biosimilars for moderate-to-severe plaque psoriasis. Br J Dermatol 2018;178:509–19.

Kragh Andersen P, Pohar Perme M, van Houwelingen HC, Cook RJ, Joly P, Martinussen T, et al. Analysis of time-to-event for observational studies: guidance to the use of intensity models. Stat Med 2021;40:185–211.

Menter A, Papp KA, Gooderham M, Pariser DM, Augustin M, Kerdel FA, et al. Drug survival of biologic therapy in a large, disease-based registry of patients with psoriasis: results from the Psoriasis Longitudinal Assessment and Registry (PSOLAR). J Eur Acad Dermatol Venereol 2016;30:1148–58.

Pina Vegas L, Penso L, Claudepierre P, Sbidian E. Long-term persistence of first-line biologics for patients with psoriasis and psoriatic arthritis in the French health insurance database. JAMA Dermatol 2022;158:513–22.

Sbidian E, Mezzarobba M, Weill A, Coste J, Rudant J. Persistence of treatment with biologics for patients with psoriasis: a real-world analysis of 16 545 biologic-naïve patients from the French National Health Insurance database (SNIIRAM). Br J Dermatol 2019;180:86–93.

Schmitt-Egenolf M, Freilich J, Stelmaszuk-Zadykowicz NM, Apol E, Hansen JB, Levin LÅ. Drug persistence of biologic treatments in psoriasis: A Swedish national population study. Dermatol Ther (Heidelb) 2021;11:2107–21.

Xu C, Teeple A, Wu B, Fitzgerald T, Feldman SR. Drug adherence and persistence of patients with moderate to severe psoriasis treated with biologic medications in a US commercially insured population. Dermatology 2022;238:438–47.

Yiu ZZN, Becher G, Kirby B, Laws P, Reynolds NJ, Smith CH, et al. Drug survival associated with effectiveness and safety of treatment with guselkumab, ixekizumab, secukinumab, ustekinumab, and adalimumab in patients with psoriasis. JAMA Dermatol 2022;158:1131–41.

Biological Tipping Point in Patients with Atopic Dermatitis Treated with Different Dosing Intervals of Dupilumab

JID Open

Journal of Investigative Dermatology (2023) **143,** 1822–1825; doi:10.1016/j.jid.2023.03.1659

TO THE EDITOR

Dupilumab, an IgG4 mAb targeting the IL-4 receptor alpha (IL-4Rα), substantially improves disease severity in patients with atopic dermatitis (AD) (Gooderham et al., 2018).

A recent daily practice study indicates that dose reduction of dupilumab might be successfully applied in patients with controlled AD (Spekhorst et al., 2022). Spekhorst et al. (2022) showed that prolongation of the injection interval up to 6 weeks resulted in sustained disease control in patients with AD with low disease activity after 52 weeks of dupilumab treatment. However, the associated immunologic effects of interval prolongation are currently unknown. Therefore, we studied the effects of interval prolongation on dupilumab serum levels, the IL-4Rα occupancy, (skin-homing) T-cell function, and serum thymus and regulated chemokine levels in patients with AD in whom the dupilumab interval was prolonged.

All included patients participated in the Dutch BioDay registry and signed informed consent for extraction of data from the registry. In addition, all included patients gave their consent for collection of blood samples during the treatment according to our biobank protocol, which has been approved by the Medical Ethical Committee of the University Medical Center Utrecht (approval number 17-884). After 1 year of treatment with the standard dose of dupilumab, the dosing interval was prolonged according to the protocol described previously (Spekhorst et al., 2022). In brief, a patient-centered dosing regimen that is guided by the Eczema Area and Severity Index (EASI) was applied, in which patients were eligible for dose reduction in cases of EASI ≤ 7, indicating mild disease activity or less, for at least 6 months. Patients with AD who prolonged the interval from 300 mg dupilumab subcutaneously every 2 weeks (Q2W) to every 4 weeks (Q4W), and eventually every 6 weeks (Q6W), were retrospectively enrolled in this study. Blood samples were collected from patients before the initiation of treatment (baseline) and during treatment at time points when patients were treated with dupilumab Q2W, Q4W, and Q6W for at least 3 months. PBMCs from a total of 11 patients and healthy controls were available for analyses. The EASI was used to assess disease severity and to evaluate clinical effectiveness. In addition, serum thymus and regulated chemokine levels, currently the best performing and most accepted biomarker for disease severity in AD (Thijs et al., 2015), were measured. PBMCs were isolated and analyzed using flow cytometry. The quantification of total dupilumab levels in serum was performed with a validated liquid chromatography-tandem mass spectrometry analysis (Amrani et al., 2021). Detailed methods related to laboratory assessments and statistical analysis are described in this letter's online repository.

