Supplementary Online Content

Vieyra-Garcia P, Fink-Puches R, Porkert S, et al. Evaluation of low-dose, low-frequency oral psoralen plus UVA (PUVA) treatment with or without maintenance in early-stage mycosis fungoides: a randomized clinical trial. *JAMA Dermatol.* Published online March 20, 2019. doi:10.1001/jamadermatol.2018.5905

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. PUVA Characteristics of Patients Who Completed the Minimum of 12 Weeks of Treatment

PUVA induction phase (n, 26)	**mean (range)**
MPD, J/cm^2	
CR	3.7 (1-7.7)
PR	3.5 (2-5)
Start dose of UVA, J/cm^2	
CR	1.0 (0.25-3.8)
PR	1.1 (0.5-2.5)
Cumulative UVA dose, J/cm^2	
CR	78.5 (17.7-160)
PR	94.4 (24.4-253.5)
PUVA maintenance phase (n, 11)	
Start dose of UVA, J/cm^2	4.0 (0.9-5.7)
Cumulative UVA dose, J/cm^2	56.1 (11.5-152)
PUVA induction and maintenance phase (n, 11)	
Total UVA dose, J/cm^2	130.3 (36.3-250)

Abbreviation: PUVA, psoralen plus UVA

eTable 2. Proinflammatory Mediators Prescreened

BAFF	IL12p70
bNGF	IL-13
CCL2	IL-15
CCL22	**IL-16**
CD30	IL-17A
CXCL11	IL-18
CXCL12	IL-20
CXCL13	IL-21
CXCL5	IL-22
CXCL9	IL-23
Eotaxin-1	IL-27
Eotaxin-2	IL-31
Eotaxin-3	IP-10
FGF-2	LIF
Fractalkine	M-CSF
G-CSF	MCP-2/CCL8
GM-CSF	MCP-3
GRO-alpha	**MIF**
HGF	MIP-1 alpha
IFN-alpha	MIP-1 beta
IFN-gamma	MIP-3 alpha
IL-1 alpha	**MMP-1**
IL-1 beta	sCD40L
IL-2	SCF
IL-2R	TNF-alpha
IL-3	TNF-beta
IL-4	**TNF-RII**
IL-5	**TNFSF13**
IL-6	**TRAIL**
IL-7	TSLP
IL-8	**TWEAK**
IL-9	VEGF-A
IL-10	

NOTE: Analytes in bold had detectable levels in MF patients' serum and were selected for further investigation throughout treatment (Procartaplex, Thermo Fisher, EPX650-10065-901, Vienna, Austria).

eTable 3. Primer List

CD3	
Forward	GGTTCGGTACTTCTGACTTGTG
Reverse	TCAGTTAGGAAGCCGATCATCT
CD4	
Forward	GTCCCTTTTAGGCACTTGCTTCT
Reverse	TCTTTCCCTGAGTGGCTGCT
Foxp3	
Forward	GTGGCCCGGATGTGAGAAG
Reverse	GGAGCCCTTGTCGGATGATG
CTLA-4	
Forward	GCCCTGCACTCTCCTGTTTTT
Reverse	GGTTGCCGCACAGACTTCA
GITR	
Forward	CAGTCCCAGGGGAAATTCAGT
Reverse	GAACACAGTGAGAAACCCGAA
TGF-beta	
Forward	CAATTCCTGGCGATACCTCAG
Reverse	GCACAACTCCGGTGACATCAA

LEGENDS OF eFIGURES

eFigure 1. Study Design

eFigure 2. Effect of PUVA Induction Treatment on mSWAT and Histologic Features
(A) Individual baseline mSWAT and percentage of body surface involvement values of PR and CR patients with and without maintenance PUVA. **(B)** Pathognomonic MF features score as described in materials and methods. Absent (0); low (1); or high (2). **(C)** Infiltrate density scored as no/low (0); medium (1); or high (2). Percentage of clonal TCR sequences of total reads in baseline biopsies of 21 patients with: **(D)** pathognomonic MF features, low (1) vs high (2)**, (E)** infiltration score, medium (1) vs high (2) and **(F)** clinical outcome PR vs CR (unpaired t-test, p-value shown). **(A, D-F)** Mean (SD) is plotted, t-test was used to evaluate statistical significance (p values are shown). **Note 1:** The infiltration density score at baseline and the clinical outcome (CR after 12-24 weeks of PUVA treatment) had a correlation with statistical significance (Fisher exact test, p=0.0377); see results section). **Note 2:** No biopsy after PUVA was available from patient 4301.08 due to an early termination of treatment (after presenting of repetitive vomiting, see results section). **Note 3:** No skin biopsy was available at the time of mSWAT of 0 from patients 2, 7, 14, 15, 7 and 8, instead, a biopsy taken during treatment near to randomization (between 12-24 weeks) was used to evaluate histological response.

