

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%

2014 and NIH/Fogarty International Center 5R25TW009340-04 (5037874).

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,*}

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

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

References

- Alvar J, Velez ID, Bern C *et al*. Leishmaniasis worldwide and global estimates of its incidence. *PLoS ONE* 2012; **7**: e35671.
- Sharquie K, Hameed A, Noaimi A. Panniculitis is a common unrecognized histopathological feature of cutaneous leishmaniasis. *Indian J Pathol Microbiol* 2016; **59**: 16–19.
- Aronson N, Herwaldt BL, Libman M *et al*. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Am J Trop Med Hyg* 2016; **63**: e202–e264.
- Harland CC, Bamber JC, Gusterson BA, Mortimer PS. High frequency, high resolution B-scan ultrasound in the assessment of skin tumours. *Br J Dermatol* 1993; **128**: 525–532.
- Sharquie KE, Noaimi AA, Saleh BA. Cutaneous leishmaniasis as imitator of skin diseases and a diagnostic challenge. *J Cosmet, Dermatol Sci Appl* 2018; **8**: 158.

6 Sharquie KE, Hameed AF. Panniculitis is an important feature of cutaneous leishmaniasis pathology. *Case Rep Dermatol Med*, 2012; **2012**: 612434.

7 Pousa-Martínez M, Sánchez-Aguilar D, Aliste C, Vázquez-Veiga H. Usefulness of ultrasound in the diagnosis and follow-up of pyoderma gangrenosum. *Actas Dermo-sifiliograficas* 2017; **108**: 962–964.

DOI: 10.1111/jdv.16111

Preclinical evidence that the PPAR γ 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 PPAR γ modulator⁸ 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, K15⁹, and protects/partially rescues HFeSCs from experimentally-induced EMT², 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

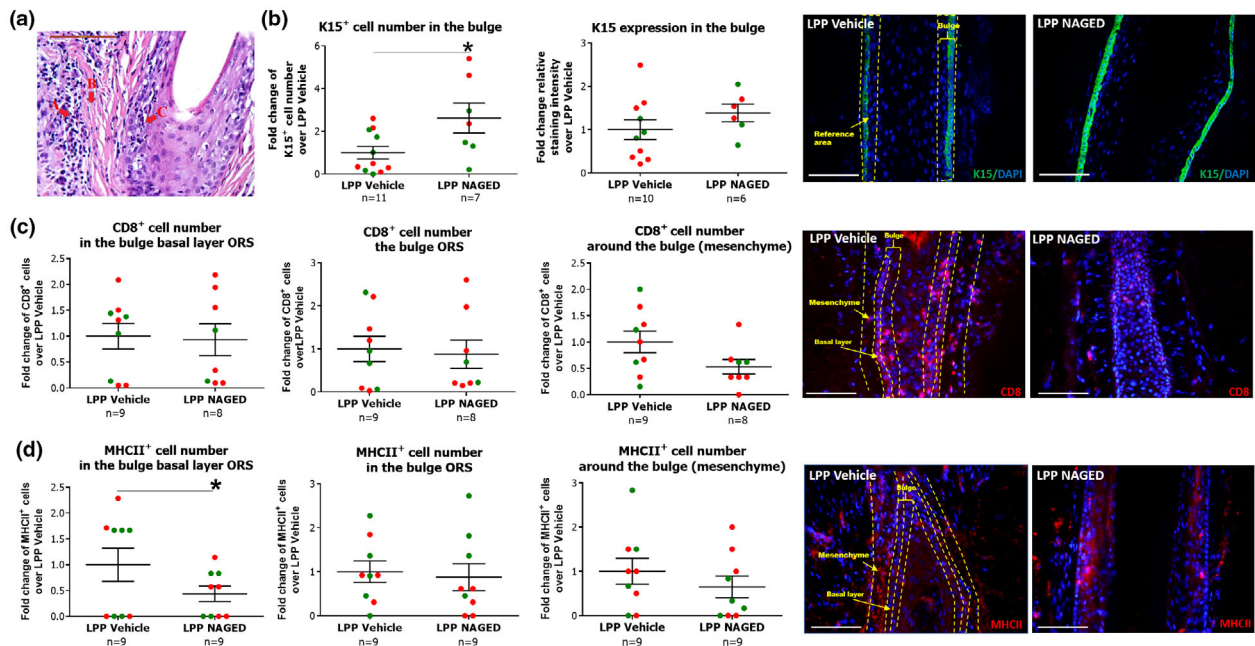


Figure 1 NAGED re-enforces K15⁺ HFeSC pool, reduces intra- and perifollicular inflammation, and may restore immune privilege in the bulge of lesional LPP HF *ex vivo*. (a) Representative image of Haematoxylin and Eosin histochemistry in lesional skin from one LPP patient showing inflammatory lymphocytic 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 HF 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 HF 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 HF 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 \pm SEM over LPP Vehicle; $n = 6$ – 11 HF/group from two different patients. Graph Pad Prism 6, Student's *t*-test, $*P < 0.05$. Scale bars: 100 μ m. 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⁺ cells⁴ 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 HF of three donors *ex vivo*.² Consistent with our previous results,² HF 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 HF, but also to pioglitazone-treated, EMT-induced HF (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 PPAR γ 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,

