APPENDIX S1

Supplementary Patients and methods

Study design and patient population

PSORITUS was a phase IIIb multicenter, randomized, double-blind, placebo-controlled trial which (NCT02362789) included both an open-label (OL) run-in phase (weeks 1–16) and a subsequent randomized withdrawal (RW) phase (weeks 16-32) with monthly injections of secukinumab 300mg s.c. (SEC) or placebo, respectively (Fig. S1a). The study was conducted in 19 centers in Germany between March 2015 and July 2016.

Patients were adults (aged ≥18 years) with moderate to severe plaque psoriasis (Psoriasis Area Severity Index (PASI)>10) and moderate to severe pruritus (≥30 on a pruritus 0-100 visual analogue scale (VAS) as part of the Patients Global Assessment of Chronic Pruritus (PGA-CP)). All patients were eligible for systemic treatment with biologics. There was a 2-4 week washout period for all systemic and non-systemic (PUVA) psoriasis treatments. Key exclusion criteria were previous exposure to secukinumab or any other biologic drug directly targeting IL17A or the IL17A receptor (e.g. brodalumab, ixekizumab), any underlying conditions other than psoriasis that could cause pruritus, and pregnancy or breastfeeding. No concomitant medication that could potentially impact pruritus, e.g. antihistamines, was permitted.

All patients received OL SEC, administered by prefilled syringe at baseline, weeks 1, 2, 3, and 4 and then every 4 weeks until week 16. Patients who did not reach PASI≥98 reduction discontinued the study after week 16. Patients who achieved a reduction in PASI≥98 at week 16 were then randomised 1:1 until week 32 to either 4-weekly placebo or SEC, using a randomization list produced by a validated system for the random assignment of treatment arms, with patients and investigators blinded to study treatment (Fig. S1b).

The primary objective of PSORITUS was superiority of SEC- versus placebo-withdrawal on pruritus intensity (pruritus VAS 0–100; worst pruritus within 24 hours (WI-VAS-24h)) at week 32. Exploratory objectives included the assessment of the treatment-dependent course of pruritus in association with absolute PASI and PASI 50/75/90/100 response, as well as histopathology and cutaneous neuroanatomy in lesional and non-lesional skin before, during and after treatment.

PSORITUS was designed and implemented in accordance with the Guidelines for Good Clinical Practice, with applicable local regulations (including European Directive 2001/20/EC), and with the ethical principles laid down in the Declaration of Helsinki. Written informed consent was obtained from all study patients.

Histological analyses

Punch skin biopsies (4-6 mm diameter) were collected from lesional psoriatic plaques (LS biopsy) and non-lesional (NL) skin preferentially at the trunk (evasively at the extremities) according to the assessment schedule and laboratory manual. Following local anaesthesia, biopsies were taken from the center of a typical sentinel psoriatic plaque judged by the site investigator to be representative of the overall PASI score and from an NL control area, with all consecutive biopsies from each patient taken in the same way from their respective body regions. The location of the biopsy on the body was recorded, with photo-documentation of each biopsy region performed according to the assessment schedule and archived at each clinical site. If a sentinel psoriasis plaque had fully resolved at any biopsy time point, the LS biopsy was obtained from the resolved sentinel plaque region using the baseline biopsy photo and/or scar to provide information about the location and orientation of the sentinel plaque. Biopsies were not to contain scar tissue from previous biopsies, confirmed by histology. Standard wound care was applied to all biopsies as routinely performed at each clinical site. Either a single 6 mm diameter biopsy or two 4 mm diameter biopsies were taken from the same LS and NL regions, the latter only if the 2 biopsies corresponded clinically to one another and the distance between them was < 3 cm. If a single 6 mm biopsy sample was collected, it was bisected perpendicular to the dermal/epidermal axis into two 3 mm pieces; one piece was frozen in OCT medium for histopathology and immunohistochemical staining, and the other piece was stored for later analyses. If two 4 mm biopsies were collected from the same site, one biopsy was used for histopathology and immunohistochemical staining and the other one stored for further investigations. Routine hematoxylin and eosin as well as PAS stained paraffin sections of all biopsies were analysed regarding histopathological changes (e.g. hyperplasia, hyperkeratosis, etc.). Additionally, immunoperoxidase staining were performed on paraffin sections with psoriasis- related antibodies against cytokeratin 16 (CK16; clone LL025, Zytomed, Berlin, Germany; 1:150 in citrate dilution buffer pH 6.0) and psoriasin (S100 A7; clone 47C1068; Abcam, Cambridge, UK; 1:4000 in citrate dilution buffer pH 6.0). Biopsies were independently scored semi-quantitavely by two investigators (SST, DM) blinded to the treatment group. Disagreements between the two scorers were resolved prior to clinical database lock and unblinding. Epidermal thickness was measured and segmented cells were counted using ImageJ (rsb.info.nih.gov/nih-imageJ), an image processing program.

