Supplementary Appendix

Supplement to: Hagen M, Bucci L, Böltz S, et al. BCMA-targeted T-cell–engager therapy for autoimmune disease. N Engl J Med 2024;391:867-9. DOI: 10.1056/NEJMc2408786

This appendix has been provided by the authors to give readers additional information about the work.

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Methods

Patient selection and enrollment

Patients with treatment-refractory autoimmune disease were recruited at the Department of Internal Medicine 3 (Rheumatology and Immunology) of the Friedrich Alexander University Erlangen-Nurnberg. Eligibility criteria were based on (i) a diagnosis of an autoimmune disease according to established classification criteria, (ii) evidence for B cell involvement based on positivity of characteristic autoantibodies, (iii) active disease and (iv) treatment resistance to at least three different targeted synthetic (ts) or biologic (b) disease modifying anti-rheumatic drugs (DMARDs). Among the 14 patients screened by specialized rheumatologists, 4 patients were selected for teclistamab therapy. The residual 10 patients did either not fulfil severity (N=6) or resistance criteria (N=3) or refused informed consent (N=1).

Teclistamab therapy was offered via a compassionate use program for critically ill patients according to the Arzneimittelgesetz, §21/2 and the Arzneimittel-Härtefall-Verordnung §2 that allows experimental treatment if (i) patients are afflicted by severe life-threatening disease such as SSc, (ii) have failed on previous treatments and (iii) a scientific rationale exists that potential efficacy of the respective treatment in the disease. Interventions are reported to the Legal Authorities (Paul Ehrlich Institute, PEI, Germany). Use of patient data and biomaterial from this study is covered by license 334_18 B of the Institutional Review Board (IRB) of the University Clinic of Erlangen. All procedures were performed in accordance with the Good Clinical Practice guidelines of the International Council for Harmonization. Self-reported and biological sexes were identical in all patients. All participants gave written informed consent for all the procedures and the data sharing according to CARE guidelines and in compliance with the Declaration of Helsinki principles. No commercial sponsor was involved.

Treatment protocol

Teclistamab was administered in an inpatient setting with premedication of 1 g Paracetamol, 16 mg Dexamethasone and 2 mg Clemastine. The treatment schedule for Teclistamab was as follows: day 1 (0.06 mg/kg), day 3 (0.3 mg/kg) and day 5 (1.5 mg/kg) (Fig. 1B). Since peripheral B cells recovered after 12 weeks in patient #1, one maintenance dose (1.5 mg/kg) was administered after 12 weeks in patient #1, while in patients #2-4 maintenance dose was given 4 weeks after dose-up. Acyclovir and Sulfamethoxazole/Trimethoprim were initiated until stable B cell recovery is reached.

All immunosuppressants were stopped. Glucocorticoids were discontinued (patients #1-3). Since patient #4 had been taking prednisolone for several years, it was substituted for

Hydrocortisone and tapered to avoid side effects from steroid withdrawal. A detailed overview of medication at baseline and end of follow-up is given in **Table S2**.

Clinical assessment

Disease activity was assessed at baseline and follow-up by the following scores: modified Rodnan skin score in systemic sclerosis [1], EULAR Sjogren's syndrome disease activity index (ESSDAI) [2] in Sjogren's syndrome, Cutaneous Disease Area and Severity Index (CDASI) [3] in Dermatomyositis and Disease Activity Score (DAS) 28-CRP[4] in rheumatoid arthritis.
In addition, function was assessed by Health Assessment Questionnaire (HAQ) in all patients[5].

Laboratory measurements

Serum level of rheumatoid factor-IgM was tested by nephelometry (Optilite Assay; The Binding Site; Birmingham). Anti-cyclic citrullinated peptide 2 (CCP2) and anti-modified citrullinated vimentin (MCV) antibodies were measured by ELISA (Orgentec, Mainz, Germany). Antibodies against dsDNA were assessed by radioimmunoassay (Tecan IBL). Anti-nuclear antibodies and antibodies against PM-Scl-70, PM-Scl-100, SS-A/Ro, SS-B/La, MDA5 and PL-7 were measured by immunoblots from Euroimmune (Lübeck, Germany). All measurements were taken from distinct samples.

