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CD19 CAR T-Cell Therapy in Autoimmune Disease — A Case Series with Follow-up

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ABSTRACT

BACKGROUND

Treatment for autoimmune diseases such as systemic lupus erythematosus (SLE), idiopathic inflammatory myositis, and systemic sclerosis often involves long-term immune suppression. Resetting aberrant autoimmunity in these diseases through deep depletion of B cells is a potential strategy for achieving sustained drug-free remission.

METHODS

We evaluated 15 patients with severe SLE (8 patients), idiopathic inflammatory myositis (3 patients), or systemic sclerosis (4 patients) who received a single infusion of CD19 chimeric antigen receptor (CAR) T cells after preconditioning with fludarabine and cyclophosphamide. Efficacy up to 2 years after CAR T-cell infusion was assessed by means of Definition of Remission in SLE (DORIS) remission criteria, American College of Rheumatology–European League against Rheumatism (ACR–EULAR) major clinical response, and the score on the European Scleroderma Trials and Research Group (EUSTAR) activity index (with higher scores indicating greater disease activity), among others. Safety variables, including cytokine release syndrome and infections, were recorded.

RESULTS

The median follow-up was 15 months (range, 4 to 29). The mean (±SD) duration of B-cell aplasia was 112±47 days. All the patients with SLE had DORIS remission, all the patients with idiopathic inflammatory myositis had an ACR–EULAR major clinical response, and all the patients with systemic sclerosis had a decrease in the score on the EUSTAR activity index. Immunosuppressive therapy was completely stopped in all the patients. Grade 1 cytokine release syndrome occurred in 10 patients. One patient each had grade 2 cytokine release syndrome, grade 1 immune effector cell–associated neurotoxicity syndrome, and pneumonia that resulted in hospitalization.

CONCLUSIONS

In this case series, CD19 CAR T-cell transfer appeared to be feasible, safe, and efficacious in three different autoimmune diseases, providing rationale for further controlled clinical trials. (Funded by Deutsche Forschungsgemeinschaft and others.)

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Chimeric antigen receptor (CAR) T cells have emerged as a powerful therapeutic tool to treat cancer.[1] In particular, targeting of malignant B cells by CAR T cells has significantly improved the treatment of hematologic neoplasms. Aside from malignant B cells, autoreactive B cells can also cause severe pathologic effects in the form of autoimmune diseases. Disorders such as systemic lupus erythematosus (SLE), idiopathic inflammatory myositis, and systemic sclerosis are characterized by chronic inflammation, organ damage, and increased mortality.[2-4] Treatment of autoimmune disease with the goal of long-term remission remains challenging. Current intervention strategies do not adequately control the underlying autoimmune process and involve long-term immunosuppressive drug treatment. Targeting B cells in autoimmune disease has long been limited to antibodies that either deplete B cells (e.g., through binding the B-cell–specific surface molecule CD20) or inhibit their activation (e.g., through binding B-cell activating factor).[5-8] Although antibody-based B-cell targeting certainly improved treatment of autoimmune disease, achieving long-lasting drug-free remission has proven elusive. CAR T cells could potentially achieve this goal[9] by deep depletion of B cells through the targeting of the surface molecule CD19, which is expressed on a wide spectrum of B cells and plasmablasts. In the past 2 years, several cases[10-13] and one small case series[14] have suggested the feasibility and short-term efficacy of CD19 CAR T-cell therapy in autoimmune disease. Here, we present data on the short- and long-term efficacy and safety of CD19 CAR T-cell treatment in 15 patients with severe forms of three distinct autoimmune diseases.

Methods

Patients

Details on patient recruitment and characteristics are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org. The protocol is also available at NEJM.org. Patients had to have received a diagnosis of SLE,[15] idiopathic inflammatory myositis,[16] or systemic sclerosis[17]; have a severe and progressive disease course; and be resistant to at least two different, standard-care immunomodulating treatments. CAR T-cell therapy was offered through an expanded-access program for critically ill patients in Germany. All the patients provided written informed consent according to CARE guidelines for case reports and in accordance with the principles of the Declaration of Helsinki.

CAR T-Cell Manufacturing and Treatment

Details on CD19 CAR T-cell manufacturing and the treatment procedure are provided in the Supplementary Appendix. The investigational medicinal product MB-CART19.1 consisted of autologous CD19 CAR–transduced CD4- and CD8-enriched T cells, derived from a leukapheresis product and processed with the use of the CliniMACS Prodigy device (Miltenyi Biotec). Patients received lymphodepleting chemotherapy with fludarabine and cyclophosphamide, as described in the Supplementary Appendix.[10]

Clinical Assessments and Follow-up

SLE disease activity was assessed by means of the score on the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K; range, 0 to 105, with higher scores indicating greater disease activity),[18] Lupus Low Disease Activity State (LLDAS) criteria,[19] and Definition of Remission in SLE (DORIS) remission criteria[20] every 3 months after CAR T-cell transfer. In addition, levels of urinary protein excretion, complement factor C3, and double-stranded DNA (dsDNA) antibodies were assessed every 3 months. In patients with idiopathic inflammatory myositis, values for the 2016 American College of Rheumatology–European League against Rheumatism (ACR–EULAR) Total Improvement Score,[21] creatine kinase level, Manual Muscle Test–8 (MMT-8) score,[22] and extramuscular disease activity score were recorded every 3 months. Values for the ACR-EULAR Total Improvement Score range from 0 to 100, with a score of 0 to 19 indicating no clinical response, 20 to 39 a minimal clinical response, 40 to 59 a moderate clinical response, and 60 to 100 a major clinical response. In patients with systemic sclerosis, clinical response was assessed by means of the score on the European Scleroderma Trials and Research Group (EUSTAR) activity index (range, 0 to 10, with higher scores indicating greater disease activity)[23] and modified Rodnan skin score (range, 0 to 51, with higher scores indicating worse skin fibrosis)[24] every 3 months.

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CLINICAL AND SAFETY ASSESSMENTS

Patients were screened daily for cytokine release syndrome and immune effector cell–associated neurotoxicity syndrome (ICANS) during the first 10 days after CAR T-cell infusion. Cytokine release syndrome and ICANS were assessed according to the American Society for Transplantation and Cellular Therapy consensus criteria.[25] Myelotoxicity was defined as grade III or IV neutropenia or leukocytopenia lasting more than 28 days after CAR T-cell therapy. Further details on adverse-event assessments are provided in the Supplementary Appendix. Monitoring of CAR T cells and leukocyte subsets, quantification of autoantibodies, vaccination-related antibodies, and B-cell analyses are also described in the Supplementary Appendix.

RESULTS

PATIENT CHARACTERISTICS

From February 2021 through May 2023, a total of 15 patients with refractory systemic autoimmune diseases (8 with SLE, 3 with idiopathic inflammatory myositis, and 4 with systemic sclerosis) were treated with CD19 CAR T cells. Preliminary short-term effects of CAR T-cell therapy on Patients 1 through 5 with SLE,[10,14] Patients 1 and 2 with idiopathic inflammatory myositis,[11,26] and Patient 1 with systemic sclerosis[12] have been previously reported.

The characteristics of the patients at baseline are summarized in Table 1. All the patients had had an inadequate response to at least 2 previous immune-suppressive treatments (median, 5; range, 2 to 14). Patients with SLE had active disease with a median SLEDAI-2K score of 13 (interquartile range, 9.3 to 16), at least one A (severe) score on the British Isles Lupus Assessment Group (BILAG) 2004 index, and the presence of histologically proven glomerulonephritis of World Health Organization grade III or IV. All the patients with idiopathic inflammatory myositis and systemic sclerosis had interstitial lung disease with reduced forced vital capacity (median, 53% [interquartile range, 49 to 64] and 36% [interquartile range, 24 to 53], respectively) and reduced diffusing capacity of the lungs for carbon monoxide (median, 67% [interquartile range, 49 to 75] and 59% [interquartile range, 34 to 72], respectively). Patients with idiopathic inflammatory myositis showed active muscle involvement (median creatine kinase level, 4298 U per liter; interquartile range, 766 to 9305), whereas in patients with systemic sclerosis, skin involvement was active (median modified Rodnan skin score, 25.5; interquartile range, 18.8 to 30.8). Eight patients had received B-cell–depleting agents, and 9 patients had received cyclophosphamide (median dose, 3 g; interquartile range, 1 to 6; mean [±SD], 5.3±6.9 g). Details of the sequence of immune-suppressive drugs received before CAR T-cell treatment are shown in Figure S1 in the Supplementary Appendix.

The proliferation of cells during manufacturing (Fig. S2A and S2B), transduction efficacy (Fig. S2C), and cellular composition of the individual autologous CD19 CAR T-cell products (Fig. S2D) were highly consistent among the 15 patients. In all 15 patients, more than 1×10^9 CD19 CAR T cells could be produced, with transduction efficiencies between 18% and 44%. Most CD19 CAR T-cell products (except in Patient 8 with SLE and Patients 1 and 4 with systemic sclerosis) contained a higher number of CD4+ cells than CD8+ T cells.

CAR T-CELL EXPANSION AND B-CELL DEPLETION

After infusion, CAR T cells rapidly expanded in vivo, reaching peak concentrations after a mean of 8.6±0.8 days (median, 9 days; interquartile range, 9 to 9) (Fig. S3A). At the peak of expansion, the median number of circulating CAR T cells was 146 per microliter (interquartile range, 61 to 697). CD19+ B cells were rapidly eliminated from peripheral blood after a mean of 5.9±2.2 days (median, 7 days; interquartile range, 3 to 7). In 14 of 15 patients, B cells reappeared after a mean of 112±47 days (median, 100 days; interquartile range, 72 to 153), and the last patient (Patient 4 with systemic sclerosis; 128 days of follow-up) still awaited B-cell reconstitution (Fig. S3B). Details on the effects on leukocyte and lymphocyte counts are shown in the Supplementary Appendix, including Figure S3C and S3D.