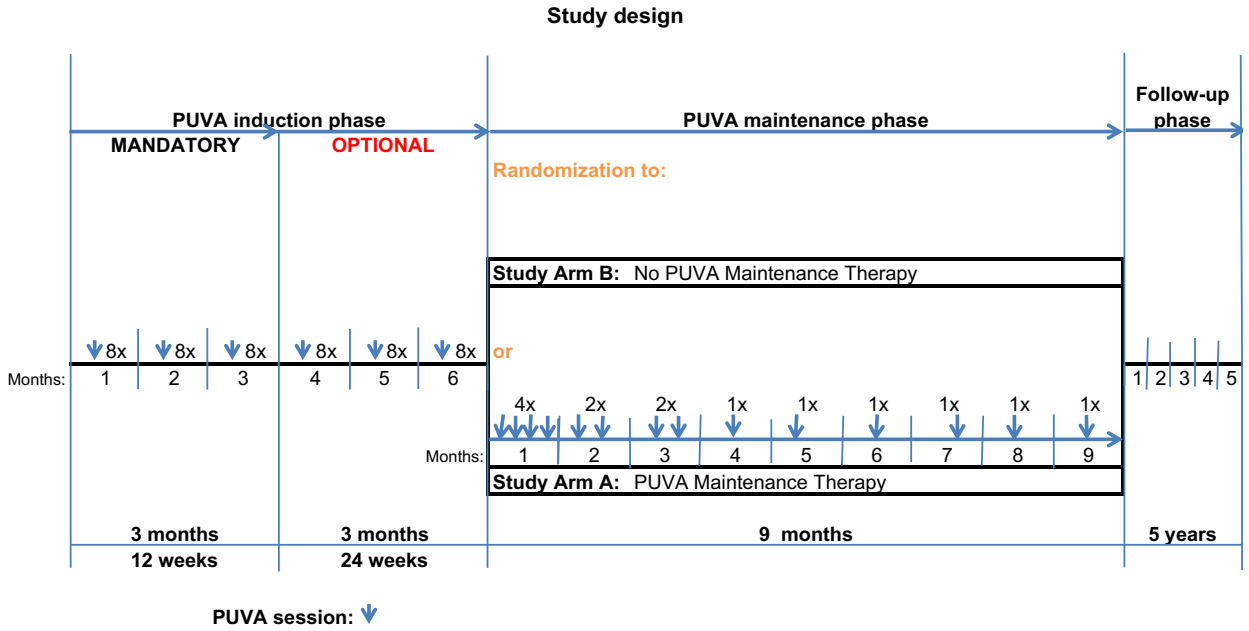
eFigure 3. Expression of Treg-related Markers in Lesional Skin
(A) Expression of CD3, CD4, and Treg-related markers quantified by qPCR in skin biopsies of MF patients taken at baseline (n=14) and 12-24 weeks of PUVA (n=13) compared with healthy controls (n=5). Paired t-test, statistical significance shown). **(B)** Expression of CD3, CD4, and Treg-related markers in post-treatment biopsies of CR versus PR patients. Mean (SD) is plotted, Mann-Whitney test was used to evaluate statistical significance (p values are shown).

eFigure 4. Proliferation Capacity of Peripheral T-cells and Treg Levels in Blood of MF Patients
Plots show results from blood samples collected during the PUVA induction phase. **(A)** Proliferative capacity of peripheral blood T-cells at baseline (n=14), 6 weeks (n=9) and 12-24 weeks (n=11) after treatment. $CD4^+CD25^-CD127^+$ cells were sorted at indicated time points and stimulated with anti-CD3/28 antibodies for 72h to determine [^{3}H]thymidine incorporation. Values from unstimulated cells were used to normalize scintillation counts. **(B)** Frequency of Treg cells in peripheral blood determined by flow cytometry at baseline (n=17), 6 weeks (n=12) and 12-24 weeks (n=16) after treatment (left plot); baseline frequency was used to calculate an index to compare fluctuations of Tregs throughout treatment (right plot). Mean (SD) is plotted, paired t-test was used to evaluate statistical significance (p values are shown).