We included a total of 11 patients with AD. All clinical characteristics are shown in Table 1. In addition, a total of 11 healthy adult volunteers were included in the analyses. An overview of the study design, peripheral blood collection, and laboratory assessments is presented in Figure 1a.

As expected, the dupilumab interval prolongation resulted in a significant decrease in dupilumab serum levels in all patients (Figure 1b). Dupilumab surface binding (anti-IgG4) to both CD19+ B cells and CD4+ T cells also gradually decreased with interval prolongation. During treatment with dupilumab Q2W and Q4W, IL-4Rα expression remained undetectable, regardless of the number of days between the last dupilumab injection and the blood draw, and despite lower levels of dupilumab in the serum for the Q4W interval. Importantly, during

Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; IL-4Rα, IL-4 receptor alpha; Q2W, once every other week; Q4W, once every 4 weeks; Q6W, once every 6 weeks

Accepted manuscript published online 28 March 2023; corrected proof published online 15 June 2023

Table 1. Baseline Characteristics

Clinical Characteristics	Tapering Patients (n = 11)	Healthy Control (n = 11)
Age at start, y, median (IQR)	37 (33–49.5)	29 (27–31)
Male, n (%)	6 (54.54)	4 (36.36)
Atopic comorbidities, n (%)		
Allergic asthma	4 (36.36)	—
Allergic rhinitis	10 (90.90)	—
Allergic conjunctivitis	7 (63.63)	—
No atopic comorbidities	1 (9.09)	—
Food allergy, n (%)	7 (63.63)	—
Age of onset, n (%)		
Children	8 (72.72)	—
Adolescent	2 (18.18)	—
Adult	1 (9.09)	—
Previous use of systemic immunosuppressive medication		
Cyclosporine	10 (90.90)	—
Methotrexate	4 (36.36)	—
Azathioprine	1 (9.09)	—
Mycophenolate mofetil	1 (9.09)	—
EASI score at baseline, median (IQR)	12.00 (9.40–24.5)	—
EASI score at prolongation to Q4W, median (IQR)	0.95 (0.57–2.87)	—
EASI score at prolongation to Q6W, median (IQR)	1.10 (0.82–1.92)	—
TARC levels at baseline, median (IQR)	1392 (779–4272)	—
Median duration on interval in days (IQR)		—
1×/2 wk	366 (112–376)	
1×/4 wk	210 (178–285)	
1×/6 wk	178 (98–191)	

Abbreviations: EASI, Eczema Area Severity Index; IQR, interquartile range; Q4W, every 4 weeks; Q6W, every 6 weeks.

treatment with dupilumab Q6W, the IL-4Rα became fully or partly detectable again if the time between the injection of dupilumab and the blood draw was 40 days or longer (Figure 1c). These results indicate that around an interval of 40 days, a tipping point occurs where dupilumab is no longer (fully) saturating IL-4Rα on circulating lymphocytes. This was accompanied by clinical and immunological changes. A significant decrease in EASI scores was seen during all dosing intervals compared with baseline, but EASI scores slightly increased in 8 of 11 patients when extending the interval from Q4W to Q6W (Figure 1d). The same applied to serum thymus and regulated chemokine levels (Figure 1e). In addition, IL-4 and IL-13 levels increased slightly during interval prolongation in some patients, and the percentage of proliferating (Ki67+) skin-homing (C-C chemokine receptor type 4 +, Cutaneous Lymphocyte Antigen +) CD4+ T cells increased significantly when extending the interval from Q4W to Q6W (Figure 1f). No significant changes were found in the production of T helper types 1, 17, and 22-related cytokines by skin-homing T cells during interval prolongation (data not shown).