Patients who were exposed to B-cell–depleting antibodies (see the Supplementary Appendix) had lower B-cell counts at baseline and a longer B-cell aplasia time than those who were not exposed to B-cell–depleting antibodies (median, 138 days [interquartile range, 66 to 189] vs. 86 days [interquartile range, 70 to 124]) (Table S1). In addition, the peak of CAR T-cell expansion

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Table 1. Characteristics of 15 Patients with Autoimmune Disease at Baseline.*

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15
Age (yr)	20	23	22	24	18	38	33	35	41	43	42	60	36	37	47
Sex	F	M	F	F	F	F	F	F	M	F	M	M	M	F	M
Disease	SLE	SLE	SLE	SLE	SLE	SLE	SLE	SLE	IIM	IIM	IIM	SSc	SSc	SSc	SSc
Disease duration (yr)	4	1	6	9	3	18	1	20	2	5	1	2	2	1	11
Follow-up (mo)	29	25	21	19	15	15	12	6	18	18	5	13	10	7	4
Autoantibodies															
Lead	dsDNA	dsDNA	dsDNA	Sm	dsDNA	dsDNA	dsDNA	dsDNA	Jo-1	Jo-1	PL-7	RNAP III	Scl70	Scl70	Scl70
Co-lead	—	Sm	—	—	Sm	Sm	—	—	—	Pm-Scl100	—	—	—	—	—
Other	—	—	PCNA	Ro60	Ku	Ro52/60	RNP	RNP	—	Ro52	Ro52	—	—	Ro60	—
Organ involvement															
Skin	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+
Kidney	+	+	+	+	+	+	+	+	0	0	0	0	0	+†	0
Nephritis (WHO grade)	III	III	IV	III–V	III–V	IV	IV	IV	0	0	0	0	0	0	0
Lungs	+	0	+	+	0	0	0	+	+	+	+	+	+	+	+
Heart	+	0	0	+	0	0	0	0	0	0	0	+	+	0	0
Bone marrow	+	0	0	0	+	+	0	0	0	0	0	0	0	0	0
Muscles	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0
Joints	0	+	+	+	+	+	0	+	0	+	0	+	+	0	0
Treatments															
Glucocorticoids	+	+	+	+	+	+	+	+	+	+	+	0	+	0	0
HCQ	+	+	+	+	+	+	+	+	0	0	0	0	+	0	0
Mycophenolate	+	+	+	+	+	+	+	+	0	+	0	+	+	+	+
Methotrexate	0	0	0	+	0	+	0	+	0	0	0	+	0	+	0
Azathioprine	0	0	0	+	+	+	0	+	0	0	0	0	0	0	0

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CPM	+	+	+	0	0	+	+	0	+	+	+	0	0	0	+
Tacrolimus	+	0	0	0	0	+	+	0	+	+	0	0	0	0	0
Rituximab	+	0	0	0	0	+	+	+	+	+	+	0	0	+	0
Belimumab	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0
IVIG	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0
Other	0	0	0	LEF	0	+‡	0	+§	0	+¶	OCR	0	0	TOC	NIN

* CPM denotes cyclophosphamide, dsDNA double-stranded DNA, HCQ hydroxychloroquine, IIM idiopathic inflammatory myositis, IVIG intravenous immunoglobulins, LEF leflunomide, NIN nintedanib, OCR ocrelizumab, PCNA proliferating-cell nuclear antigen, Pm-Scl100 polymyositis and scleroderma 100, RNAP III RNA polymerase III, RNP ribo-nucleoprotein, Scl70 scleroderma 70, SLE systemic lupus erythematosus, Sm Smith, SSc systemic sclerosis, TOC tocilizumab, and WHO World Health Organization.

† Patient 14 had grade IV chronic kidney insufficiency due to renal crisis.

‡ Patient 6 received leflunomide, bortezomib, upadacitinib, immunoadsorption, and ustekinumab.

§ Patient 8 received photopheresis, lenalidomide, thalidomide, ustekinumab, and interleukin-2.

¶ Patient 10 received tofacitinib, tocilizumab, and ocrelizumab.

was lower in patients who had previously received rituximab than in those who had not previously received rituximab (median, 91 cells per microliter [interquartile range, 38 to 270] vs. 461 cells per microliter [interquartile range, 61 to 1275]). The duration of CAR T-cell presence in the blood was shorter in patients who had previously received rituximab than in those who had not previously received rituximab (median, 40 days [interquartile range, 30 to 61] vs. 58 days [interquartile range, 26 to 79]).

SHORT- AND LONG-TERM EFFICACY

After CD19 CAR T-cell administration, patients were regularly monitored for their disease activity. All eight patients with SLE met LLDAS criteria and had DORIS remission after 6 months (Fig. 1A). Their SLEDAI-2K score was equal to zero after 6 months. Analysis of BILAG status at baseline and follow-up showed resolution of SLE in all major disease categories after 6 months (Fig. S4). Long-term follow-up of up to 29 months showed that SLE disease activity remained absent in all eight patients (SLEDAI-2K score, 0) (Fig. 1B). Furthermore, anti-dsDNA antibodies disappeared and remained negative, complement factor C3 levels normalized, and proteinuria disappeared during the entire observation period. Patient 4 with SLE, who had a rebound of proteinuria 4 months after CAR T-cell therapy and in whom follow-up renal biopsy did not reveal any histopathological signs of lupus nephritis (but concomitant podocytopathy[14]), did not have a relapse and had only very mild proteinuria at the last follow-up (24 months).

All three patients with idiopathic inflammatory myositis had an ACR–EULAR major clinical response and normalization of creatine kinase levels after 3 months and maintained these responses (Fig. 1A and 1C). Muscular function as measured by MMT-8 normalized in all three patients, and extramuscular disease activity ceased (Fig. 1C). In the four patients with systemic sclerosis, global disease activity as measured by the score on the EUSTAR activity index and skin activity as measured by the modified Rodnan skin score decreased in all four patients. In the three patients with at least 6 months of follow-up, the median change in the EUSTAR activity index was −4.2 points (interquartile range, −4.7 to −2.3) and in the modified Rodnan skin score was −9 points (interquartile range,

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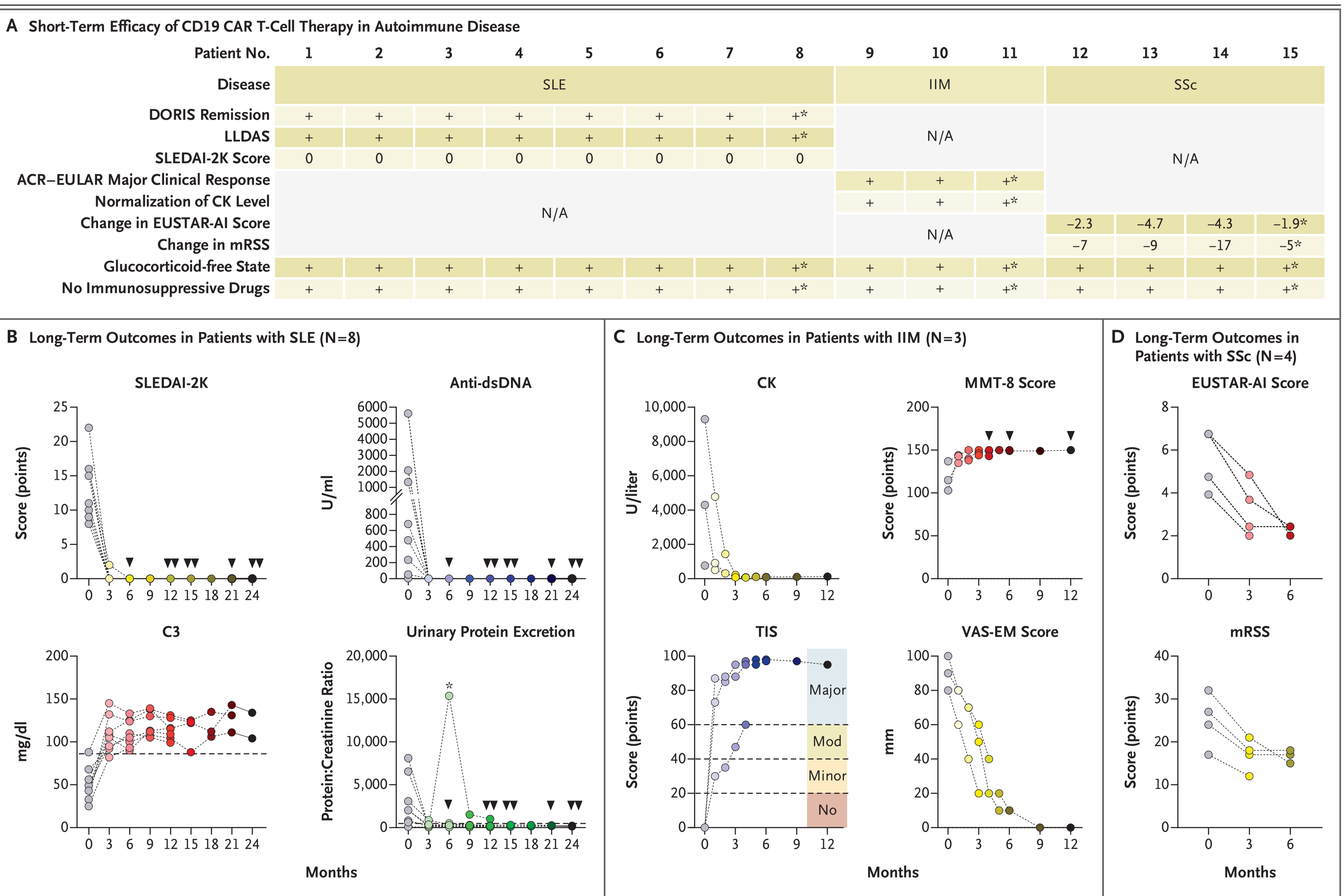

A Short-Term Efficacy of CD19 CAR T-Cell Therapy in Autoimmune Disease

Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Disease	SLE								IIM			SSc			
DORIS Remission	+	+	+	+	+	+	+	+*	N/A			N/A			
LLDAS	+	+	+	+	+	+	+	+*							
SLEDAI-2K Score	0	0	0	0	0	0	0	0							
ACR–EULAR Major Clinical Response	N/A								+	+	+*				
Normalization of CK Level									+	+	+*				
Change in EUSTAR-AI Score									N/A			−2.3	−4.7	−4.3	−1.9*
Change in mRSS												−7	−9	−17	−5*
Glucocorticoid-free State	+	+	+	+	+	+	+	+*	+	+	+*	+	+	+	+*
No Immunosuppressive Drugs	+	+	+	+	+	+	+	+*	+	+	+*	+	+	+	+*

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Figure 1 (facing page). Clinical Efficacy of CD19 CAR T-Cell Therapy in Autoimmune Disease.

Panel A summarizes outcomes in 8 patients with systemic lupus erythematosus (SLE), 3 patients with idiopathic inflammatory myositis (IIM), and 4 patients with systemic sclerosis (SSc) treated with CD19 chimeric antigen receptor (CAR) T cells after 6 months. The asterisks in the columns for Patients 8, 11, and 15 reflect data at 3 months. Scores on the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) range from 0 to 105, with higher scores indicating greater disease activity. Scores on the European Scleroderma Trials and Research Group activity index (EUSTAR-AI) range from 0 to 10, with higher scores indicating greater disease activity. Values for the modified Rodnan skin score (mRSS) range from 0 to 51, with higher scores indicating worse skin fibrosis. ACR–EULAR denotes American College of Rheumatology–European League against Rheumatism, CK creatine kinase, DORIS Definition of Remission in SLE, LLDAS Lupus Low Disease Activity State, and N/A not applicable. Panel B shows long-term outcomes in patients with SLE, including SLEDAI-2K scores and levels of anti–double-stranded DNA (dsDNA) antibodies, complement factor 3 (C3), and urinary protein excretion. Arrowheads indicate observation periods of up to 24 months. For the C3 level, the dashed horizontal line indicates the lower limit of the normal range. For urinary protein excretion, the dashed horizontal line near the x axis indicates the upper limit of the normal range, and the asterisk indicates a bout of proteinuria that led to kidney biopsy with evidence of lupus nephritis (podocytopathy). The protein:creatinine ratio was calculated with urinary protein measured in milligrams and urinary creatinine in grams. Panel C shows long-term outcomes in patients with IIM. Scores on the Manual Muscle Test–8 (MMT-8) range from 0 to 150, with lower scores indicating weaker muscles. Values for the ACR–EULAR Total Improvement Score (TIS) range from 0 to 100, with 0 to 19 indicating no clinical response, 20 to 39 a minimal clinical response, 40 to 59 a moderate clinical response, and 60 to 100 a major clinical response. Scores on the visual-analogue scale of extramuscular symptoms (VAS-EM) range from 0 to 100 mm, with higher scores indicating greater disease activity. Arrowheads indicate observation periods of up to 12 months. Panel D shows long-term outcomes in patients with systemic sclerosis.

−17 to −7) (Fig. 1A and 1D). All 15 patients successfully discontinued glucocorticoids and all other immunosuppressive medications as of the final follow-up (median, 15 months; interquartile range, 7 to 19).