Flow cytometry

Flow cytometry was used to quantify lymphocytes in whole blood during therapy. Absolute cell counts were determined with BD Trucount tubes (BD Biosciences) according to the manufacturer's instructions. The following antihuman antibodies were used for flow cytometry for monitoring T cells and B cells: anti-CD3 (clone SK7), anti-CD19 (clone SJ25C1), anti-CD45 (clone 2D1). Data were acquired on an LSRFortessa (BD Biosciences) and analyzed by FlowJo v10 software (Tree Star). All measurements were taken from distinct samples.

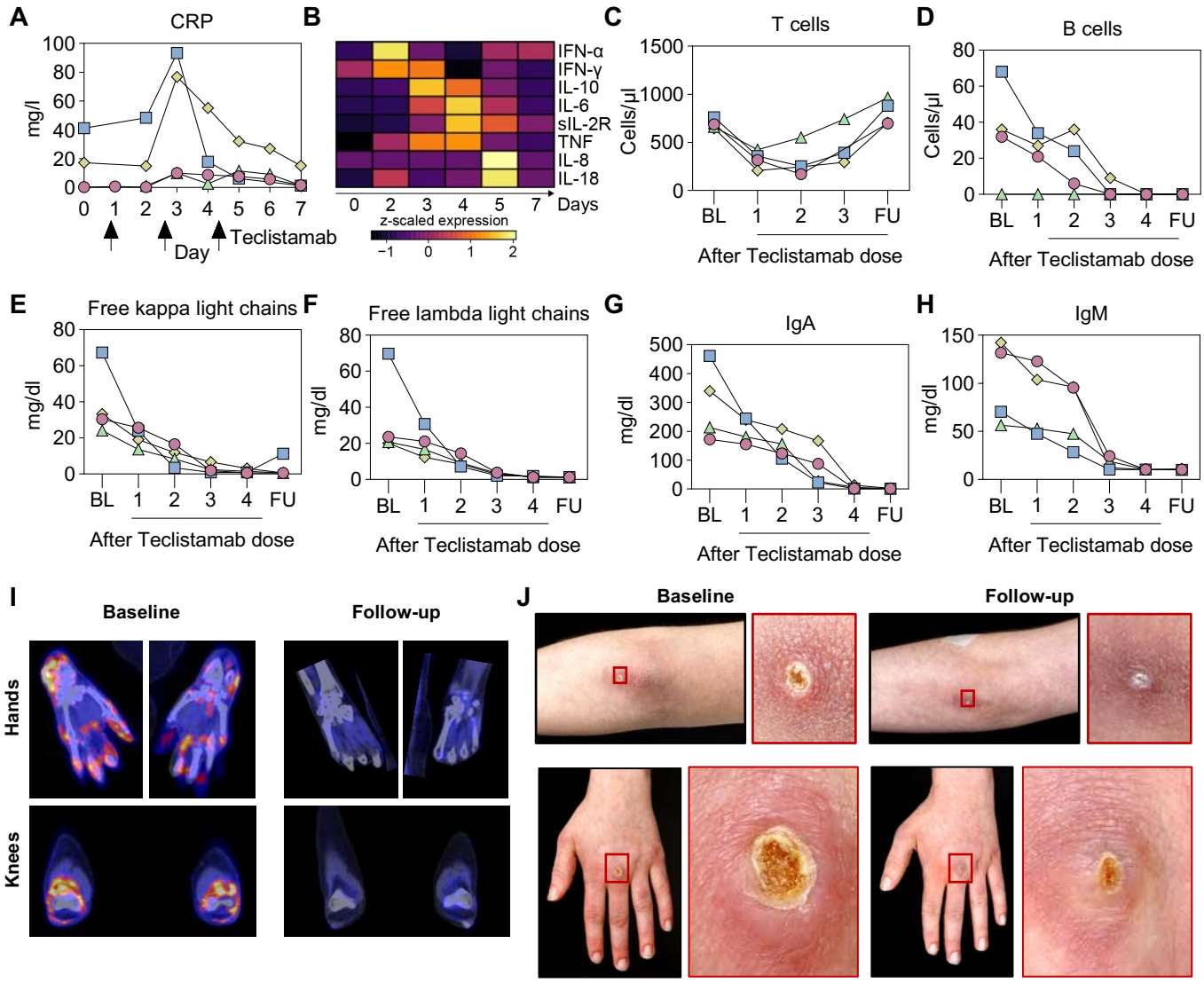
B cells were further phenotyped as follows: Peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation and stained with fluorescently labeled antibodies as shown in **Table S3**. Zombie staining was used for exclusion of dead cells. PBMCs were acquired on a Cytek Northern Lights spectral analyzer (Fremont) and analyzed using FlowJo v10 (Tree Star).