This study confirms the strong functional immunological effects of dupilumab treatment as previously presented by Bakker et al. (2021). Complementary to this study, we showed that the IL-4Rα was still undetectable when prolonging the interval from Q2W to Q4W, regardless of the number of days since the latest injection of dupilumab. There seems to be a clear tipping point within Q4W to Q6W at which the receptor becomes detectable again. This is likely the result of reduced dupilumab binding, but additional changes in surface IL-4Rα receptor expression upon dupilumab withdrawal cannot be ruled out. Although no signs of receptor internalization or reduced expression of the receptor were observed in the previous study by Bakker et al. (2021), the recently published study by Heeb and Boyman (2023) shows that engagement of IL-4Rα by dupilumab may result in the internalization of the antibody and decreased total IL-4Rα expression. IL-4Rα surface expression may therefore be restored when dupilumab serum levels drop. In the 3 patients of our cohort in whom the IL-4Rα became detectable again during Q6W, the lowest levels of dupilumab in serum were measured. This suggests that a certain concentration of dupilumab in the serum is required for IL-4Rα saturation. Our clinical experience after using this protocol for several years is that patients treated with dupilumab Q6W often report symptoms such as redness and itch in the week before the new dupilumab injection should be given. This is in line with the returning detectability of the IL-4Rα after 40 days. In addition, there are some patients in whom it seems impossible to extend the interval to longer than 2 weeks to maintain good clinical effects. It could be hypothesized that one of the mechanisms for the lack of response includes subtherapeutic drug concentrations secondary to enhanced clearance. For future research, it would be interesting to examine these patient groups to further optimize biologic therapy.

In conclusion, our study supports the idea to consider interval prolongation of dupilumab from Q2W to Q4W in patients with AD in whom the disease is well-controlled. The transition from dupilumab Q4W to Q6W seems to be an important biological tipping point and marks the window through which disease activity may show the first signs of relapse, at least in some patients.

Ethics statement

All included patients participated in the Dutch BioDay registry and signed informed consent for extraction of data from the registry. In addition, all included patients gave their consent for collection of blood samples during the treatment according to our biobank protocol, which has been approved by the Medical Ethical Committee of the University Medical Center Utrecht (approval number 17-884).

Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request. No large datasets were generated and/or analyzed during the current study.