Analysis of Antibody Repertoire

Analysis of autoantibody repertoire in patients with SLE showed seroconversion of antibodies against dsDNA, single-stranded DNA, secondary necrotic cells, nucleosomes, and Smith protein (Fig. 2A). Long-term analysis of autoantibodies in patients with SLE with at least 1 year of follow-up (seven of eight patients) confirmed a sustained absence of SLE-specific autoantibodies (Fig. S5A). Assessment of antinuclear and anti-Ro60 antibodies is shown in Fig. 2B, and detailed analysis of disease-associated autoantibodies in patients with idiopathic inflammatory myositis or systemic sclerosis is shown in Figure 2C.

Protective IgG responses to standard vaccines — including tetanus, measles, mumps, rubella, varicella–zoster virus, and Epstein–Barr virus — remained stable, whereas moderate decreases, but not loss, of antibodies against pneumococci and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were noted in the long-term observation (Fig. 2D). Vaccinations against SARS-CoV-2 (in two patients), pneumococcus (in one patient), and tetanus (in two patients) that occurred after CD19 CAR T-cell therapy and B-cell reconstitution resulted in a subsequent increase in antigen-specific IgG levels. The results of long-term analysis of antivaccine antibody responses in patients with SLE are shown in Figure S5B.

Analysis of B-Cell Phenotype

Analysis of B-cell subsets was performed at baseline, 4 months, and 12 months after CAR T-cell therapy. Gating strategies for B cells in fluorescence-activated cell-sorting analyses are shown in Figure S6. Reconstituted B cells at 4 and 12 months showed a naive B-cell phenotype, whereas CD19+CD27+ memory B cells were drastically reduced (Fig. 3A). There was a limited increase in CD19+CD27+ memory B cells between 4 and 12 months, which was predominantly seen in preswitched IgD+ CD27+ B cells. CD27+CD38+ plasmablasts and SLE-associated activated CD11c+ memory B cells were not found after B-cell reconstitution. Immature CD38+ B cells, indicating reconstitution of B cells from the bone marrow, increased at 4 months and decreased thereafter. Similar results were seen in patients with idiopathic inflammatory myositis and systemic sclerosis, although depletion of memory B cells appeared to be not as deep as in patients with SLE at 4 and 12 months (Fig. 3B and 3C). Details of single-cell sequencing–based

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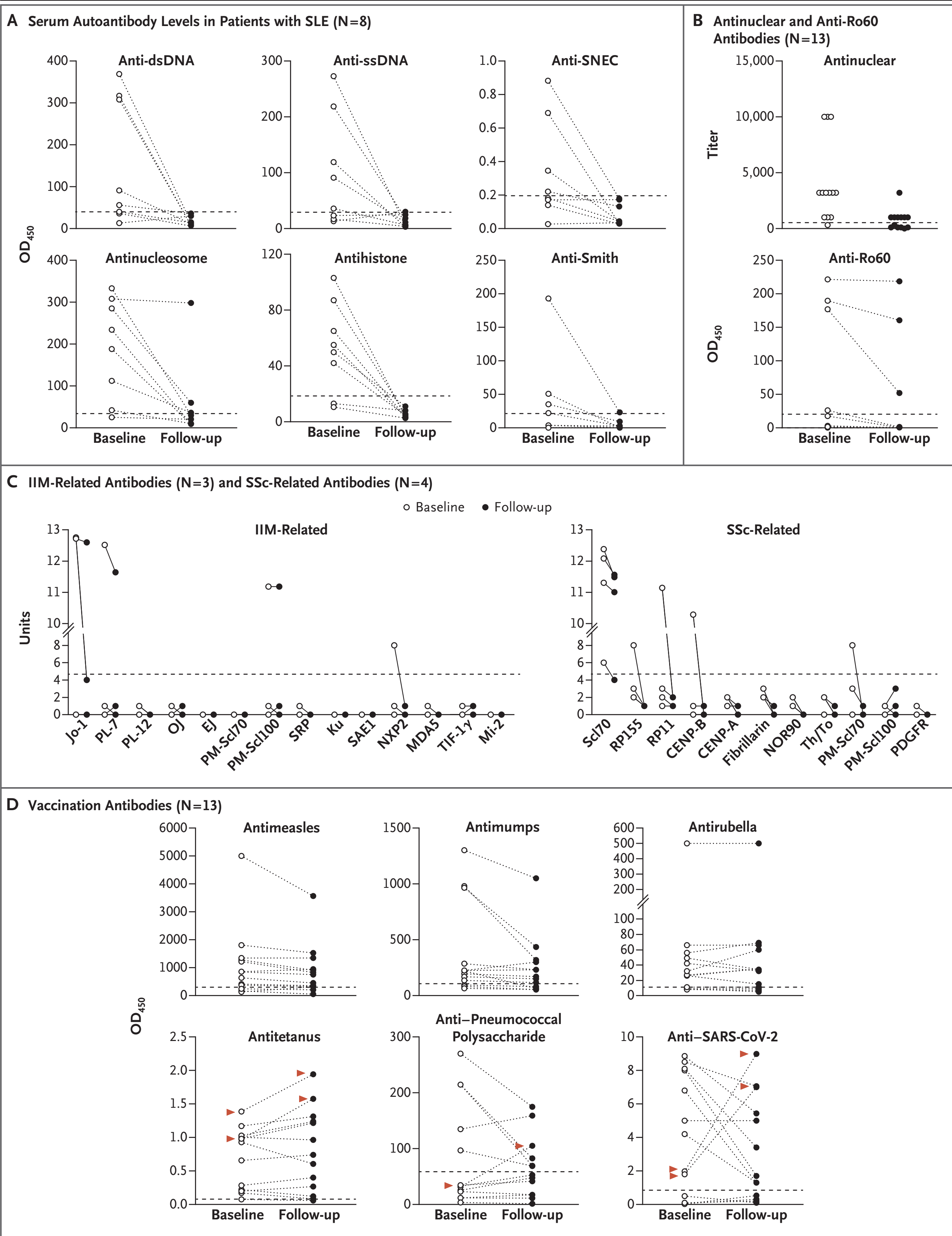

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Figure 2 (facing page). Analysis of Antibody Repertoires after CAR T-Cell Therapy in Autoimmune Diseases.
Panel A shows serum autoantibody levels in 8 patients with SLE treated with CD19 CAR T cells at baseline and 6 months of follow-up. OD_{450} denotes optical density at a wavelength of 450 nm, SNEC secondary necrotic cells, and ssDNA single-stranded DNA. Panel B shows titers of antinuclear antibodies and levels of anti-Ro60 antibodies in 13 patients (except Patients 11 and 15) with autoimmune diseases treated with CD19 CAR T cells at baseline and 6 months of follow-up. Panel C shows profiling of IIM-related and SSc-related antibodies in 3 patients with IIM and 4 patients with SSc before and 6 months after CAR T-cell treatment. CENP-A denotes centromere protein A, CENP-B centromere protein B, MDA5 melanoma differentiation–associated protein 5, NOR90 nucleolus organizing region 90, NXP2 nuclear matrix protein 2, PDGFR platelet-derived growth factor receptor, PM-Scl70 polymyositis and scleroderma 70, PM-Scl100 polymyositis and scleroderma 100, RP RNA polymerase, SAE1 SUMO-activating enzyme subunit 1, SRP signal recognition particle, and TIF-1γ transcriptional intermediary factor 1γ. Panel D shows vaccination antibodies in 13 patients with autoimmune diseases treated with CD19 CAR T cells at baseline and 6 months of follow-up. SARS-CoV-2 denotes severe acute respiratory syndrome coronavirus 2. Red arrowheads indicate vaccination responses. Throughout the figure, the dashed horizontal lines indicate cutoffs between positive and negative responses.

analysis of immunoglobulin heavy and light chains are shown in the Supplementary Appendix, including Figures S7 and S8.

Short- and Long-Term Safety

No moderate- or high-grade cytokine release syndrome or ICANS occurred. With respect to short-term safety, 11 of 15 patients had cytokine release syndrome, with 10 patients having grade 1 (fever) and 1 additional patient having grade 2 (Patient 3 with idiopathic inflammatory myositis) (Table 2). Cytokine release syndrome started after a median of 1 day (interquartile range, 1 to 7) and lasted for a median of 5 days (interquartile range, 2 to 7). Tocilizumab was administered in 6 of 15 patients (3 of 8 with SLE, 3 of 3 with idiopathic inflammatory myositis, and 0 of 4 with systemic sclerosis). Details on body temperature, blood pressure, heart rate, and C-reactive protein levels are shown in Figure S9. Dizziness that was interpreted as possible mild ICANS resolved after glucocorticoid therapy in 1 patient (Patient 2 with idiopathic inflammatory myositis) 2 weeks after the infusion. No prolonged (>28 days) or biphasic bone marrow suppression occurred in any of the patients. One patient had grade 4 neutropenia (leukocytes, 1710 per microliter, and neutrophils, 200 per microliter) 120 days after CD19 CAR T-cell treatment, which resolved after cessation of sertraline, pregabalin, and doxazosin, and after three injections of 30 million units of granulocyte colony-stimulating factor. Details on the recovery of lymphocytes, CD4 T cells, CD8 T cells, and natural killer cells are summarized in Figure S10.

With respect to long-term safety, infections were recorded (Table 3). Patient 8 with SLE was admitted to the hospital owing to pneumonia 7 weeks after CAR T-cell therapy, which resolved with antibiotic treatment. All other infections were mild and mostly manifested as upper respiratory tract infections. The occurrence of new-onset hypogammaglobulinemia was uncommon. Details on immunoglobulins are presented in the Supplementary Appendix, including Figure S11.

Discussion

These data provide evidence for the feasibility, preliminary efficacy, and side-effect profile of CD19 CAR T-cell therapy in patients with severe autoimmune disease. Despite differences in disease entities and previous treatments, the dynamics of CAR T-cell expansion and of B-cell ablation were highly consistent among patients. CD19 CAR T-cell therapy was effective independent of previous B-cell targeting by monoclonal antibodies. Because B-cell targeting by monoclonal antibodies is not approved as treatment for SLE, idiopathic inflammatory myositis, and systemic sclerosis, previous exposure to such agents was not a prerequisite for treatment. In patients with SLE and idiopathic inflammatory myositis, complete resolution of disease symptoms was observed, whereas patients with systemic sclerosis showed reduced severity of skin and lung disease. It is notable that all the patients could successfully stop their immunosuppressive medication without having relapses or worsening of their disease.

Given that patients had full B-cell reconstitution for up to 2 years without having relapses, it seems that a single injection of CD19 CAR T-cell therapy can lead to a long-lasting remission.