68Ga-FAPI-04 PET-CT scan

68Ga-labeled fibroblast activation protein inhibitor (FAP)I-04 tracer accumulation was detected by a dedicated PET/CT system (Biograph Vision 600, Siemens Healthineers,

Erlangen, Germany)[6]. The covered PET field-of- View (FOV) was from skull to the toes with an additional bed position of the hands (3 min per bed, axial FOV per bed 26.3 cm). PET data were corrected for random and scattered coincidences, as well as for decay during scanning. PET attenuation correction was carried out by the CT portion of the multimodal acquisition. All corrections and reconstructions were obtained using the PET/CT manufacturer's software. PET/CT datasets were analysed with commercially available software (Syngo. via, Siemens Molecular Imaging, Hoffman Estates, Illinois, USA), allowing review of PET, CT and fused imaging data. Visual evaluation was performed by two experienced nuclear medicine physicians and one radiologist. Datasets were analysed by visual interpretation of coronal, sagittal and transverse slices.

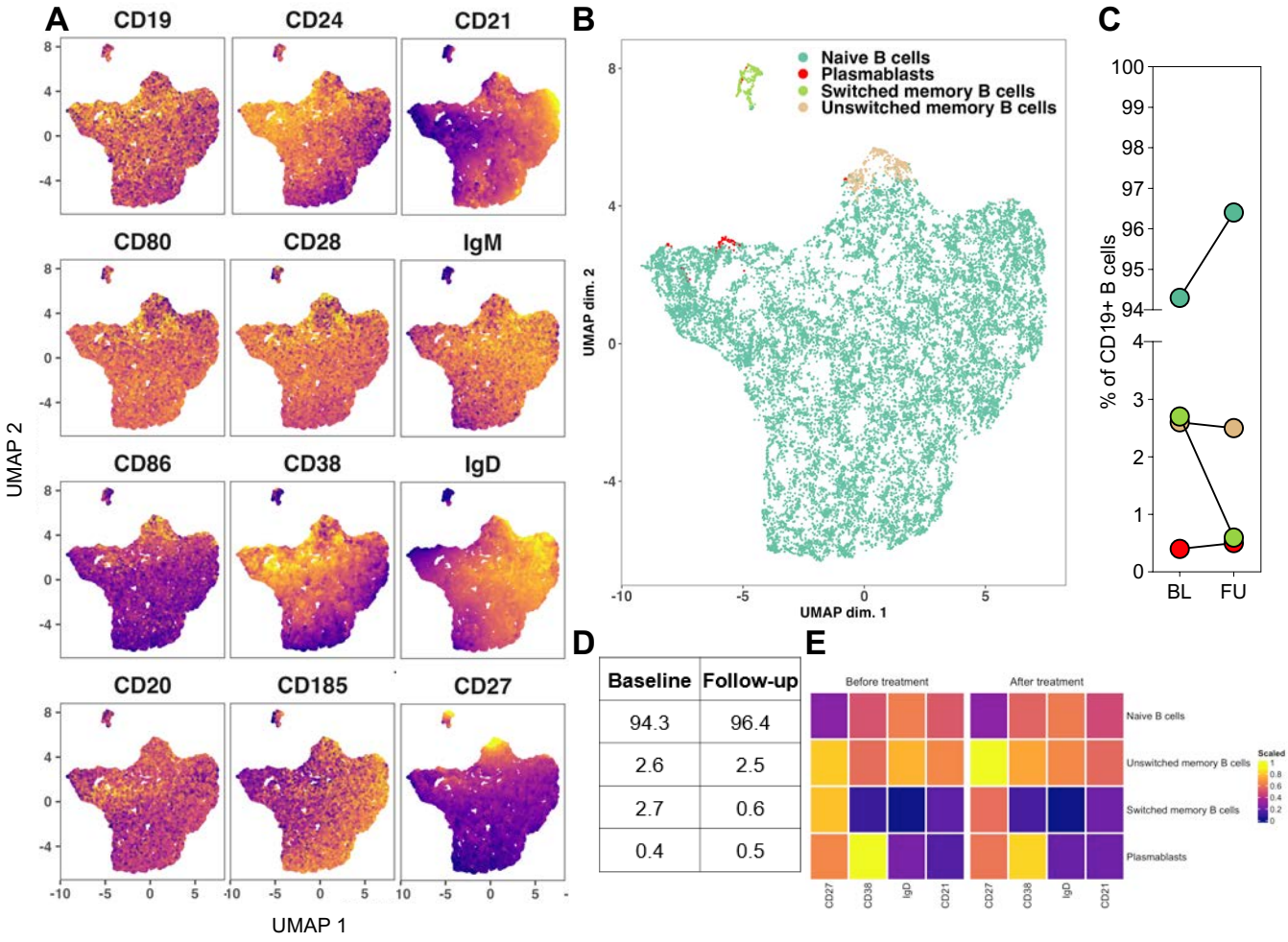
Figures and Tables



Supplementary Figure 1. Laboratory findings and imaging follow-up in autoimmune patients treated with Teclistamab.