ORCIDs

Coco Dekkers: http://orcid.org/0000-0002-2344-6860

M. Marlot van der Wal: http://orcid.org/0000-0003-4554-9059

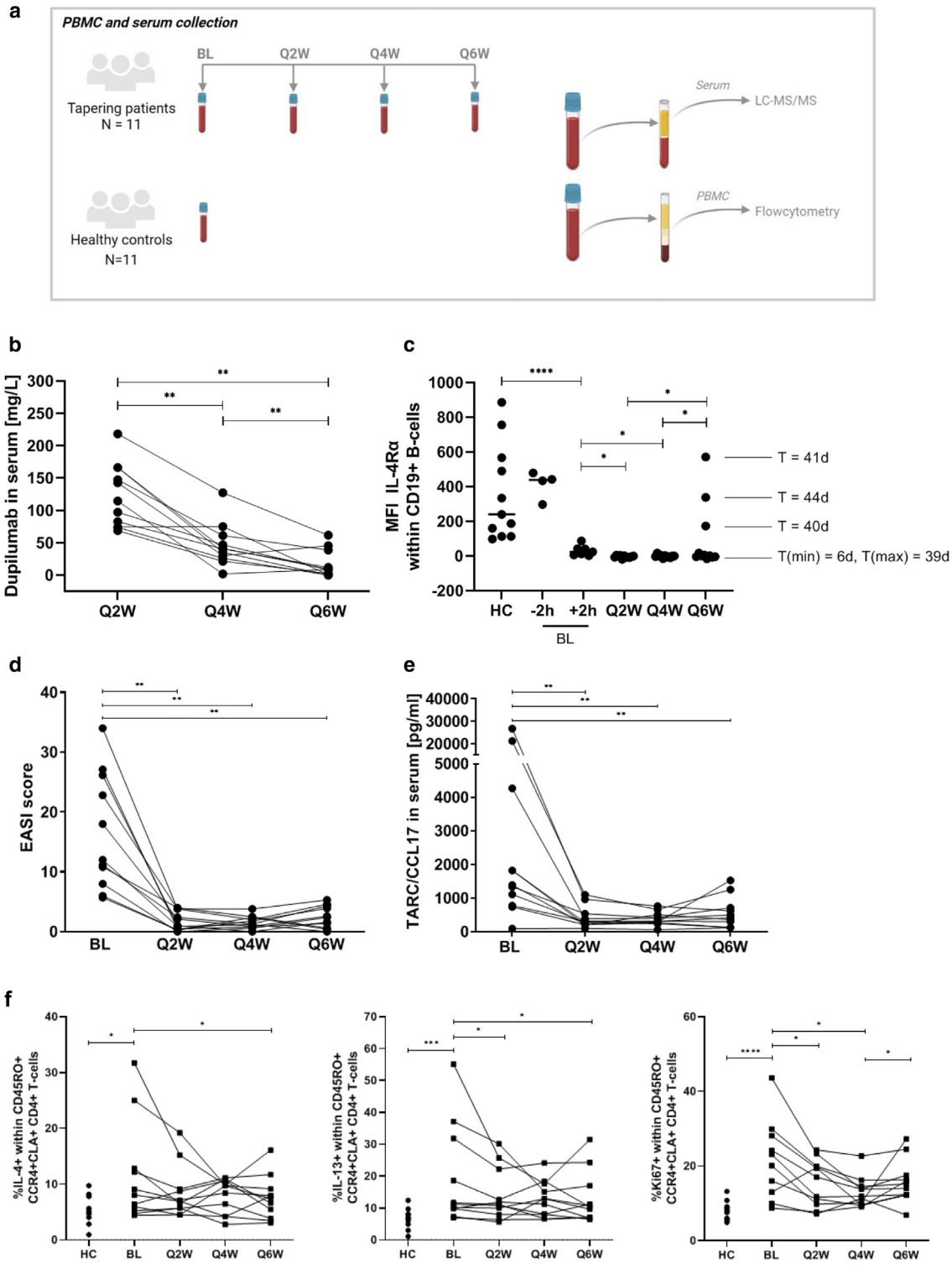

Figure 1. Effects of dupilumab dosing interval prolongation on immunologic levels and clinical parameters. (**a**) Overview of study design, peripheral blood collection, and laboratory assessments. (**b**) Dupilumab levels in serum measured during treatment with dupilumab Q2W, Q4W, and Q6W. (**c**) The MFI of IL4-Rα on CD19+ B cells in 11 HCs and 11 patients before dupilumab treatment (BL) and when treated with dupilumab Q2W, Q4W, and Q6W. (**d**) The EASI in 11 HCs and 11 patients at BL and when treated with dupilumab Q2W, Q4W, and Q6W. (**e**) TARC/CCL17 level (in pg/ml) in 11 HCs and 11 patients at BL and when treated with dupilumab Q2W, Q4W, and Q6W. (**f**) Percentage of IL-4+ (left), IL-13+ (middle), and proliferation (Ki67+) (right) within memory (CD45RO+) skin-homing (CCR4+CLA+) CD4+ T cells in 11 HCs and 11 patients at BL and when treated with dupilumab Q2W, Q4W, and Q6W. Significance levels correspond to the following *P*-values: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, and $^{****}P < 0.001$. BL, baseline; CCR4, C-C chemokine receptor type 4; CLA, cutaneous lymphocyte antigen; EASI, Eczema Area and Severity Index; HC, healthy control; IL-4Rα, IL-4 receptor alpha; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MFI, mean fluorescence intensity; Q2W, once every other week; Q4W, once every 4 weeks; Q6W, once every 6 weeks; TARC/CCL17, thymus and regulated chemokine.