eFigure 5. Assessment of Serum Levels of Proinflammatory Mediators

Plots show values for each patient and mean (SEM) is plotted, paired t-test was used to evaluate statistical significance (p values are shown). The p value printed in bold (CD30) remained significant after Bonferroni multiple endpoint testing.

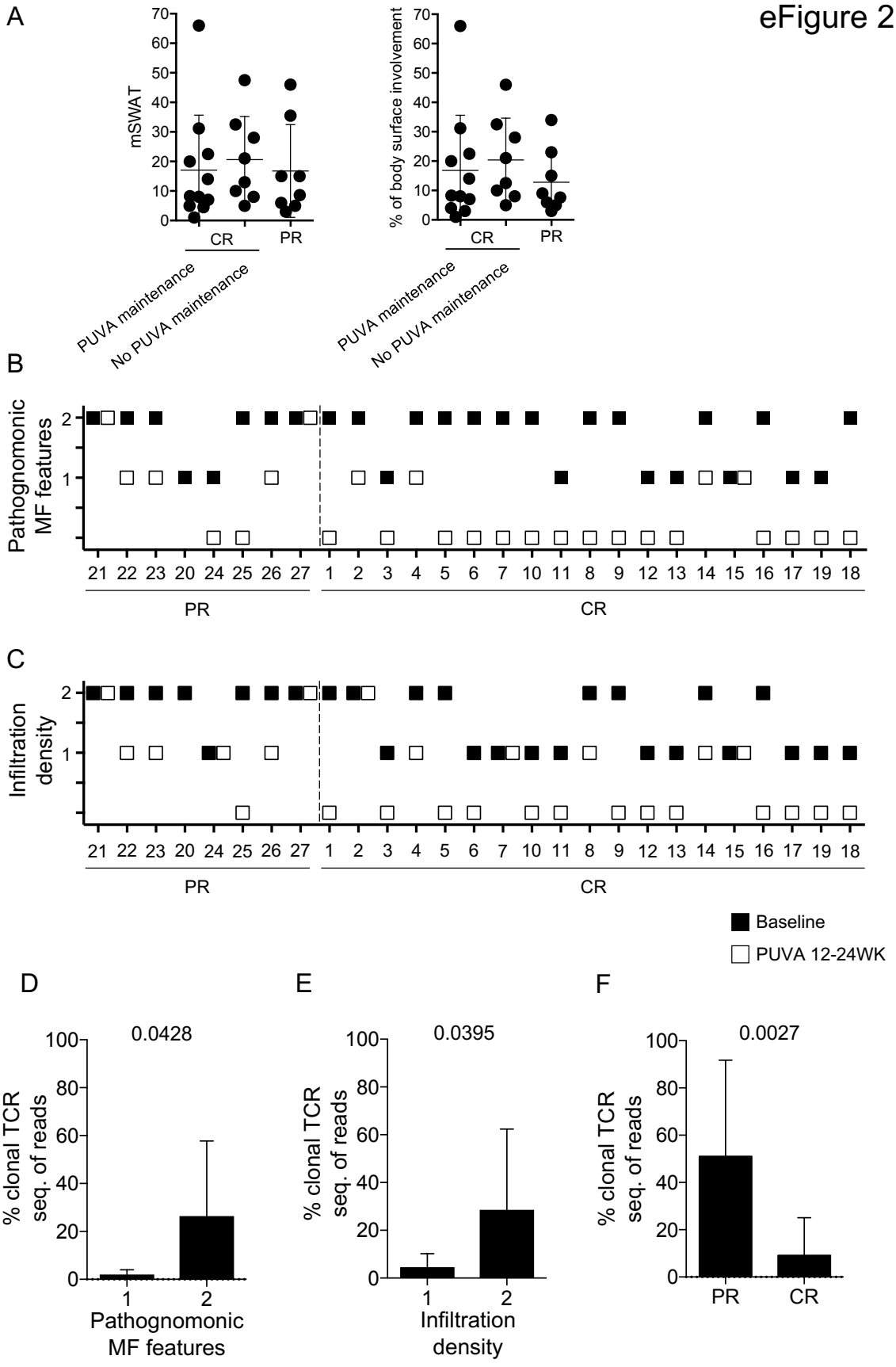
eFigure 1



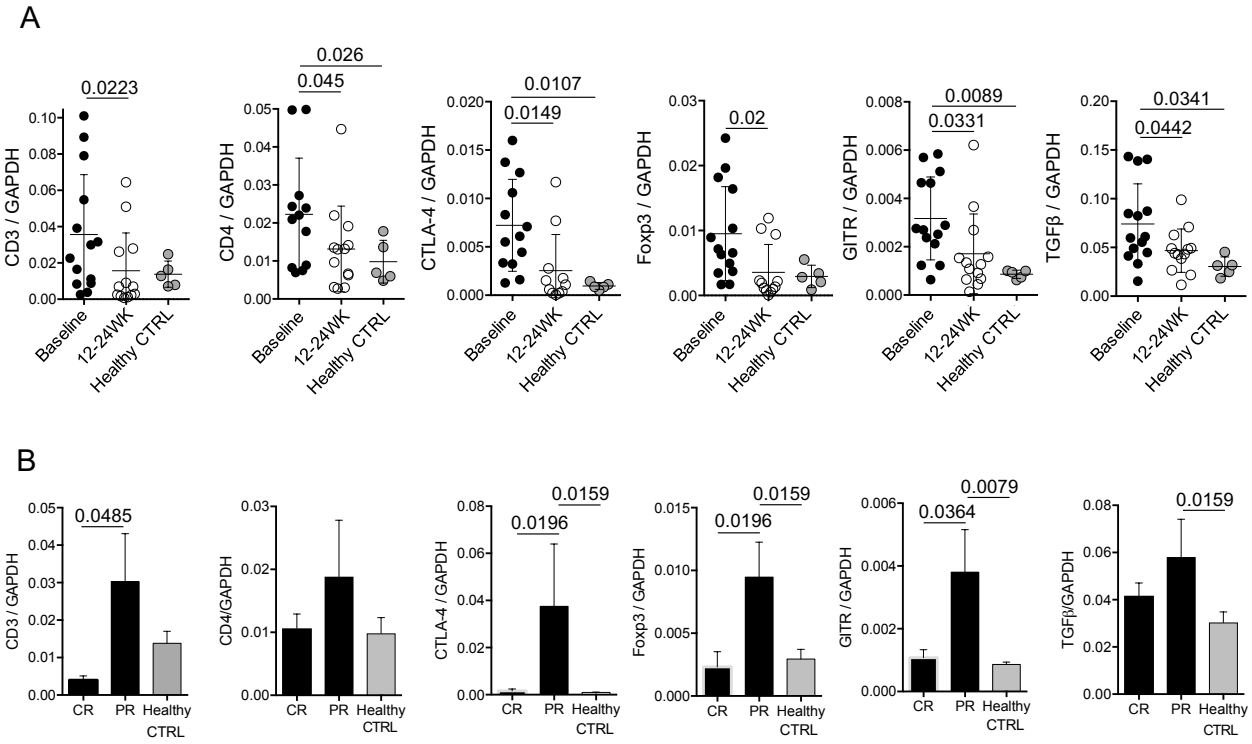
PUVA induction treatment was administered for a minimum of 12 weeks and a maximum of 24 weeks until modified severity-weighted assessment tool (mSWAT) was reduced to 0 and (whenever consented to by the patient) confirmed by complete histological response. Response evaluation for potential randomization started at week 12. In case of non-CR, PUVA treatment was continued and patients were reassessed for randomization every 4 weeks (16, 20 and 24 weeks) until CR occurred. After 24 weeks of PUVA, study participation was terminated in PR patients.

Randomized patients were evaluated 4, 12, 24 and 36 weeks after randomization and thereafter 4 times a year (1st year), twice a year (2nd year) and then once a year (3rd to 5th year).