A.-H. Longitudinal laboratory changes including **A**. C-reactive protein, **B**. Serum cytokines, **C**. Quantification of CD3+ T cells and **D**. CD19+ B cells in blood via flow cytometry. **E**. Free kappa light chains in serum. **F**. Free lambda light chains in serum. **G**. Immunoglobulin M (IgM) in serum. **H**. Immunoglobulin A (IgA) in serum.

I. Fibroblast activation protein inhibitor (FAPI) positron emission tomography/computed tomography (PET/CT) of patient #2 at baseline and follow-up. **J**. Skin changes in patient #3 between baseline and follow-up.



Baseline	Follow-up
94.3	96.4
2.6	2.5
2.7	0.6
0.4	0.5

Supplementary Figure 2. High dimensional flow cytometry of B cells in patient #1 at baseline and at 12 weeks.

A. Uniform Manifold Approximation and Projection (UMAP) representation of B cell markers in patient #1 at baseline (BL) (CD19+ B cells / nl: 32) and follow-up (FU) at week 12 (CD19+ B cells / nl: 23). **B**. Annotation of B cells according to surface markers. **C.** Quantification of B cells before Teclistamab therapy and at week 12. **D.** Table with B cell frequencies at baseline and follow-up. **E.** Heatmap indicating B cell lineage marker expression before and after Teclistamab therapy.