Mohsin El Amrani: http://orcid.org/0000-0001-8276-0860
Matthijs van Luin: http://orcid.org/0000-0001-6172-887X
Daphne S. Bakker: http://orcid.org/0000-0002-0193-5794
Marjolein de Bruin-Weller: http://orcid.org/0000-0002-1249-6993
Femke van Wijk: http://orcid.org/0000-0001-8343-1356

CONFLICT OF INTEREST

DSB is a speaker for Sanofi Genzyme, Janssen, and Leo Pharma. MB is a consultant, advisory board member, and/or speaker for AbbVie, Almirall, Arena, Aslan, Eli Lilly, Galderma, Janssen, Leo Pharma, Pfizer, Regeneron, and Sanofi Genzyme. FW is a speaker and/or consultant for Janssen, Johnson & Johnson, and Takeda. She has received research funding from Leo Pharma, Takeda, Galapagos, Sanofi, and Bristol-Myers Squibb, all unrelated to this research. The remaining authors state no conflict of interest.

ACKNOWLEDGMENTS

CD, MMVDW, MEA, MVL, DSB, MDBW, and FVW have made substantial contributions to conception and design, the acquisition of data, or the analysis and interpretation of data. All authors have been involved in drafting the manuscript or revising it critically and have given their final approval of the version to be published. Patients included in this manuscript participated in the BioDay registry sponsored by Sanofi Genzyme.

AUTHOR CONTRIBUTIONS

Conceptualization: CD, MMVDW, DSB, MDBW, FVW; Formal Analysis: CD, MMVDW, MEA; Funding Acquisition: FVW, MDBW; Investigation: CD, MMVDW, MEA; Methodology: MMVDW, MEA; Resources: CD, MMVDW, MEA; Supervision: MDBW, FVW; Validation: FVW; Visualization: CD, MMVDW; Writing - Original Draft Preparation: CD, MMVDW; Writing - Review and Editing: MEA, MVL, DSB, MDBW, FVW

Coco Dekkers[1,2,4], M. Marlot van der Wal[2,4], Mohsin El Amrani[3], Matthijs van Luin[3], Daphne S. Bakker[1], Marjolein de Bruin-Weller[1,4] and Femke van Wijk[2,4,*]

[1]Department of Dermatology and Allergology, National Expertise Center for Atopic Dermatitis, University Medical Center Utrecht, Utrecht, The Netherlands; [2]Center for Translational Immunology, University Medical Center Utrecht, Utrecht, The Netherlands; and [3]Department of Clinical Pharmacy, Division Laboratories, Pharmacy and Biomedical Genetics, University Medical Centre Utrecht, Utrecht, The Netherlands

[4]These authors have contributed equally to this work.

[*]Corresponding author. e-mail: f.vanwijk@umcutrecht.nl

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2023.03.1659.

REFERENCES

Amrani ME, Gerencser L, Huitema ADR, Hack CE, van Luin M, van der Elst KCM. A generic sample preparation method for the multiplex analysis of seven therapeutic monoclonal antibodies in human plasma or serum with liquid chromatography-tandem mass spectrometry. J Chromatogr A 2021;1655:462489.

Bakker DS, van der Wal MM, Heeb LEM, Giovannone B, Asamoah M, Delemarre EM, et al. Early and long-term effects of dupilumab treatment on circulating T-cell functions in patients with moderate-to-severe atopic dermatitis. J Invest Dermatol 2021;141:1943–1953.e13

Heeb LEM, Boyman O. Comprehensive analysis of human IL -4 receptor subunits shows compartmentalization in steady state and dupilumab treatment. Allergy 2023;78:1073–87.

