Appendix SI

SUPPLEMENTARY MATERIALS AND METHODS

Patients

Patients with PN were recruited from the Department of Dermatology and Allergy, Herlev and Gentofte Hospital, University of Copenhagen, Denmark. Inclusion criteria were age above 18 years, clinically verified moderate-to-severe PN, inadequate response to local anti-inflammatory treatment and to UV therapy. Pregnant or lactating women, patients with serious infections or active psychiatric diseases were not allowed to participate in the study.

Study approval was obtained from the Danish Data Protection Agency (int. ref. HGH-0212-58-004, I-Suite 05785), the Danish Medicines Agency (ref. 2017020398, EudraCT no. 2016-003018-29), and the research ethics committees of the Capital Region of Denmark (ref. H-17003973). The study was conducted according to the Declaration of Helsinki and was registered at ClinicalTrials.gov (ref. ID. NCT03576287).

Treatment

Enrolled patients were treated with apremilast, 30 mg twice daily for 12 weeks. As recommended, dose-titration was used for the initial 6 days. At week 16, a follow-up visit was performed to monitor any relapse. Use of topical or systemic anti-inflammatory treatment for 2 and 4 weeks prior to baseline was prohibited, and also was not allowed during the trial.

Definition of efficacy and safety assessment

The primary objective of this 16-week phase II study was to evaluate the efficacy of 12 weeks' treatment of apremilast in patients with PN using the visual analogue scale (VAS) pruritus score (range 0–10). Secondary endpoints were to evaluate the efficacy of apremilast using Physician Global Assessment (PGA, range 0–4), Patient Assessed Global Assessment (PaGA, range 0–5), QoL using Dermatology Life Quality Index (DLQI, range 0–30), and Pittsburgh Sleep Quality Index (PSQI, range 0–21).

Responders were considered as those receiving the minimally important difference/minimally clinically important difference at week 12 compared with baseline, defined as a difference in VAS pruritus ≥ 3 points, in PGA ≥ 2 points, in PaGA ≥ 2 points, in DLQI ≥ 4 points, and in PSQI ≥ 3 points (S1–3).

In addition, a secondary endpoint was to evaluate changes in expression of cytokine and chemokine detected by real-time quantitative polymerase chain reaction (RT qPCR) analyses. Safety was assessed using Becks Depression Inventory (BDI, range 0–63) score. All efficacy and safety parameters were monitored at weeks 0, 2, 4, 12 and 16.

RNA purification and RT qPCR

To investigate the efficacy of apremilast on the immune system, biopsies from lesional skin were taken at baseline, weeks 4 and 12, and at each time-point analysed for RT qPCR of IL-6, IL-10, IL-17, IL-22, IL-31, IFN-ɣ, TNF-α, CCL2, and CCL3 mRNA expression.

All skin biopsies were immediately transferred to RNAlater™ stabilization solution. After 24 h at 5°C they were stored at −80°C until RNA purification. RNA extraction, cDNA synthesis, and RT qPCR analysis were undertaken by Eurofins Genomics Europe Genotyping A/S, Aarhus, Denmark. Total cellular RNA was extracted and purified from skin biopsies from baseline, week 4 and week 12, using the automated process on a QIAsymphony SP robot using the QIAsymphony RNA kit (QIAGEN, Hilden, Germany). cDNA synthesis was performed by means of the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems; ThermoFisher Scientific, Waltham, MA, USA) using 100 ng total RNA. A specific target amplification was performed using a pool of the TaqMan assays; IL-6: Hs00174131_m1; IL-10: Hs00961622_m1; IL-17: Hs00174383_m1; IL-22: Hs01574154_m1; IL-31: Hs01098710_m1; IFN-γ: Hs00989291_m1; TNF-α: Hs00174128_m1; CCL3: Hs00234142_m1: Hs00234142_m1; CCL2: Hs00234140_m1; RPLP0: Hs99999902_m1; PPIA: s99999904_m1; TBP: Hs99999910_m1 (TaqMan; ThermoFisher Scientific), which were also used in the subsequent qPCR. The pre-amplified cDNA and the assays were loaded on a 48×48 dynamic array and run under standard conditions on the Fluidigm BioMark (Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark) system according to the manufacturer's protocol. Data were analysed using Fluidigm BioMark software version 4.1.3 with linear (derivative) baseline correction and the user (detectors) method for Ct threshold settings. Mean Ct values were calculated from the raw data, along with assay standard curve linearity and amplification efficiency. mRNA levels were determined using the relative quantification method (2^-2(ΔΔCt)). An algorithm was used (NormFinder, Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark) for the validation of stability of the 3 candidate reference genes (S4). Based on this algorithm, PPIA and RPLP0 were chosen as reference genes.

Statistical analysis

Data from all included patients were evaluated and analysed. For patients who dropped out before end-of-trial, last observation was carried forward.

A statistical power calculation was not performed, as this was a proof-of-concept study to determine the anti-pruritic efficacy of apremilast in patients with PN. Descriptive analyses were presented as means ± standard deviations (SD).

Cytokine and chemokine concentrations at weeks 4 and 12 were compared with baseline concentrations by use of Wilcoxon signed-rank test. Concentration of IL-31 at week 4 and week 12 was compared with baseline concentration by use of Mann–Whitney test due to missing data for IL-31. Values of $p < 0.05$ were considered significant.

Statistical analyses were performed using SPSS (version 22.0.0.0) and GraphPad Prism version 6.07 (GraphPad Software, La Jolla, CA, USA).

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