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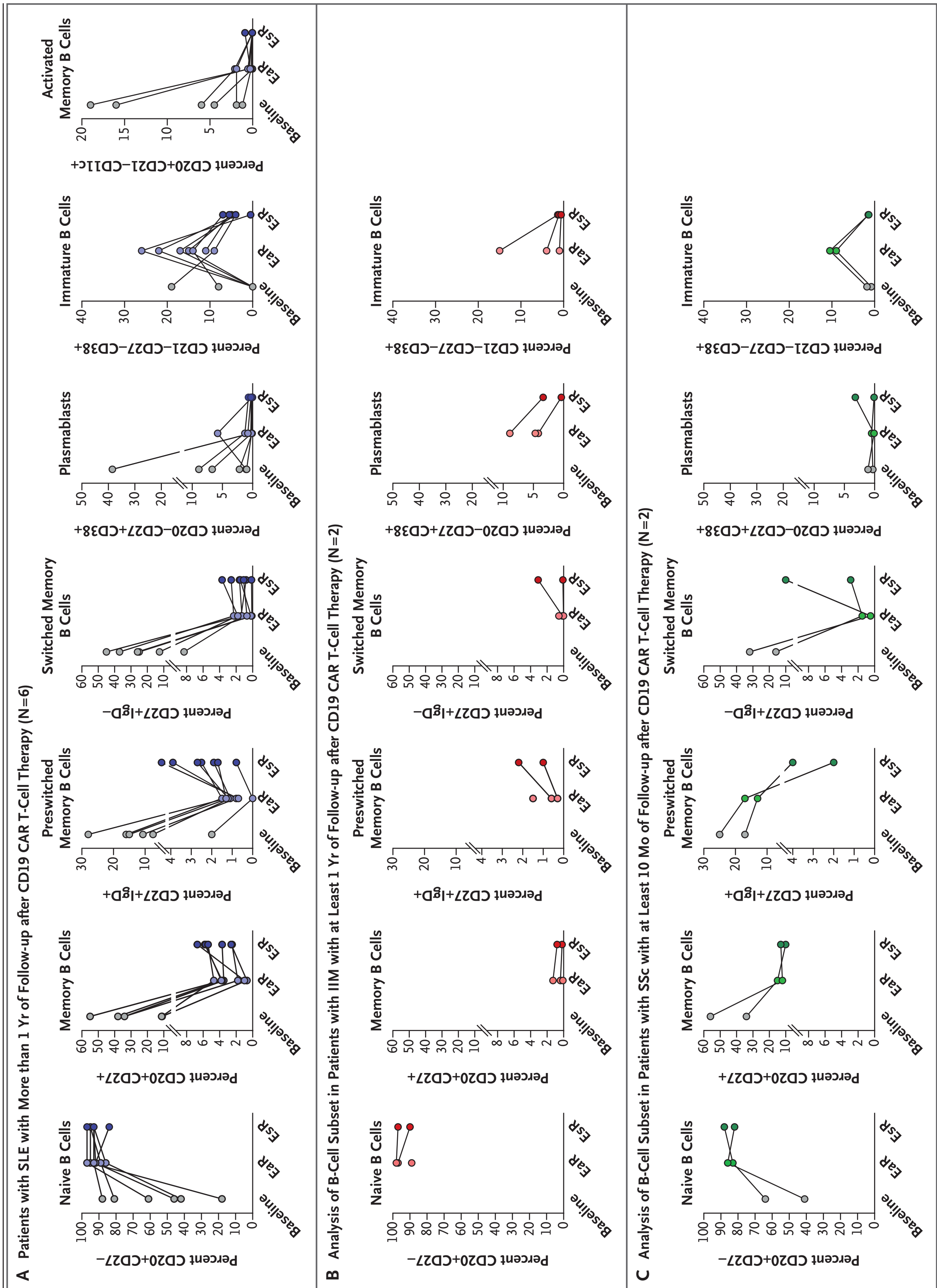

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Figure 3 (facing page). Analysis of B-Cell Subsets after CAR T-Cell Therapy in Autoimmune Diseases.

Panel A shows naive (CD20+CD27−), memory (CD20+CD27+), preswitched memory (CD27+IgD+), and switched memory (CD27+IgD−) B cells, CD20−CD27+CD38+ plasmablasts, immature CD21−CD27−CD38+ B cells, and CD20+CD21−CD11c+ activated memory B cells in six patients with SLE with more than 1 year of follow-up after CD19 CAR T-cell therapy; EaR denotes early reconstitution approximately 4 months after treatment, and EsR established reconstitution 1 year after treatment. Panel B shows an analysis of B-cell subset in two patients with IIM with at least 1 year of follow-up after CD19 CAR T-cell therapy; baseline values are not available because patients had previously been exposed to rituximab before CD19 CAR T-cell therapy. Panel C shows an analysis of B-cell subset in two patients with SSc with at least 10 months of follow-up after CD19 CAR T-cell therapy. Values indicate the percentages of total B cells.

This stable remission state is also supported by the sustained diminution of autoantibodies, which usually precede disease onset in autoimmune disease.[27] In addition, the appearance of a naive non–class-switched B-cell system, the disappearance of circulating plasmablasts, and the down-regulation of specific disease-associated heavy and light chains support the concept that CD19 CAR T-cell therapy may have induced a reset of pathologic autoimmunity in these patients. Hence, although CAR T-cell therapy is directed to both pathogenic and nonpathogenic B cells, the rebooting of the B-cell system after the disappearance of CAR T cells obviously occurs in the absence of the pathogenic B cells that trigger autoantibody production.

All the patients received lymphodepleting chemotherapy with fludarabine and cyclophosphamide. It cannot be ruled out that this treatment contributed to the short-term effects of CD19 CAR T-cell treatment. However, complete B-cell depletion, abrogation of autoantibodies, and sustained drug-free remission are highly unlikely to be induced by mere lymphodepleting chemotherapy. Many patients were exposed to substantially higher doses (>5 g) of cyclophosphamide before CAR T-cell administration and showed no clinical effects. Fludarabine has been tested in small case series of patients with SLE and idiopathic inflammatory myositis.[28,29] Doses were 6 times as high as the lymphodepleting regimen used in our study but were not effective in patients with idiopathic inflammatory myositis

Table 2. Short-Term Safety of CD19 CAR T-Cell Therapy in Autoimmune Disease.*

Variable	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15
Disease	SLE	SLE	SLE	SLE	SLE	SLE	SLE	SLE	IIM	IIM	IIM	SSc	SSc	SSc	SSc
CRS (grade)	0	1	1	1	0	1	0	1	1	1	2	1	1	1	0
ICANS (grade)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Bone marrow toxicity†	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOC treatment	0	0	0	+	0	+	0	+	+	+	+	0	0	0	0
GLC treatment	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0
Low IgG	+	+	+	0	0	0	0	+‡	+‡	0	0	0	0	0	0
IgG substitution	0	+	0	0	0	0	0	+	0	0	0	0	0	0	0

* CAR denotes chimeric antigen receptor, CRS cytokine release syndrome, GLC glucocorticoid, and ICANS immune-effector cell–associated neurotoxicity syndrome.
† Bone marrow toxicity was defined as persisting grade 2 or higher thrombocytopenia, leukopenia, or granulocytopenia at or beyond day 28 after CD19 CAR T-cell therapy.
‡ Patients 8 and 9 had preexisting hypogammaglobulinemia due to previous rituximab exposure.

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Table 3. Long-Term Safety of CD19 CAR T-Cell Therapy in Autoimmune Disease.*

Patient No.	Disease	<3 Months	3–6 Months	6–12 Months	>12 Months
1	SLE	UTI	0	0	URTI (nonspecified)
2	SLE	0	0	URTI (SARS-CoV-2†)	URTI (nonspecified)
3	SLE	URTI (SARS-CoV-2)	0	URTI (nonspecified)	URTI (SARS-CoV-2) and herpes zoster
4	SLE	0	0	0	Otitis
5	SLE	0	URTI (SARS-CoV-2†)	0	0
6	SLE	0	URTI (SARS-CoV-2† and RSV)	URTI (SARS-CoV-2†)	URTI (nonspecified)
7	SLE	0	0	0	
8	SLE	Pneumonia	0		
9	IIM	0	Enteritis (nonspecified)	0	0
10	IIM	0	Herpes simplex	0	0
11	IIM	URTI (nonspecified)	0		
12	SSc	0	URTI (*Haemophilus influenzae*)	0	0
13	SSc	0	Cellulitis	Herpes zoster	
14	SSc	URTI (SARS-CoV-2†)	0		
15	SSc	0			

* Shown is the infection profile of patients with autoimmune disease undergoing CD19 CAR T-cell therapy. Data are presented chronologically and according to site of infection. RSV denotes respiratory syncytial virus, SARS-CoV-2 severe acute respiratory syndrome coronavirus 2, URTI upper respiratory tract infection, and UTI urinary tract infection.
† The infection was treated with nirmatrelvir–ritonavir (Paxlovid).

and induced severe bone marrow toxic effects in patients with SLE. In addition, the fast recovery of all leukocyte lineages, except B cells, within 3 weeks after lymphodepleting chemotherapy make sustained effects of this regimen very unlikely. However, whether and what doses of lymphodepletion are needed in autoimmune disease need to be further explored.

The adverse effects of CD19 CAR T-cell treatment were minimal, with no high-grade cytokine release syndrome or ICANS and no prolonged bone marrow toxic effects. In addition, the incidence of infections was low, and total immunoglobulin levels were only moderately decreased. Nonetheless, careful monitoring of these patients is warranted because immunoglobulin levels decreased to some extent and infections occurred during the follow-up. Although a decrease of immunoglobulin levels after CD19 CAR T-cell therapy is expected owing to the elimination of antibody-producing plasmablasts, a substantial proportion of serum immunoglobulins, such as vaccination-related antibodies, were not eliminated, which implies that CD19-negative plasma cells may not be substantially affected by the therapy. Similar results, which suggest different sources (plasmablasts vs. plasma cells) of antibody production, have also been obtained from studies using B-cell–depleting antibodies in SLE.[30] With long-lived plasma cells less affected by CD19 CAR T-cell therapy, it remains an open question whether new vaccinations are necessary and how they will affect the recovery of the memory B-cell compartment.

Although these data provide new evidence for the short- and long-term safety and efficacy of CD19 CAR T-cell therapy in autoimmune disease, controlled clinical studies are needed. Even though it is premature to judge whether these patients are indeed cured from their autoimmune disease, CD19 CAR T cells at least appear to be able to achieve sustained disease- and drug-free remission.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