Previous Treatments	SSc1	PSS2	IIM3	RA4
Glucocorticoids [yes/no]	+	+	+	+
Hydroxychloroquine [yes/no]	+	+	+	0
Methotrexate [yes/no]	+	+	+	+
Azathioprine [yes/no]	0	+	+	0
Leflunomide [yes/no]	0	0	0	+
Mycophenolate [yes/no]	+	+	+	0
Cyclophosphamide [yes/no]	0	0	0	0
Intravenous immunoglobulins [yes/no]	0	0	+	0
TNF-Inhibitor	0	0	0	+
JAK inhibitor [yes/no]	0	0	+	+
Tocilizumab [yes/no]	+	0	0	+
Abatacept [yes/no]	0	0	0	+
Rituximab [yes/no]	+	+	+	+
Blinatumomab [yes/no]	0	0	0	+

Table S1. Overview of previous therapies of patients treated with Teclistamab.
IIM, Idiopathic Inflammatory Myositis; JAK, Janus Kinase; PSS, Primary Sjoegrens Syndrome; RA, Rheumatoid Arthritis; SSc, Systemic Sclerosis; TNF, Tumor Necrosis Factor

Patient	Baseline		Follow-up	
SSC1	Tocilizumab	162 mg/week	0	0
	Prednisolone	5 mg/day	0	0
	Mirtazapin	15 mg/day		
	Vitamin D3	20.000 units/week		
	Vitamin supplements			
	Tenofovir	245 mg/day	Tenofovir	245 mg/day
			Acyclovir	800 mg/day
			Sulfamethoxazole/Trimethoprim	960 mg 3x/week
PSS2	Mycophenolate mofetil	2,000 mg/day	0	0
	Prednisolone	5 mg/day	0	0
	Calcium	500 mg/day		
	Pantoprazole	40 mg/day	Pantoprazole	40 mg/day
	Vitamin D3	1,000 units/day	Vitamin D3	20,000 units/week
			Risedronate	35 mg/week
			Sulfamethoxazole/Trimethoprim	960 mg 3x/week
			Acyclovir	800 mg/day
IIM3	Rituximab	Last dose 12/2023	0	0
	Methotrexate	10 mg/week	0	0
	Prednisolone	7.5 mg/day	0	0
	Folic acid	10 mg/week		
	Vitamin D3	1,000 units/day	Vitamin D3	1,000 units/day
			Pantoprazol	20 mg/day
			Levetiracetam	1000 mg/day
			Salmeterol	25/250 µg as needed
			Isoniazid-comp	360 mg/day
			Sulfamethoxazole/Trimethoprim	960 mg 3x/week
			Aciclovir	800 mg/day
RA4	Methotrexate	20 mg/week	0	0
	Prednisolone	5 mg/day	Hydrocortisone	10 mg/day (2.5 mg Prednisolone equivalent)
	Folic acid	5 mg/week		
	Pantoprazole	40 mg/day		
	VitaminD3	20,000 units/week	Vitamin D3	20,000 units/week
	Diclofenac	As needed	Metamizol	As needed
			Sulfamethoxazole/Trimethoprim	960 mg 3x/week
			Acyclovir	400 mg/day

Legend

Reduced immunosuppression

Discontinued immunosuppression

New prophylaxis until stable B cell recovery

Table S2. Overview of the change in immunosuppression and comedication of patients treated with Teclistamab.

Marker	Fluorochrome (clone)	Company	Dilution	Cat. #
CD19	BV421 (HIB19)	BioLegend	1/400	302233
CD20	AF700 (2H7)	BioLegend	1/1000	302322
CD38	PerCP-Cy5.5 (HIT2)	BD Biosciences	1/200	551400
CD27	PE-Cy7 (M-T271)	BioLegend	1/100	356412
IgD	PE (IA6-2)	BioLegend	1/400	348204
CD24	BV605 (ML5)	BioLegend	1/200	311124
CD185	APC-Fire 810 (J52D4L243)	BioLegend	1/200	356956
CD21	PE-Dazzle 594 (Bu32)	BioLegend	1/200	354922
CD3 (dump)	SparkBlue 550 (SK7)	BioLegend	1/400	344852
Viability	Fixable Viability Stain 780	ThermoFisher	1/2000	65-0865-14

Table S3. Flow cytometry panel used for B cell phenotyping.

Supplementary appendix references

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