Spekhorst LS, Bakker D, Drylewicz J, Rispens T, Loeff F, Boesjes CM, et al. Patient-centered dupilumab dosing regimen leads to successful dose reduction in persistently controlled atopic dermatitis. Allergy 2022;77:3398–407.

Thijs J, Krastev T, Weidinger S, Buckens CF, de Bruin-Weller M, Bruijnzeel-Koomen C, et al. Biomarkers for atopic dermatitis: a systematic review and meta-analysis. Curr Opin Allergy Clin Immunol 2015;15:453–60.

Gooderham MJ, Hong HC, Eshtiaghi P, Papp KA. Dupilumab: a review of its use in the treatment of atopic dermatitis. J Am Acad Dermatol 2018;78(Suppl. 1):S28–36.

The Long Noncoding RNA *PRANCR* Is Associated with Alternative Splicing of Fibronectin-1 in Keratinocytes

Journal of Investigative Dermatology (2023) **143,** 1825–1830; doi:10.1016/j.jid.2023.01.038

TO THE EDITOR

We recently identified a long noncoding RNA (lncRNA), *PRANCR,* that regulates epidermal renewal and stratification in cell culture and organoid models (Cai et al., 2020), but its mechanism of action is not fully known. We initially hypothesized that *PRANCR* may alter the expression of genes associated with keratinocyte (KC) cell fate (Wu et al., 2012). However, when comparing control with *PRANCR*-depleted KCs, we observed no significant expression changes in KC cell fate genes (Supplementary Figure S1a). This led us to consider post-transcriptional mechanisms. Emerging evidence suggests that lncRNAs can regulate alternative splicing by controlling the level or activity of splicing factors, forming RNA:RNA duplexes with pre-mRNA molecules (Yang et al., 2014), or altering chromatin remodeling (Romero-Barrios et al., 2018). Alternative splicing generates multiple isoforms of the same gene and is involved in cell differentiation, development, and diseases of the epidermis (Tanis et al., 2018). However, whether lncRNAs affect alternative splicing in the skin remains largely unknown.

To detect the alternative splicing events associated with *PRANCR*, we

Abbreviations: EDA, extra-domain A; FN1, fibronectin-1; KC, keratinocyte; lncRNA, long noncoding RNA; SRSF, serine/arginine-rich family of splicing factor

Accepted manuscript published online 9 March 2023; corrected proof published online 23 April 2023

SUPPLEMENTARY MATERIALS AND METHODS

Study design

All included patients participated in the Dutch BioDay registry, which is a large prospective registry that contains daily practice data regarding dupilumab for the treatment of atopic dermatitis (ClinicalTrials.gov identifier: NCT03549416, retrospectively registered 08 June 2018). All patients were treated with dupilumab in daily practice under our biobank protocol, which has been approved by the Medical Ethical Committee of the University Medical Center Utrecht (approval number 17-884), and gave their consent for the collection of blood samples during treatment. At baseline, patients received a loading dose of 600 mg dupilumab, followed by a standard maintenance dose of 300 mg dupilumab every 2 weeks during the first year of treatment. After 1 year of treatment with the standard dose of dupilumab, the dosing interval was prolonged according to the protocol described previously (Spekhorst et al., 2022). The injection intervals were stepwise prolonged and guided by the Eczema Area and Severity Index score. In the case of an Eczema Area and Severity Index ≤ 7, indicating mild disease activity or less, for at least 6 months, patients were eligible for dose reduction. If patients remained in a state of controlled disease (Eczema Area and Severity Index ≤ 7), the dosage was further reduced. At any time, the actual decision for dose reduction of dupilumab was based on shared decision-making between the patient and the physician. Patients with moderate-to-severe atopic dermatitis prolonged the interval from 300 mg dupilumab subcutaneously every 2 weeks to 300 mg dupilumab subcutaneously every 4 weeks, and eventually 300 mg dupilumab subcutaneously every 6 weeks, were retrospectively enrolled in this longitudinal study.