APPENDIX

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REFERENCES

1. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science 2018;359:1361-5.
2. Dörner T, Furie R. Novel paradigms in systemic lupus erythematosus. Lancet 2019;393:2344-58.
3. Lundberg IE, Fujimoto M, Vencovsky J, et al. Idiopathic inflammatory myopathies. Nat Rev Dis Primers 2021;7:86.
4. Denton CP, Khanna D. Systemic sclerosis. Lancet 2017;390:1685-99.
5. Furie RA, Aroca G, Cascino MD, et al. B-cell depletion with obinutuzumab for the treatment of proliferative lupus nephritis: a randomised, double-blind, placebo-controlled trial. Ann Rheum Dis 2022;81:100-7.
6. Smith V, Van Praet JT, Vandooren B, et al. Rituximab in diffuse cutaneous systemic sclerosis: an open-label clinical and histopathological study. Ann Rheum Dis 2010;69:193-7.
7. Oddis CV, Reed AM, Aggarwal R, et al. Rituximab in the treatment of refractory adult and juvenile dermatomyositis and adult polymyositis: a randomized, placebo-phase trial. Arthritis Rheum 2013;65:314-24.
8. Blair HA, Duggan ST. Belimumab: a review in systemic lupus erythematosus. Drugs 2018;78:355-66.
9. Kansal R, Richardson N, Neeli I, et al. Sustained B cell depletion by CD19-targeted CAR T cells is a highly effective treatment for murine lupus. Sci Transl Med 2019;11:eaav1648.
10. Mougiakakos D, Krönke G, Völkl S, et al. CD19-targeted CAR T cells in refractory systemic lupus erythematosus. N Engl J Med 2021;385:567-9.
11. Müller F, Boeltz S, Knitza J, et al. CD19-targeted CAR T cells in refractory antisynthetase syndrome. Lancet 2023;401:815-8.
12. Bergmann C, Müller F, Distler JHW, et al. Treatment of a patient with severe systemic sclerosis (SSc) using CD19-targeted CAR T cells. Ann Rheum Dis 2023;82:1117-20.
13. Pecher A-C, Hensen L, Klein R, et al. CD19-targeting CAR T cells for myositis and interstitial lung disease associated with antisynthetase syndrome. JAMA 2023;329:2154-62.
14. Mackensen A, Müller F, Mougiakakos D, et al. Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. Nat Med 2022;28:2124-32.
15. Aringer M, Costenbader K, Daikh D, et al. 2019 European League against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Ann Rheum Dis 2019;78:1151-9.
16. Bohan A, Peter JB. Polymyositis and dermatomyositis — (second of two parts). N Engl J Med 1975;292:403-7.
17. van den Hoogen F, Khanna D, Fransen J, et al. 2013 Classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum 2013;65:2737-47.
18. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol 2002;29:288-91.
19. Franklyn K, Lau CS, Navarra SV, et al. Definition and initial validation of a Lupus Low Disease Activity State (LLDAS). Ann Rheum Dis 2016;75:1615-21.
20. van Vollenhoven RF, Bertsias G, Doria A, et al. 2021 DORIS definition of remission in SLE: final recommendations from an international task force. Lupus Sci Med 2021;8(1):e000538.
21. Aggarwal R, Rider LG, Ruperto N, et al. 2016 American College of Rheumatology/European League against Rheumatism criteria for minimal, moderate, and major clinical response in adult dermatomyositis and polymyositis: an International Myositis Assessment and Clinical Studies Group/Paediatric Rheumatology International Trials Organisation collaborative initiative. Arthritis Rheumatol 2017;69:898-910.
22. Rider LG, Koziol D, Giannini EH, et al. Validation of manual muscle testing and a subset of eight muscles for adult and juvenile idiopathic inflammatory myopathies. Arthritis Care Res (Hoboken) 2010;62:465-72.
23. Valentini G, Iudici M, Walker UA, et al. The European Scleroderma Trials and Research group (EUSTAR) task force for the development of revised activity criteria for systemic sclerosis: derivation and validation of a preliminarily revised EUSTAR activity index. Ann Rheum Dis 2017;76:270-6.
24. Khanna D, Furst DE, Clements PJ, et al. Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis. J Scleroderma Relat Disord 2017;2:11-8.
25. Lee DW, Santomasso BD, Locke FL, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. Biol Blood Marrow Transplant 2019;25:625-38.
26. Taubmann J, Knitza J, Müller F, et al. Rescue therapy of antisynthetase syndrome with CD19-targeted CAR-T cells after failure of several B-cell depleting antibodies. Rheumatology (Oxford) 2024;63(1):e12-e14.
27. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies

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before the clinical onset of systemic lupus erythematosus. N Engl J Med 2003;349:1526-33.
28. Illei GG, Yarboro CH, Kuroiwa T, et al. Long-term effects of combination treatment with fludarabine and low-dose pulse cyclophosphamide in patients with lupus nephritis. Rheumatology (Oxford) 2007;46:952-6.
29. Adams EM, Pucino F, Yarboro C, et al. A pilot study: use of fludarabine for refractory dermatomyositis and polymyositis, and examination of endpoint measures. J Rheumatol 1999;26:352-60.
30. Cambridge G, Leandro MJ, Teodorescu M, et al. B cell depletion therapy in systemic lupus erythematosus: effect on autoantibody and antimicrobial antibody profiles. Arthritis Rheum 2006;54:3612-22.
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Supplementary Appendix

Supplement to: Müller F, Taubmann J, Bucci L, et al. CD19 CAR T-cell therapy in autoimmune disease — a case series with follow-up. N Engl J Med 2024;390:687-700. DOI: 10.1056/NEJMoa2308917

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

Supplementary Material

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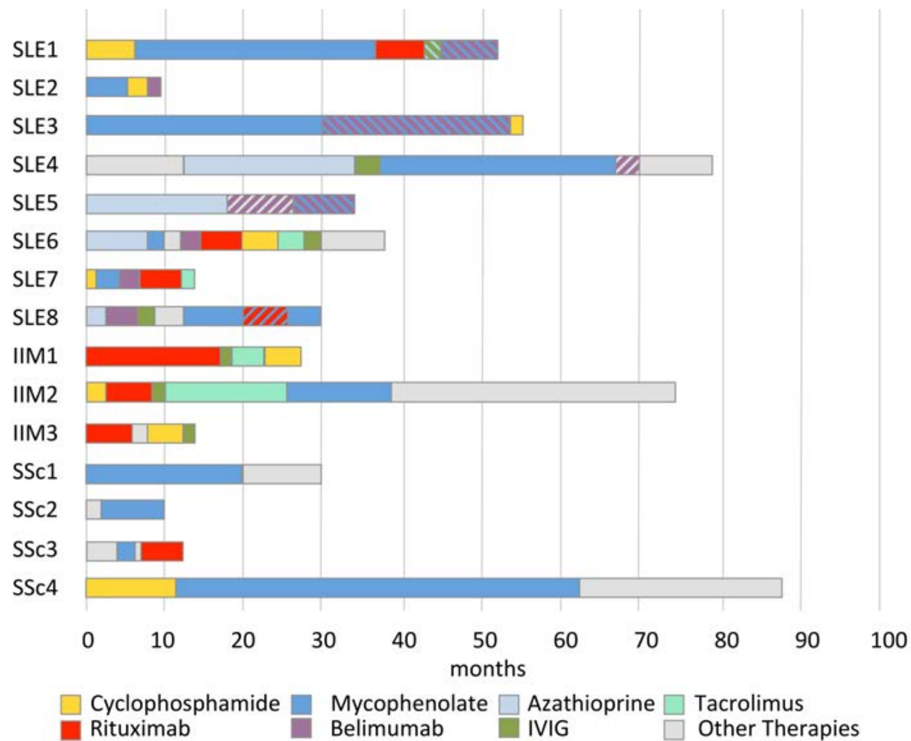
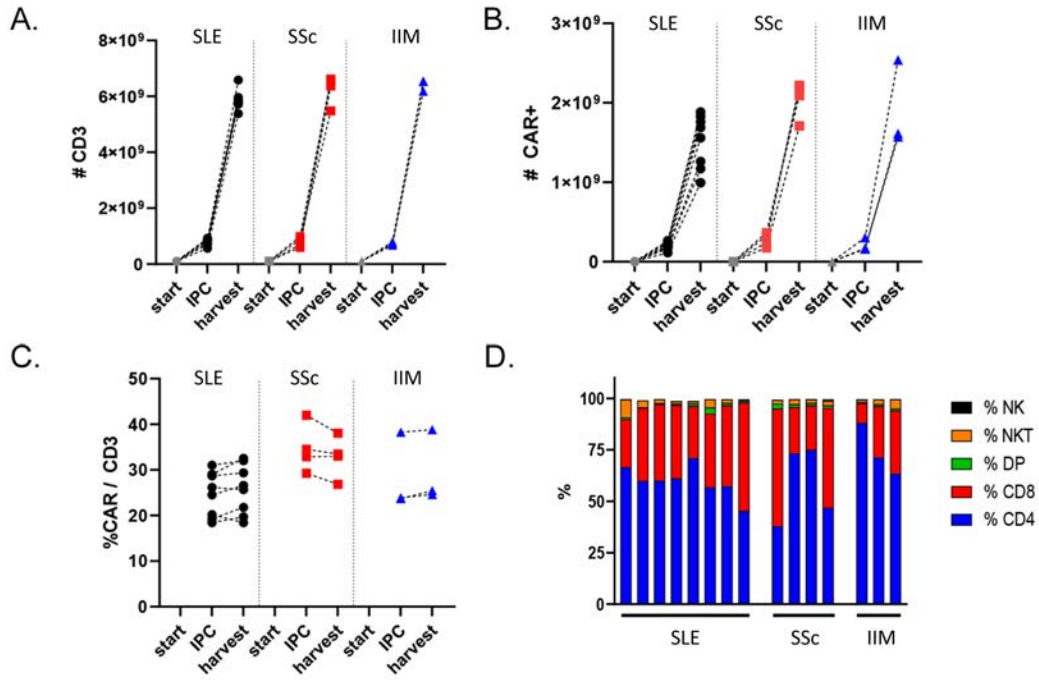
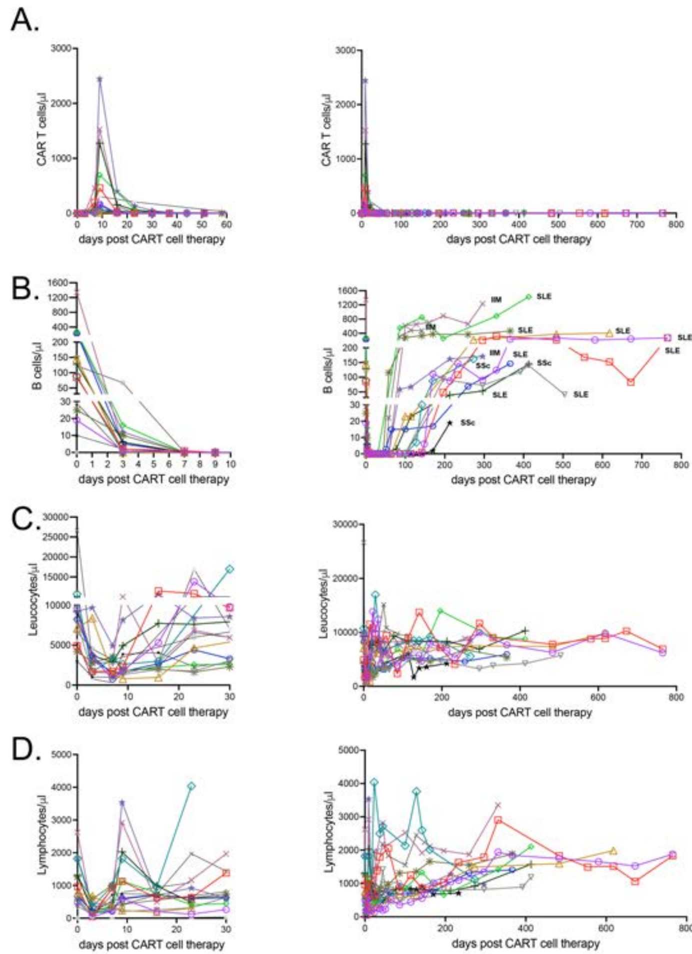


Suppl. Figure 1. Treatment history with immunosuppressive drugs
Bars indicate the time of treatment (months) with key immunosuppressive drugs in patients with systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and idiopathic inflammatory myositis (IIM) subjected to treatment with CD19 chimeric antigen receptor (CAR) T cells. IVIG, intravenous immunoglobulins. All SLE patients received concomitant hydroxychloroquine (not depicted). All patients (except SSc patients 1, 3 and 4 also received oral glucocorticoids. Hatched parts of the bars indicate combination treatment with indicated drugs. Grey parts of the bars symbolize treatment with other immunosuppressive drugs (baricitinib, leflunomide, lenabasum, methotrexate, ocrelizumab, secukinumab, tocilizumab, tofacinitib), which are listed in Table 1 of the manuscript.