Cutaneous neuronal architecture

For determination of intraepidermal nerve fibre density (IENFD), counting of PGP 9.5-positive nerve fibres crossing the basement membrane per mm epidermis, samples were processed according the validated and previously reported protocol (1). Biopsies were fixed in paraformaldehyde (PFA 4%) overnight. Afterwards, we cryopreserved the biopsies by sucrose immersion (5%, 10% and 20% with 10% glycerine) before biopsies were embedded in Neg-50 Frozen Section Medium (Epredia, Kalamazoo). Three cryosections (30 µm) per biopsy were first incubated with a primary antibody against PGP 9.5 (polyclonal rabbit, 1:2000; Chemicon,

Temecula, CA, USA) and subsequently stained with a fluorescein isothiocyanate isomer 1 (FITC)-conjugated secondary antibody (Swine Anti-Rabbit, 1:200; Dako, Cytomation, Glostrup, Danmark). The determination of IENFD was independently performed by two investigators under blinded conditions at 400x magnification using a fluorescence microscope (Olympus, Modell BX43F, Tokyo, Japan). Due to localization-specific differences in IENFD (Fig. S6c), only localization-matched biopsies were analysed.

To determine the absolute nerve fibre length, we generated 3 z-stacks (26 individual images within a 20 µm range) per patient per time point at 200x magnification using the same microscope as described above. Subsequent processing included the open source software DeconvolutionLab2 (2) to deconvolve the stacks into Tagged Image File Format (TIFF) files for further processing (Fig. S6a). Next, the epidermis height was assessed by determination of the distance between the basement membrane and the stratum corneum. Morphometric tracing of nerve fibres within the epidermis was performed using the software NeuroM/Blue Brain Project (3) by measuring the length of fibres penetrating the basement membrane (Fig. S6b). *Statistical analyses*

The study sample size was calculated based on previous pruritus results from the FIXTURE Study (CAIN457A2303) (4). Assuming an effect size of 0.79 (mean difference between SEC and placebo) with the pruritus VAS at the end of the RW phase (week 32), a sample size of 27 was deemed sufficient in each group for a power of 80% and a 5% significance level with a 2-sided test. According to Phase III data, approximately 40% of patients treated with SEC were expected to achieve PASI≥98 reduction at week 16. Thus, a sample size of 150 patients was chosen to enter the single-arm OL part of the study.

The Full Analysis and Safety Sets for the OL/RW phase consisted of all patients that received at least one dose of OL or blinded study drug during the study.

The primary analysis method was testing differences between the placebo and the SEC-group for significance using a t-test. Missing values of VAS or PASI were imputed with the median value of the entire group at the corresponding timepoint.

Non-parametric tests were used for data without normal distribution. Wilcoxon's rank sum test was used for comparison of paired samples and data of independent groups were tested with the Kruskal-Wallis test. Correlations were calculated with Spearman's correlation coefficient test. All calculations were performed using SPSS 27 software (SPSS, Chicago, IL, USA) and two-sided p-values of <0.05 were considered statistically significant.

References

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