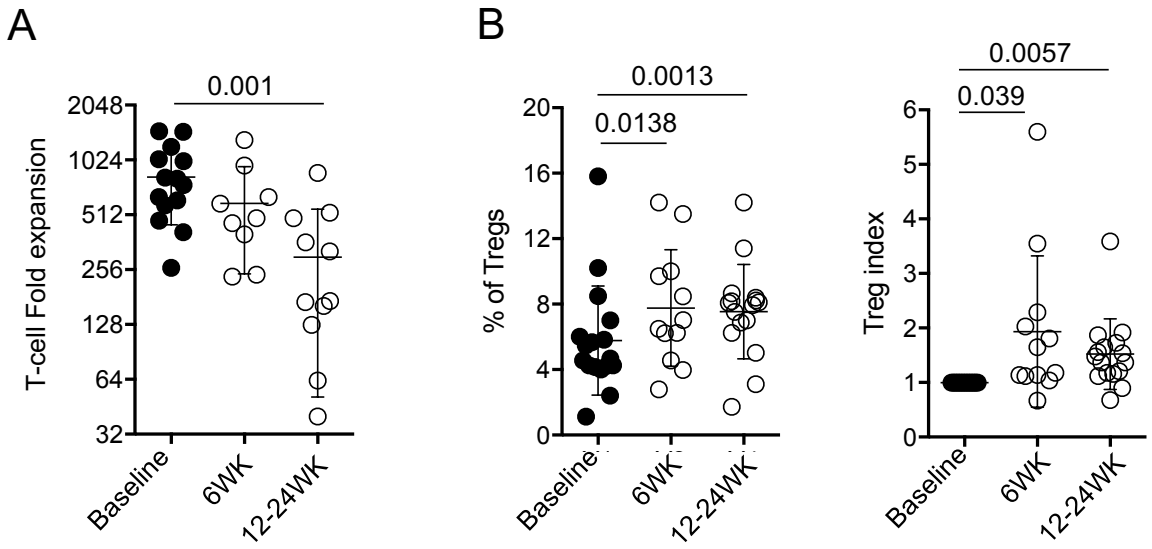
eFigure 2



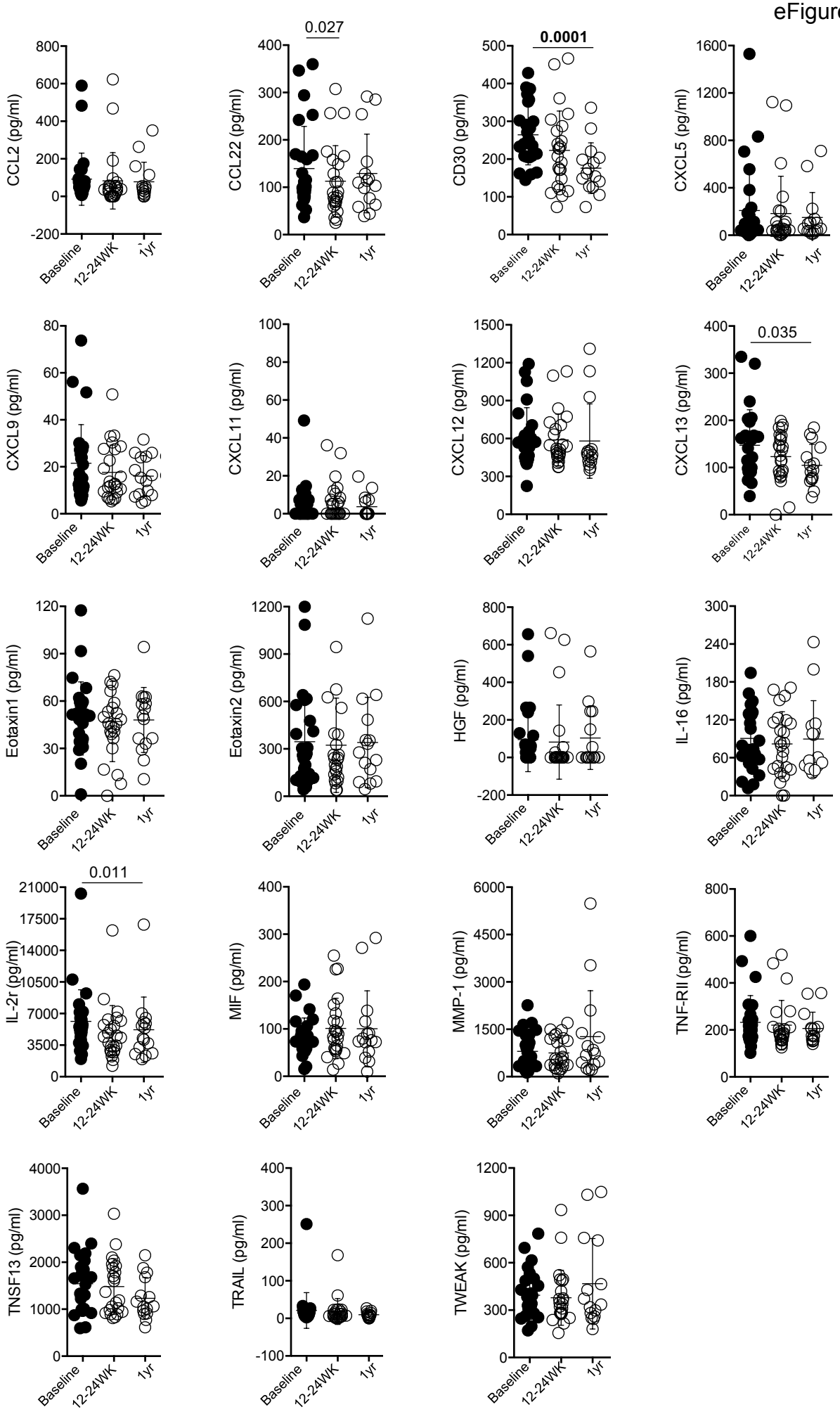
eFigure 3



eFigure 4



eFigure 5



eMethods. Patients inclusion and exclusion criteria, MPD determination, PUVA treatment, sample collection and processing, histologic assessment, qPCR, flow cytometry and CD3/28 cell stimulation.

Patient Inclusion and Exclusion Criteria

Eligible patients had histologically confirmed early-stage MF (stage IA-IIA),[28,29] were between 18 and 85 years old; had no anti-dsDNA and anti-Ro/La auto antibodies; had AST and SPGT levels < 2.5 × the upper limit of normal (ULN) and creatinine levels < 2 × ULN; had no evidence of severe cardiac insufficiency; had no serious illness/infection that could interfere with the planned treatment; and were in good health with a Karnofsky score > 60. All women of reproductive age had a pregnancy test; those who had a positive test result or were lactating were excluded from the study. Also excluded were patients who had received PUVA within 3 months before screening, had a photosensitive disease (including lupus erythematosus), or had a skin cancer syndrome such as basal cell nevus syndrome or xeroderma pigmentosum.

PUVA treatment

8-methoxypsoralen (8-MOP) was administered as a liquid preparation in soft gelatine capsules (Oxsoralen®; G.L. Pharma GmbH, Lannach, Austria). The standard dose was 1 capsule (10 mg 8-MOP) per 20 kg body weight (0.5mg/kg of bodyweight; maximal dose 70mg) given with food (preferentially dairy products) 