Suppl. Figure 2. Characteristics of CAR T cell production and products
(A, B) Proliferation of T cells (A) and of chimeric antigen receptor (CAR) T cells (B) during the production process at start (day 0= $1x10^8$ T cells), at in-process control (IPC) on day 5 and at harvest on day 12. Data from 15 patients are shown, 8 with systemic lupus erythematosus (SLE), 4 with systemic sclerosis (SSc) and 3 with idiopathic inflammatory myositis (IIM). (C) Transduction efficacy (%) in the products from SLE, SSc and IIM patients. (D) Cellular composition of the CAR T cells products with respect to the fraction of CD4 T cells, CD8 T cells, CD4/CD8 double positive T cells, natural killer (NK) cells and natural killer T cells (NKT) cells.



Suppl. Figure 3. Cell dynamics after CD19 CAR-T cell therapy in autoimmune disease

(A) Left: Expansion of circulating CD19 chimeric antigen receptor (CAR) T cells shortly after administration (day 0; N=15); right; numbers of circulating CD19 CAR-T cells monitored up to 800 days; (B) Left: Depletion of B cells shortly after administration of CD19 CAR-T cells (day 0; N=15); right: numbers of circulating B cells monitored up to 800 days showing recurrence of B cells; (C, D) Left: Spurious decrease of circulating leucocyte (C) and lymphocyte (D) numbers shortly after administration of CD19 CAR-T cells (day 0; N=15) in conjunction with preceding lymphodepletion; right: circulating leucocyte numbers monitored up to 800 days.

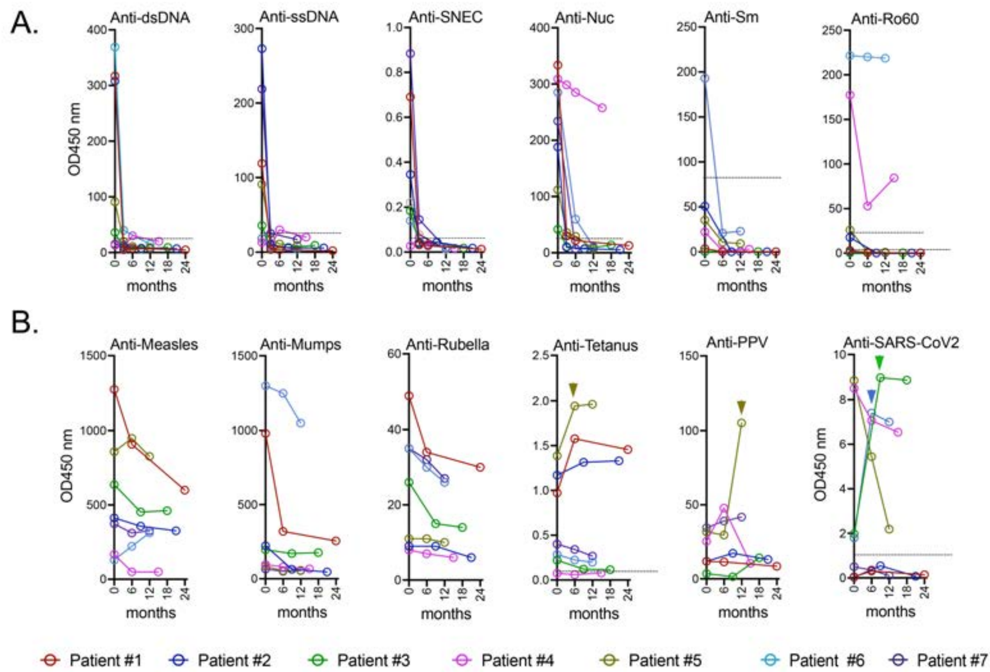
Baseline

	#1	#2	#3	#4	#5	#6	#7	#8
Mucocutaneous	B	B			B	B	A	A
Musculoskeletal	B	C	B	A		C		C
Cardiorespiratory								A
Neuropsychiatric								
Hematologic	C	C	C	C		C	C	
Constitutional	C	C	C					C
Renal	A	A	A	A	A	A	B	B
Score	24	20	19	25	17	19	18	31

6-month Follow Up

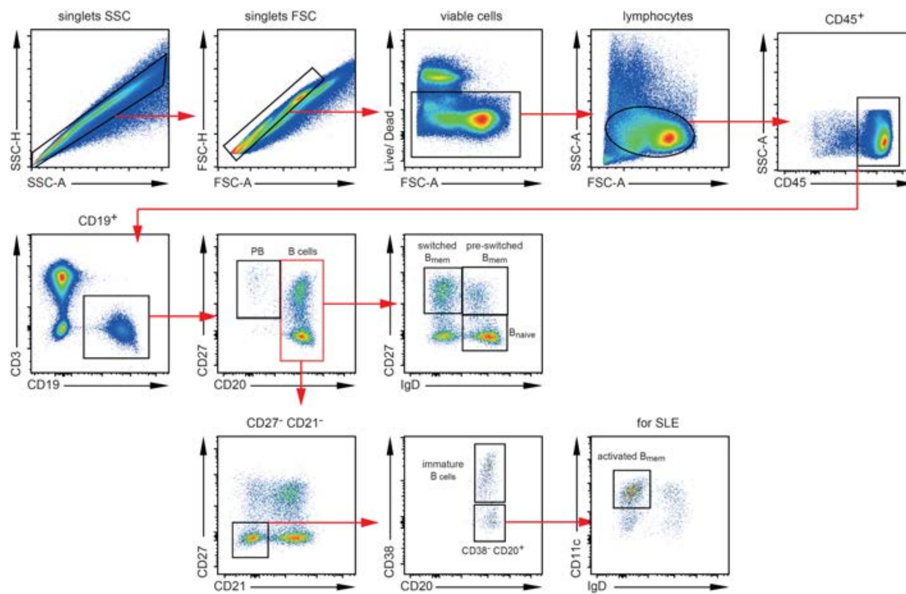
	#1	#2	#3	#4	#5	#6	#7	#8
Mucocutaneous								
Musculoskeletal								
Cardiorespiratory								
Neuropsychiatric								
Hematologic								
Constitutional								
Renal								
Score	0	0	0	0	0	0	0	0

Suppl. Figure 4. BILAG indices in SLE patients treated with CD19 CAR-T cells
Baseline (left) and 6-months follow-up (right) British Isles Lupus Assessment Group (BILAG) indices of 8 systemic lupus erythematosus (SLE) patients treated with CD19 chimeric antigen receptor (CAR) T cells. BILAG A represents very severe disease activity requiring immunosuppressive treatment and high doses of glucocorticoids. BILAG B represents moderate disease activity requiring hydroxychloroquine treatment and lower doses of glucocorticoids. BILAG C represents low disease activity requiring non-steroidal ant-inflammatory drugs or analgesics.

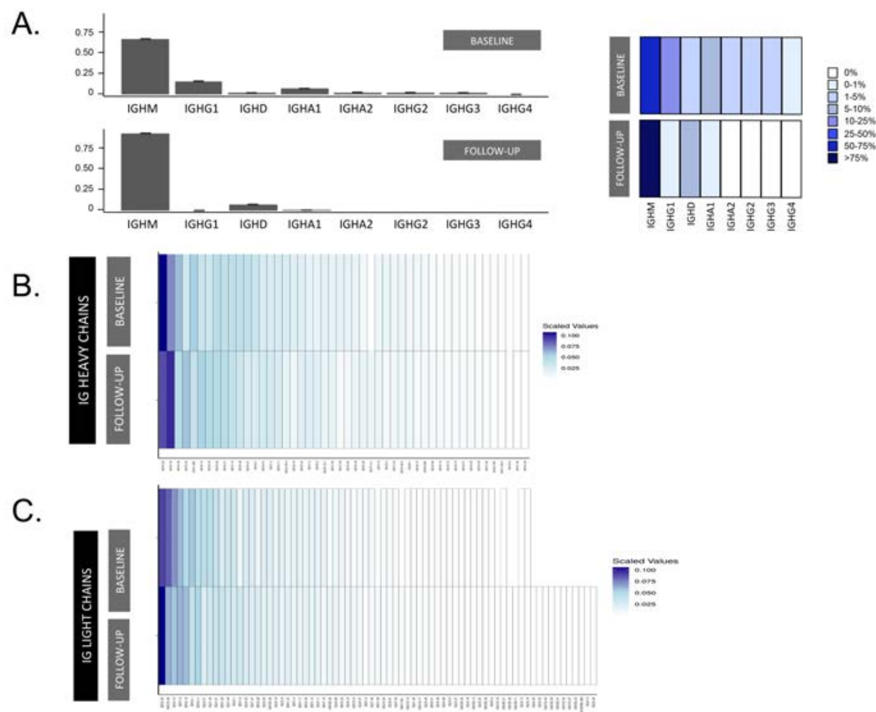


Suppl. Figure 5. Long-term effects of CD19 CAR-T cell therapy on autoantibody and vaccination-related antibody levels in SLE

(A) Serum autoantibody levels in 7 systemic lupus erythematosus (SLE) patients treated with CD19 chimeric antigen receptor (CAR) T cells and at least one year follow-up; dsDNA, double stranded DNA; NUC, nucleosomes; SNEC, secondary necrotic cells; ssDNA, single stranded DNA; Sm, Smith antigen; (B) Vaccination antibodies in 7 SLE patients treated with CD19 CAR-T cells and at least one year follow-up; PPV, pneumococcal polysaccharide vaccine; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2. Arrowheads indicate vaccinations that occurred after CAR-T cell therapy and B cell reconstitution.

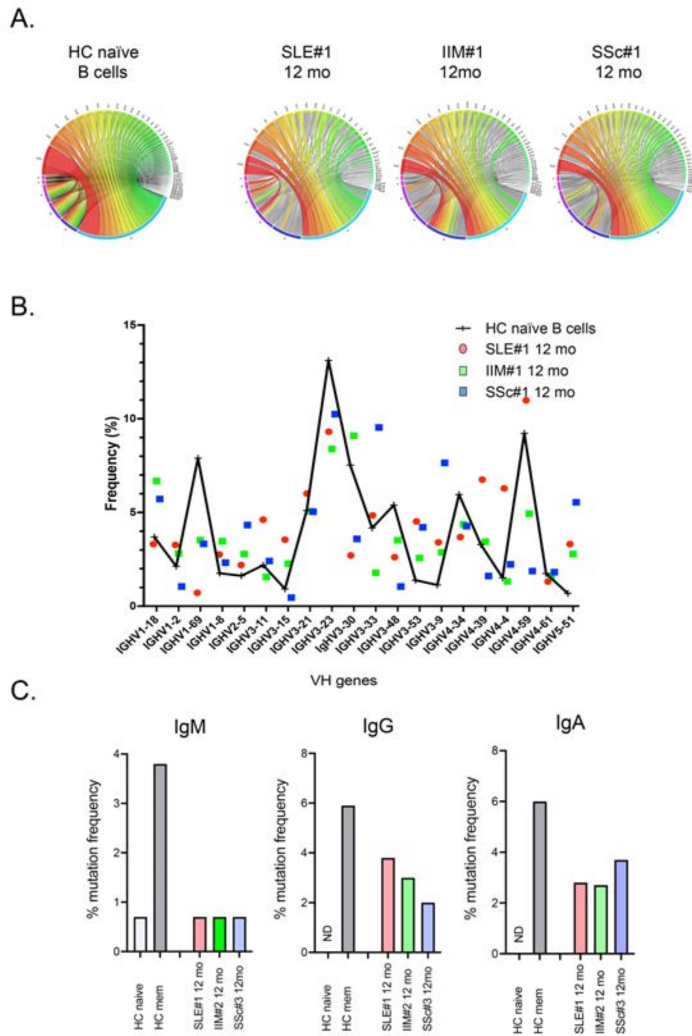


Suppl. Figure 6. Gating strategies for analysis of peripheral B cells
Fluorescence activated cell scan (FACS)-based gating strategies for naïve (CD20+, CD27-), memory (CD20+CD27+), pre-class-switched (CD27+IgD+) and class-switched (CD27+IgD-) memory B cells (Bmem). In addition, gating strategies for immature CD27-CD21-CD38+ B cells, CD20-CD27+ plasmablasts (PB) and CD11c+ activated memory B cells (the later in patients with systemic lupus erythematosus) are shown. FSC, forward scatter; SSC, sideward scatter.