Blood samples were collected from patients before initiation of dupilumab treatment (baseline) and during dupilumab treatment at time points when patients were treated with dupilumab every 2, 4, and 6 weeks for at least 3 months. From a total of 11 patients, PBMCs as well as serum samples were available for analyses. Blood samples from 11 healthy adult volunteers without atopic dermatitis or any other atopic disease were obtained from the Mini Donor Service at the University Medical Center Utrecht, The Netherlands.

Clinical data collection and outcome measures

Clinical data were extracted from the BioDay registry. All patients signed written informed consent, adhering to the principles of the Declaration of Helsinki.

The Eczema Area and Severity Index score was used to assess disease severity and to evaluate the clinical effectiveness. In addition, thymus and regulated chemokine levels, currently the best performing and most accepted biomarker for disease severity in atopic dermatitis (Thijs et al., 2015), were measured in routine care using Quantikine ELISA immunoassays (R&D Systems, Minneapolis, MN).

Cell isolation

PBMCs were isolated using Ficoll-Paque (GE Healthcare, Eindhoven, The Netherlands) density gradient centrifugation. PBMCs were frozen in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 IU/ml penicillin-streptomycin, 20% fetal bovine serum, and 10% DMSO (Sigma-Aldrich, Saint Louis, MO), and stored at −196 °C until use.

Flow cytometry

PBMCs were thawed in a 37 °C water bath, washed, and resuspended in RPMI 1640 medium (Gibco, Grand Island, NY) containing 10% fetal bovine serum with the addition of L-glutamine and penicillin-streptomycin. 1,000,000 PBMCs were plated in round-bottom 96-well plates. To determine cell death, eBioscience Fixable Viability Dye eFluor 506 (Invitrogen, Carlsbad, CA) in PBS was used. Surface staining of multiple T- and B-cell markers (Supplementary Tables S1 and S2) was performed for 25 minutes at 4 °C. Surface staining of IL-4 receptor alpha (CD124) phycoerythrin (PE) was performed for 25 minutes at 37 °C. For intracellular and nuclear staining, cells were fixed and permeabilized using eBioscience Fixation and Permeabilization buffers (Invitrogen) and stained for Ki67 AF467.

For intracellular cytokine production, cells were first stimulated with phorbol 12-myristate 13-acetate (20 ng/ml) (Sigma-Aldrich, Saint Louis, MO) and ionomycin (1.0 mg/ml) (Sigma-Aldrich, Saint Louis, MO) for a total of 4 hours. Golgistop (1/1,500) (BD Biosciences, San Jose, CA) was added for the last 3.5 hours of cell culture. Afterward, cells were incubated with the fixable viability dye and surface antibodies (Supplementary Tables S3 and S4) and then fixed, permeabilized, and intracellularly stained with IFN-γ PE-Cy7, IL-4 BV711, IL-5 PE, IL-13 PerCP-Cy5.5, IL-17A APC, IL-22 APC, and TNF-α PE-Cy7.

Stained cells were resuspended in PBS containing 2% FBS and 0.1% sodium azide (Sigma-Aldrich). Data acquisition was performed on a FACS LSR Fortessa (BD Biosciences), and data were analyzed using FlowJo Software (version 10.8) (Tree Star, Ashland, OR).

Liquid chromatography-tandem mass spectrometry

The quantification of total dupilumab levels in serum was performed with a validated liquid chromatography-tandem mass spectrometry analysis at the department of Clinical Pharmacy (UMC Utrecht, The Netherlands) (Amrani et al., 2021).

Statistical analyses

Statistical analyses were performed using SPSS (for Windows, version 25.0, SPSS, Chicago, IL) and Prism (version 9.3.463, GraphPad Software, San Diego, CA). For the comparison of healthy controls with tapering patients or disease controls, the chi-square test was used for categorical variables and the Mann-Whitney *U* test was used for continuous variables. The Wilcoxon signed-rank test was used to compare two continuous variables in the same patients. *P*-values < 0.05 were considered statistically significant.