1 h before UVA exposure. Before starting total body PUVA treatment, each patient's individual minimal phototoxic dose (MPD) was determined after ingestion of 8-MOP by applying a standard series of UVA doses to skin areas 1.5 cm in diameter (on the buttocks), as previously described.[30]

The MPD was defined as the UVA dose producing slight homogenous erythema at the site of exposure at 72 h. The standard initial UVA dose of PUVA induction therapy was set to <50% of MPD. Patients were treated twice a week (on treatment weekdays at least 2 days apart). UVA radiation was delivered using Waldmann F85/100 W-PUVA fluorescent bulbs (Waldmann Medizintechnik, Villingen-Schwenningen, Germany) mounted in Waldman 7001 or similar

cabinets. The UVA dose was increased weekly by 0-30%, depending on the absence or presence of erythema after preceding treatments. Dose was adjusted according to the skin's erythema response. Depending on the duration of the PUVA induction phase (3-6 months until CR or not), the patients received a total of 24-48 PUVA exposures according to the protocol. In Arm A, PUVA maintenance therapy was administered for 9 months in fixed UVA doses (each dose equal to the last dose before randomization) once weekly for 4 weeks, then once every 2 weeks for 8 weeks, and finally once every 4 weeks for 24 weeks. According to this protocol, patients were scheduled to receive a total of 14 PUVA exposures during the maintenance phase. Patients in Arm B did not receive PUVA maintenance.