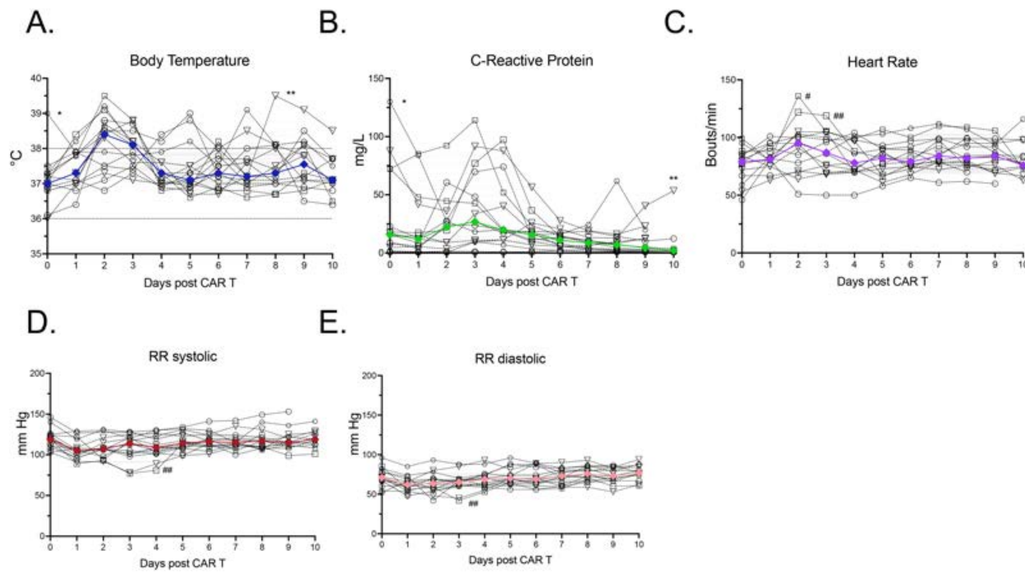
Suppl. Figure 7. Single cell sequencing of B cells showing immunoglobulin chain expression before and after CD19 CAR-T cell therapy

(A) Frequency of immunoglobulin heavy chain expression in 7 systemic lupus erythematosus (SLE) before and one year after CD19 chimeric antigen receptor (CAR) T cell therapy. IGHA (1,2), immunoglobulin α heavy chain (1,2); IGHD, immunoglobulin δ heavy chain, IGHG (1-4), immunoglobulin γ (1-4) heavy chains; IGHM, immunoglobulin μ heavy chain. (B) Heat map of expression of immunoglobulin heavy chains in 7 SLE patients before and one year after CD19 CAR-T cell therapy; (C) Heat map of expression of immunoglobulin light chains in 7 SLE patients before and one year after CD19 CAR-T cell therapy.

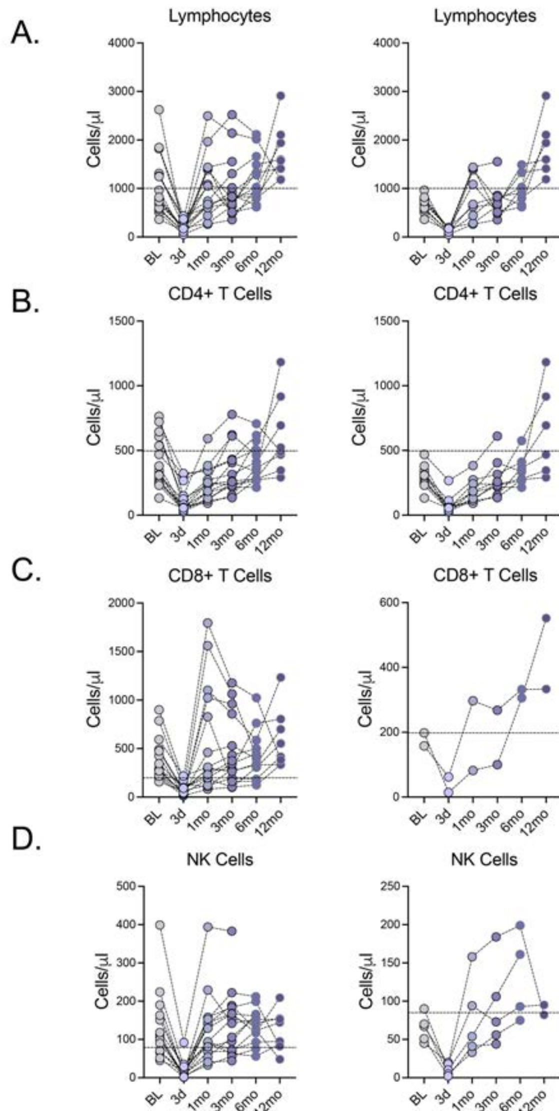


Suppl. Figure 8. Analysis of B cell repertoire in patients after B cell reconstitution

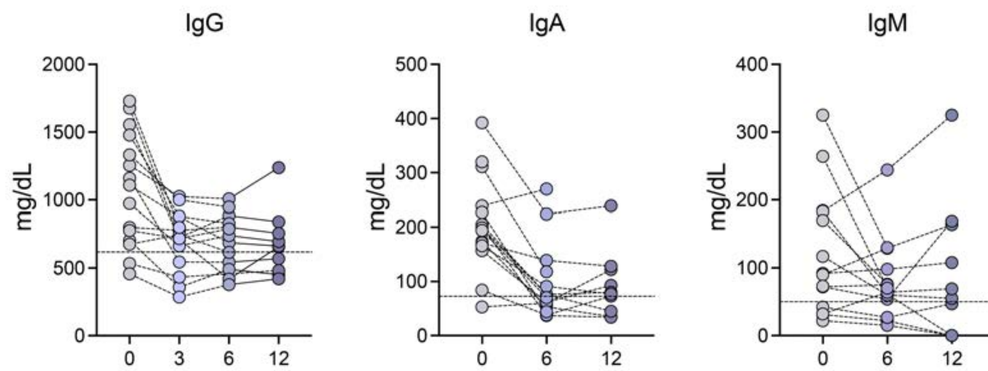
(A) VJ recombination in naïve B cells from a healthy control (HC) and in B cells from each one patient with systemic lupus erythematosus (SLE#1), idiopathic inflammatory myositis (IIM#1) and systemic sclerosis (SSc#1) 12 months after CD19 chimeric antigen receptor (CAR) T cell therapy. Circos plots depicting the frequencies of V_HJ_H usage and combinations of productive sequences. (B) V_H gene usage of top 20 most frequent V_H genes in the human repertoire. Results for libraries from SLE#1, IIM#1 and SSc#1 patients overlaid with the frequency obtained from the V_H repertoire from sorted naïve B cells from two healthy controls (mean values from > $3x10^5$ individual sequences each); (C) Analysis of mutation frequency of V_H genes for immunoglobulin (Ig)M, IgG and IgA from SLE#1, IIM#1 and SSc#1 patients 12 months after CAR-T cell therapy. Mutations frequencies for sorted naïve and memory B cells from a healthy control are shown for comparison.



Suppl. Figure 9. Course of clinical parameters after CAR-T cell infusion
Spaghetti plots showing body temperature (A), C-reactive protein levels (B), heart rate (C), systolic (D) and diastolic (E) blood pressure in 15 patients with systemic lupus erythematosus (SLE; N=8), idiopathic inflammatory myositis (IIM, N=3) and systemic sclerosis (SSc; N=4) treated with CD19 chimeric antigen receptor (CAR) T cells. Medians are shown as solid colored line. *elevated body temperature and C-reactive protein due to SLE activity in SLE patient with no evidence of infection; **spurious elevation of body temperature and C-reactive protein level in SSc patient #3 due to hematopoietic regeneration with no evidence of infection; #increased heart rate in IIM patient #3 due to cytokine release syndrome; ##increased heart rate and hypotension in SSc patient #3 due to hypovolemia in the context of chronic hemodialysis.



Suppl. Figure 10. Regeneration of T cells after CD19-CAR-T cell treatment
Numbers of circulating lymphocytes (A), CD4+ T cells (B), CD8+ T cells (C) and NK cells (D) in 15 patients with systemic lupus erythematosus (SLE; N=8), idiopathic inflammatory myositis (IIM, N=3) and systemic sclerosis (SSc; N=4) between baseline and one year after CD19 chimeric antigen receptor (CAR) T cell therapy. Left graphs show all 15 patients, right graphs show those with cytopenia at baseline.



Suppl. Figure 11. Immunoglobulin levels after CD19-CAR-T cell treatment
Immunoglobulin G (A), immunoglobulin A (B), and immunoglobulin M (C) levels in 15 patients with systemic lupus erythematosus (SLE; N=8), idiopathic inflammatory myositis (IIM, N=3) and systemic sclerosis (SSc; N=4) between baseline and one year after CD19 chimeric antigen receptor (CAR) T cell therapy.

Suppl. Table 1. Effects of pretreatment with B cell depleting antibodies

	Previous B cell depletion	No Previous B cell depletion
Numbers	8	7
Age (ys)	37.5 [33.5; 41.7]	24.0 [22.0; 47.0]
Female	7/8	3/7
Male	1/8	4/7
SLE	4/8	4/7
IIM	3/8	0/7
SSc	1/8	3/7
Rituximab	8/8	-
Ocrelizumab	2/8	-
B cells (baseline); cells/ml	57 [12; 223]	145 [123; 249]
B cells (recovery); days	138 [66; 189]	86 [70; 124]*
CART maximum; days	9 [9; 9]	9 [7; 9]
CART cell maximum; cells/ml	91 [38;270]	461 [61; 1275]
CART cell <1 cells/ml; days	40 [30;61]	58[26; 79]*
CRS incidence; N	6/8	2/7
CRS grade (0-4)	1 [0.25;1]	1[1; 1]
ICANS incidence; N	1/8	0/7
ICANS grade (0-4)	0 [0.;0]	0 [0.;0]
Leukopenia > grade 3, days	14 [8;20]	9 [0; 22]
Leukocytes, nadir; cells/ml	1402 [833;1809]	841 [420; 2357]
Neutropenia, > grade 3, days	14 [8;20]	16 [0; 22]
Neutropenia, nadir; cells/ml	571[276;986]	1711 [795; 3152]

CAR, chimeric antigen receptor; CRS, Cytokine Release Syndrome; ICANS, Immune-effector Cell Associated Neurotoxicity Syndrome; IIM, idiopathic inflammatory myopathy; SLE, systemic lupus erythematosus; SSc, systemic sclerosis

Supplementary Results

Effects on leukocyte and lymphocyte counts

Following lymphodepletion, leukocytes reached their nadir at 6.8± 2.2 days after CAR-T cell administration. At nadir, the median [IQR] number of leukocytes was 1,490 cells/µl [795; 2708] and the median [IQR] number of neutrophils was 739 cells/µl [334; 1567]. Patients regained leukocyte counts >2,000/µl after a mean of 12.2 ± 9.5 days and neutrophil counts over 1,000/µl after a median of 12.5 ± 9.3 days (Suppl. Figure 3C). Lymphocyte counts showed similar dynamics as leukocytes with a nadir of 172 cells/µl [108; 597] 3.5± 3.3 days after CAR-T cell administration (Suppl. Figure 3D).