SUPPLEMENTARY REFERENCES

Amrani ME, Gerencser L, Huitema ADR, Hack CE, van Luin M, van der Elst KCM. A generic sample preparation method for the multiplex analysis of seven therapeutic monoclonal antibodies in human plasma or serum with liquid chromatography-tandem mass spectrometry. J Chromatogr A 2021; 1655:462489.

Spekhorst LS, Bakker D, Drylewicz J, Rispens T, Loeff F, Boesjes CM, et al. Patient-centered dupilumab dosing regimen leads to successful dose reduction in persistently controlled atopic dermatitis. Allergy 2022;77:3398–407.

Thijs J, Krastev T, Weidinger S, Buckens CF, de Bruin-Weller M, Bruijnzeel-Koomen C, et al. Biomarkers for atopic dermatitis: a systematic review and meta-analysis. Curr Opin Allergy Clin Immunol 2015;15: 453–60.

Supplementary Table S1. Flowcytometry Panel Overview (Panel 3)

Antigen/Target	Fluorochrome	Clone	Dilution	Company	Catalog Number
Fixable Viability Dye	eF506	—	1000	Thermo Fisher Scientific	15560607
CD3	BV605	UCHT1	100	BioLegend	300460
CD4	BV785	RPA-T4	50	BioLegend	300554
CD8	APC-Cy7	SK1	50	BD	557834
CD45RO	ECD	UCHL1	12.5	Beckman Coulter	B49192
CLA	PB	HECA-452	200	BioLegend	321308
CCR4	FITC	205410	16.7	R&D	FAB1567F
Ki67	AF647	B56	50	BD	558615

Supplementary Table S2. Flowcytometry Panel Overview (Panel 4)

Antigen/Target	Fluorochrome	Clone	Dilution	Company	Catalog Number
Fixable Viability Dye	eF780	—	1000	Thermo Fisher Scientific	13539140
CD3	BV605	UCHT1	100	BioLegend	300460
CD4	BV785	RPA-T4	50	BioLegend	300554
CD8	PE-Cy7	SK1	200	BD	335822
CD19	AF700	HIB19	50	eBioscience	56-0199-42
IL-4Ra (CD124)	PE	G077F6	50	BioLegend	355004
IgG4	Biotin	HP6025	50	Invitrogen	A10663
Streptavidine	APC	—	100	Thermo Fisher Scientific	17-4317

Supplementary Table S3. Flowcytometry Panel Overview (Panel 1)

Antigen/Target	Fluorochrome	Clone	Dilution	Company	Catalog Number
Fixable Viability Dye	eF506	—	1000	Thermo Fisher Scientific	15560607
CD3	BV605	UCHT1	100	BioLegend	300460
CD4	BV785	RPA-T4	50	BioLegend	300554
CD8	APC-Cy7	SK1	50	BD	557834
CLA	PB	HECA-452	200	BioLegend	321308
CCR4	FITC	205410	16.7	R&D	FAB1567F
CCR10	PE	314305	50	R&D	FAB3478P
IL-4	BV711	MP4-25D2	12.5	BD	564112
IL-13	PerCP-Cy5.5	JES10-5A2	50	Sony Biotechnology	3109555
IL-22	APC	IL22JOP	40	Thermo Fisher Scientific	17-7222
IFNy	PE-Cy7	4S.B3	200	BD	557844

Supplementary Table S4. Flowcytometry Panel Overview (Panel 2)

Antigen/Target	Fluorochrome	Clone	Dilution	Company	Catalog Number
Fixable Viability Dye	eF506	—	1000	Thermo Fisher Scientific	15560607
CD3	BV605	UCHT1	100	BioLegend	300460
CD4	BV785	RPA-T4	50	BioLegend	300554
CD8	APC-Cy7	SK1	50	BD	557834
CLA	PB	HECA-452	200	BioLegend	321308
CCR4	FITC	205410	16.7	R&D	FAB1567F
IL-5	PE	JES1-39D10	50	BioLegend	500904
IL-17A	APC	BL168	50	BioLegend	512333
TNFa	PE-Cy7	Mab11	400	eBioscience	25-7349-82