Tissue sample collection and processing

When possible, biopsy samples were taken from a representative lesion at baseline (within 4 weeks before start of treatment) and after 12-24 weeks of PUVA treatment. A biopsy was taken from another area of the body if the clinical response to treatment on that side was ambiguous. Skin samples were fixed in 4% formaldehyde and paraffin-embedded for histological and immunohistochemical analysis. A part of each skin sample was dispersed in RNAlater (Merck, Vienna, Austria) and thereafter stored at -80° C and transferred to the coordinating center for processing. Serum samples were collected at baseline and at 12-24 weeks and 1 year after start of PUVA, transferred to the coordinating center and analyzed simultaneously after completion of the study.

Histological assessment

Paraffin-embedded skin samples were sectioned (3.5 μm) and stained with H&E. Samples from all study centers were examined at the Dermatopathology Unit, Medical University of Graz (as the coordinating center) by an experienced histopathologist (LC) during the course of study and reports transmitted to the respective subinvestigators of the other centers. After completion of the study, available H&E skin sections were reevaluated in blinded, consensus-based fashion by three of the investigators (PV, PW, and LC). Cellular infiltration of lesional skin was assessed in biopsy samples taken at baseline (before PUVA was started) and at 12-24 weeks after PUVA at a body site, along with samples of normal skin available from the tissue bank of the Department

of Dermatology (approval number: 18-068 ex 06/07). Pathognomonic MF features including epidermotropic cells and band-like dermal infiltrate with cerebriform cells were scored as absent (0) or present and, if present, as either low (1) or high (2). Infiltrate density was scored as no/low (0), medium (1), or high (2).

Immunobead assay

Serum cytokine levels were determined using a custom-made panel of 19 analytes (ThermoFisher, Procartaplex PPX-19; Vienna, Austria). This panel was chosen after we had done a prescreening assay of 65 analytes **(sTable2)** in baseline serum samples from a subcohort of MF patients with known TCR clonality in the skin and found those 19 analytes at levels within the detection range of the assay. The Luminex 100/200 system (LX200-XPON3.1; Luminex Corp, Austin, TX, USA) was used to measure fluorescence and calculate absolute concentration of each analyte according to the manufacturer's instructions.

T-cell characterization

High-throughput TCR (HT-TCR) sequencing was used to determine clonality on DNA extracted from lesional skin, as described previously.[31] For patients recruited in the coordinating center, T-cell proliferative capacity was determined by a tritiated thymidine ([^{3}H]thymidine) incorporation assay in which sorted blood cells (obtained from samples taken shortly before 8-MOP ingestion) with phenotype $CD4^+CD25^-CD127^+$ (stained with the antibodies from Biolegend, San Diego, CA, USA [317408, 356108] and BD Pharmingen, San Diego, CA, USA [557938]) were stimulated for 72 h with anti-CD3 and anti-CD28 antibodies (Biolegend 344802 and 302902). At 16 h before harvesting, [^{3}H] thymidine (Amersham Biosciences, Little Chalfont, UK) was added and quantified in a scintillation beta-counter (Wallac 1450 TriLux, Perkin Elmer, Shelton, CT, USA). T-cell proliferation was expressed by the fold-expansion of stimulated cells normalized against unstimulated cells. Regulatory T-cell percentage was determined in blood by flow cytometry of cells with phenotype $CD3^+CD4^+CD45RA^-CD25^+$ (eBioscience 56-0037-42, 11-0049-42, 48-0458-42, San Diego, CA, USA; and Biolegend 356108). qPCR was performed using Power SYBR Green (Applied Biosystems, 4367659, Foster City, CA, USA) to quantify the expression of Treg-related molecules (CD3, CD4, CTLA4, Foxp3, GITR, and TGFβ; see primer list in **sTable-3**) in

samples from lesional skin taken at baseline and 12-24 weeks after start of PUVA. The expression of these markers was also determined in normal skin as reference controls. The results were normalized to GAPDH and relative expression was calculated by the ΔC_t method.