2a–d). Importantly, NAGED treatment *after* EMT induction partially reversed the EMT phenotype, as indicated by a significant decrease in the number of vimentin$^+$ or SLUG$^+$ cells within the bulge, compared not only to EMT cocktail-treated HFs, but also to pioglitazone-treated, EMT-induced HFs (Fig. 2a,b). Thus, NAGED is more effective than pioglitazone in reversing experimentally induced EMT in human HFeSCs *ex vivo*. However, neither agent could counteract the EMT-induced down-regulation of E-cadherin expression, confirming previously published results,[2] nor the reduction in the number of K15$^+$ HFeSCs in the bulge (Fig. 2c,d).

While further evidence from additional LPP patients is needed for confirmation, this supports that NAGED interferes with all key phases of LPP pathobiology, that is bulge IP collapse, cytotoxic peri- and intra-bulge inflammation, pathological EMT and depletion of HFeSCs.[3] Collectively, these pilot data raise the possibility that the PPARγ modulator, NAGED, which is topically applicable and has an advantageous toxicological profile, as revealed in ongoing clinical trials for acne vulgaris and psoriasis vulgaris (EudraCT2014-005244-17,

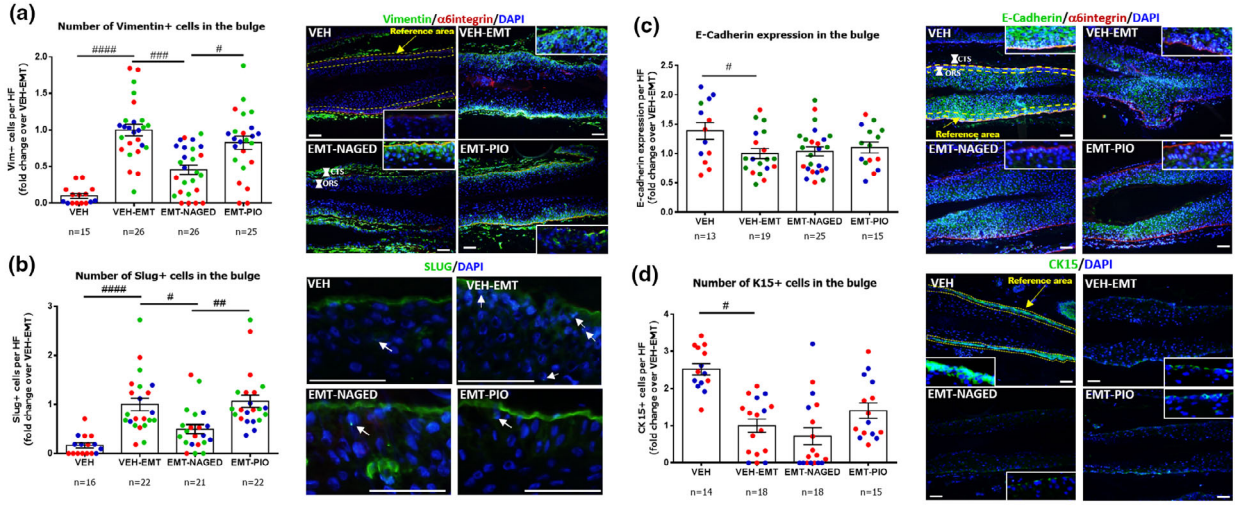

Figure 2 NAGED, but not pioglitazone, partially reverses experimentally induced EMT in 'clinically healthy' HFs *ex vivo*. (a–d) Quantitative analyses and corresponding representative images of Vimentin (a), and SLUG (b) positive cells, E-cadherin expression (c) and K15 positive cells (d) in the bulge of healthy HFs treated *ex vivo* with vehicle (VEH), EMT cocktail (VEH-EMT), EMT cocktail and 0.01 mmol/L NAGED (EMT-NAGED), or EMT cocktail and 0.01 mmol/L Pioglitazone (EMT-PIO). Data are expressed as fold change of mean ± SEM over VEH-EMT, $N = 13$–26 HFs/group from 2 to 3 different healthy donors. Graph Pad Prism 6, Kruskal–Wallis test, $P < 0.0001$, followed by Dunn's multiple comparisons test, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, $^{\#\#\#\#}P < 0.0001$. Scale bars: 100 μm.

EudraCT2016-000540-33, EudraCT2017-003796-58), may be more effective than classical PPARγ agonists (such as pioglitazone) to halt LPP progression and may even partially reverse defined aspects of LPP-associated bulge damage in HFs where the latter has not yet become irreversible.

J. Chéret,[1,2,†] I. Piccini,[1,†] J. Hardman-Smart,[3,‡] S. Ghatak,[1] M. Alam,[1,4,5,§,¶] J. Lehmann,[1] F. Jimenez,[5,6] H. Erdmann,[7] E. Poblet,[8] N. Botchkareva,[1] R. Paus,[1,2,3,†] M. Bertolini[1,†,*]

[1]Monasterium Laboratory GmbH, Münster, Germany, [2]Dr Phillip Frost Department of Dermatology & Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, FL, USA, [3]Centre for Dermatology Research, University of Manchester, and NIHR Biomedical Research Centre, Manchester, UK, [4]Mediteknia Skin & Hair Lab, Las Palmas de Gran Canaria, Gran Canaria, Spain, [5]Universidad Fernando Pessoa Canarias, Las Palmas de Gran Canaria, Gran Canaria, Spain, [6]Mediteknia Dermatology Clinic, Las Palmas de Gran Canaria, Gran Canaria, Spain, [7]Kosmed Klinik, Hamburg, Germany, [8]Department of Pathology, University General Hospital of Murcia, Murcia, Spain

*Correspondence: M. Bertolini. E-mail: m.bertolini@monasteriumlab.com

[†]Equal contribution.

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