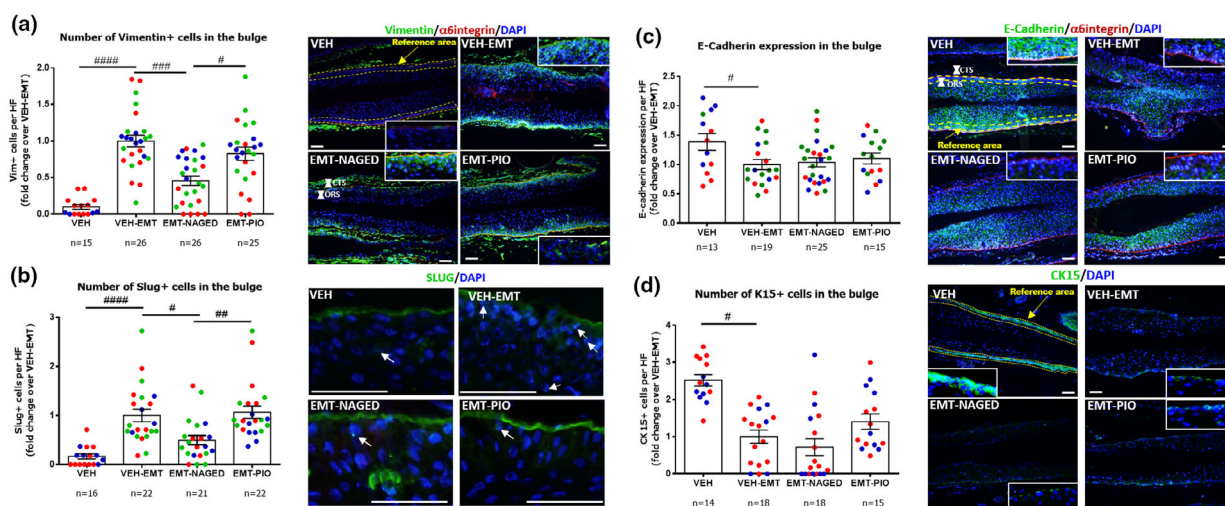



Figure 2 NAGED, but not pioglitazone, partially reverses experimentally induced EMT in ‘clinically healthy’ HF^s *ex vivo*. (a–d) Quantitative analyses and corresponding representative images of Vimentin (a), and SLUG (b) positive cells, E-cadherin expression (c) and K15 positive cells (d) in the bulge of healthy HF^s 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, $N = 13–26$ HF^s/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 PPAR γ agonists (such as pioglitazone) to halt LPP progression and may even partially reverse defined aspects of LPP-associated bulge damage in HF^s 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,†,*} 

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

*Correspondence: M. Bertolini. E-mail: m.bertolini@monasteriumlab.com
[†]Equal contribution.

References

- Bolduc C, Sperling LC, Shapiro J. Primary cicatricial alopecia: lymphocytic primary cicatricial alopecias, including chronic cutaneous lupus erythematosus, lichen planopilaris, frontal fibrosing alopecia, and Graham-Little syndrome. *J Am Acad Dermatol* 2016; **75**: 1081–1099.

- Imanishi H, Ansell DM, Chéret J *et al*. Epithelial-to-mesenchymal stem cell transition in a human organ: lessons from lichen planopilaris. *J Invest Dermatol* 2018; **138**: 511–519.
- Harries MJ, Jimenez F, Izeta A *et al*. Lichen planopilaris and frontal fibrosing alopecia as model epithelial stem cell diseases. *Trends Mol Med* 2018; **24**: 435–448.
- Harries MJ, Meyer K, Chaudhry I *et al*. Lichen planopilaris is characterized by immune privilege collapse of the hair follicle’s epithelial stem cell niche. *J Pathol* 2013; **231**: 236–247.
- Errichetti E, Figini M, Croatto M, Stinco G. Therapeutic management of classic lichen planopilaris: a systematic review. *Clin Cosmet Investig Dermatol* 2018; **11**: 91–102.
- Harnchoowong S, Suchonwanit P. PPAR- γ agonists and their role in primary cicatricial alopecia. *PPAR Res* 2017; **2017**: 2501248.
- Ramot Y, Mastrofrancesco A, Camera E, Desreumaux P, Paus R, Picardo M. The role of PPAR γ -mediated signalling in skin biology and pathology: new targets and opportunities for clinical dermatology. *Exp Dermatol* 2015; **24**: 245–251.
- Pirat C, Farce A, Lebègue N *et al*. Targeting peroxisome proliferator-activated receptors (PPARs): development of modulators. *J Med Chem* 2012; **55**: 4027–4061.
- Ramot Y, Mastrofrancesco A, Herczeg-Lisztes E *et al*. Advanced inhibition of undesired human hair growth by PPAR γ modulation? *J Invest Dermatol* 2014; **134**: 1128–1131.
- Mastrofrancesco A, Kovacs D, Sarra M *et al*. Preclinical studies of a specific PPAR γ modulator in the control of skin inflammation. *J Invest Dermatol* 2014; **134**: 1001–1011.

DOI: 10.1111/jdv.16114