Patients pre-exposed to B cell depleting antibodies

Of the 8 patients previously exposed to rituximab, 7 experienced primary failure, one experienced secondary failure. Median [IQR] B cells numbers in these patients were 0 [0; 1,5] after a median [IQR] of 3 [3; 4,75] months after rituximab infusion. Two patients (IIM patient #2 and #3) had additionally received ocrelizumab and experienced a primary failure.

Anti-nuclear antibodies and anti-SS-A/Ro responses

Anti-nuclear antibodies decreased from a median titre of 3200 [IQR: 1000; 6600] at baseline to 1000 [IQR: 100; 1000] at follow up. In contrast, anti-SS-A/Ro60 responses, which were found in three patients, remained stable after CAR-T cell treatment (Figure 2B).

Single cell sequencing-based analysis of immunoglobulin heavy and light chains

Single cell sequencing-based analysis of immunoglobulin heavy and light chains was performed in 7 SLE patients (SLE#1-7) before and 1 year after C19-CAR-T cell therapy. Analysis of heavy chains showed virtual disappearance of IGHG1, IGHG2, IGHG3, IGHG4, IGHA1 and IGHA2 chains, while the expression of IGHM and IGHD increased, resembling a non-class switched B cell receptor phenotype (Suppl. Figure 7A). Furthermore, deeper sequencing of immunoglobulin heavy and light chain usage in B cells at baseline and follow-up (Suppl. Figure 7B and 7C) showed down-regulation in the expression of distinct chains associated with SLE and autoimmunity, such as immunoglobulin kappa variable (IGKV) 4-1 and immunoglobulin heavy variable (IGHV) 4-59 chains associated with SLE (1,2) and immunoglobulin lambda variable (IGLV) 3-21 chain associated with autoimmunity (3).

Next-generation sequencing (NGS) of the V_H gene repertoire showed a broad and balanced V_H – J_H usage and a normal naïve V_H repertoire in SLE, IIM and SSc patients treated with CAR-T cells without any noticeable clonal expansions, very similar to a normal B cell repertoire of naïve B cells from healthy control donors (Suppl. Figure 8A and 8B). Analysis of the frequency of somatic mutations in the V_H genes revealed that all IgM sequences had a very low mutation frequency similar to a repertoire from sorted naïve cells from a healthy adult (Suppl. Figure 8C). IgM memory cells from healthy donors show a significantly higher frequency of somatic mutations. The presence of low numbers of IgA and IgG sequences in our libraries

allowed us to analyze the isotype - switched repertoire presumably derived from immune responses after B cell reconstitution. Also for IgA and IgG sequences mutation frequency was much lower as compared to a dataset from sorted memory cells from a healthy adult (Suppl. Figure 8C).

Immunoglobulin levels

Two patients (SLE pat#2 and 8), who had low IgG levels already before treatment, received IgG infusions after CAR-T cell treatment. Longitudinal assessment of immunoglobulins after CAR-T cell therapy showed a decrease of IgG by 41% from 1108 [704; 1478] mg/dL at baseline to 659 [474; 777] mg/dL at one year (Suppl. Figure 11). Decrease was observed within the first three months but then remained stable without immunoglobulin substitution. IgA levels decreased by 61% (baseline: 199 [166; 239] mg/dL vs. 1 year: 78 [42; 123] mg/dL) and IgM levels by 32% (baseline: 90 [42;183] mg/dL; 1 year: 62 [0;165] mg/dL) during the first 3 months after CAR-T cell therapy but remained stable thereafter.

Supplementary Methods

Patients

Patients with treatment-refractory SLE, IIM and SSc were recruited at the Department of Internal Medicine 3 (Rheumatology and Immunology) of the Friedrich Alexander University Erlangen-Nurnberg between February 2021 and May 2023. Eligibility criteria were as described in the main text and were based on (i) presence of SLE, IIM or SSc, (ii) severe and progressive disease course and (iii) resistance to treatment. Regarding severity and progression, patients had to have histology proven and clinically active glomerulonephritis (SLE), active myositis and radiographic evidence of interstitial lug diseases (IIM) or severe skin involvement (mRSS >10) and radiographic evidence of interstitial lung disease (SSc). Regarding resistance, patients had to have failed at least two immunosuppressive drug treatments. Among the 35 patients screened, an Interdisciplinary Specialist Board consisting of rheumatologists and hematooncologists selected 15 for CAR T cell treatment. The residual 20 patients did either not fulfil severity (13) or resistance criteria (N=5) or had concomitant uncontrolled disease (N=1) or refused informed consent (N=1). CAR T cell 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 SLE, (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 patients 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 and covered by license 334_18 B of the Institutional Review Board (IRB). Self-reported and biological sexes were identical in all patients. All participants gave written informed consent according to CARE guidelines and in compliance with the Declaration of Helsinki principles. No commercial sponsor was involved.

CAR T cell production

The investigational medicinal product MB-CART19.1 consisted of autologous CD19 Chimeric Antigen Receptor (CAR) transduced CD4/CD8 enriched T cells, derived from a leukapheresis product and processed by using the CliniMACS Prodigy® device (Miltenyi Biotec, Bergisch Gladbach, Germany). Aphereses were processed on day -13 without prior cryopreservation within their shelf life by enrichment of CD4+ and CD8+ CD3+ T cells. The cell count used in enrichment was limited to an upper level of 1.5 × 109 cells. On day -12 cells were cultured in TexMACS media supplemented with IL-7 and IL-15 (Miltenyi Biotec) and human AB serum (ZKT; Tübingen, Tübingen, Germany). All materials used were fully GMP compliant. For lentiviral transduction, a total cell count of 1 × 108 cells was used as starting material. T cells were activated for transduction with polymeric nanomatrix conjugated to humanized CD3 and CD28 (T Cell TransAct™; Miltenyi Biotec). Cells were transduced with a self-inactivating (SIN) lentiviral vector expressing a CAR directed against human CD19. The second-generation lentiviral vector was kindly provided by Miltenyi Biotec. The vector encodes for a single-chain variable fragment (scFv), derived from the murine anti-human CD19 antibody FMC63, that binds to exon 4 of human CD19. Furthermore, it contains the information for a CD8- derived hinge region, a TNFRSF19-derived transmembrane domain, a CD3ζ intracellular domain, and a 4-1BB co-stimulatory domain. Cells were expanded for 12 days under cleanroom conditions at the certified GMP laboratory of the Universitätsklinikum Erlangen (Department of Medicine 5, Hematology and Oncology) using the CliniMACS Prodigy® system (Miltenyi Biotec, Bergisch Gladbach, Germany) that performs all manufacturing steps in a single automated and functionally closed system. Final release tests and in-process controls included cellular composition, transduction rate, viability, microbiological control, endotoxin, and mycoplasma testing according to Ph. Eur. MB-CART19.1 was produced for each patient individually (personalized therapy).

CAR T cell treatment procedure

Before leukapheresis T cell targeted therapy was stopped 3 weeks and prednisolone dose was reduced to less than 10 mg/day. Patients received lymphodepleting chemotherapy (LD) with fludarabine 25 mg/m^2/d intravenously (i.v.) on days -5, -4, -3 and cyclophosphamide 1000 mg/m^2/d i.v. on day -3 before CAR-T-cell transfer. Pat #7 and patient #14 (due to dialysis) received 50% dose reduced LD.. On day 0, all patients received the investigational medicinal product MB-CART19.1, consisting of autologous CD19-CAR-transduced T cells at a dose of 1x10^6 CAR-T cells / kg body weight. CAR T-cells were administered as a short infusion (at day 0) after prophylactic application of anti-histamines and acetaminophen. Following CD19 CAR T cell therapy, all patients received oral prophylaxis with acyclovir and cotrimoxazol for at least three months following CAR T cell therapy. Once CD4 T cells were stable above 200/µl prophylaxis was stopped. Patients were monitored every day for signs of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) over a period of 10 days.

Monitoring of CAR T cells and leucocyte subsets

Absolute cell counts were determined with BD Trucount tubes (BD Biosciences) according to manufacturer's instructions. For monitoring of CAR T-cells, peripheral blood mononuclear cells were isolated by density centrifugation, stained with CD19 CAR detection reagent, washed twice and stained with Biotin antibody, 7-AAD (BD

Biosciences) and a standardized panel of antibodies against CD45, CD3, CD4, and CD8. The following anti-human antibodies were used for flow cytometry for monitoring leukocytes and CAR T cells after treatment: anti-CD3 (clone SK7), anti-CD4 (clone SK3), anti-CD8 (clone SK1), anti-CD14 (clone MϕP9), anti-CD19 (clone SJ25C1), anti-CD45 (clone 2D1), anti-CD56 (clone NCAM16.2; all BD Biosciences, Heidelberg, Germany), CD19 CAR Detection Reagent, and Biotin antibody (clone REA746; both Miltenyi Biotec, Bergisch-Gladbach, Germany). Data were acquired on a LSR Fortessa (BD Biosciences) and analyzed by FlowJo v10 software (Treestar, Ashland, USA). All measurements were taken from distinct samples.

Adverse event assessments

Patients were screened daily during the first 10 days for the presence of cytokine-release syndrome (CRS) and immune-effector cell associated neurotoxicity syndrome (ICANS). Grading of CRS and ICANS was done according to the American Society for Transplantation and Cellular Therapy (ASTCT) system differentiating between mild, moderate, severe and life-threatening events. To test for potential myelotoxicity, white blood cell counts, neutrophils, lymphocytes, CD4+ T cells, CD8+ T cells and natural killer (NK) cells were measured every 3 days for the first 9 days, thereafter weekly until month 1, monthly until month 3 and then every 3-6 months. Myelotoxicity was defined as grade III or IV neutropenia or leucocytopenia of more than 28 days after CAR T cell therapy. At each visit the patients were also assessed for potential flares of their AID. Immunoglobulin (Ig) G levels were measured every three months, while IgA and IgM levels were measured every 6 months after the treatment. In addition, all infections occurring after CAR-T cell infusion were recorded.

Monitoring of CAR T cells and leucocyte subsets

Absolute cell counts were determined with BD Trucount tubes (BD Biosciences) according to manufacturer's instructions. For monitoring of CAR T-cells, peripheral blood mononuclear cells were isolated by density centrifugation, stained with CD19 CAR detection reagent, washed twice and stained with Biotin antibody, 7-AAD (BD Biosciences) and a standardized panel of antibodies against CD45, CD3, CD4, and CD8. The following anti-human antibodies were used for flow cytometry for monitoring leukocytes and CAR T cells after treatment: anti-CD3 (clone SK7), anti-CD4 (clone SK3), anti-CD8 (clone SK1), anti-CD14 (clone MϕP9), anti-CD19 (clone SJ25C1), anti-CD45 (clone 2D1), anti-CD56 (clone NCAM16.2; all BD Biosciences, Heidelberg, Germany), CD19 CAR Detection Reagent, and Biotin antibody (clone REA746; both Miltenyi Biotec, Bergisch-Gladbach, Germany). Data were acquired on a LSR Fortessa (BD Biosciences) and analyzed by FlowJo v10 software (Treestar, Ashland, USA). All measurements were taken from distinct samples.

Quantification of autoantibodies against nuclear antigens

Antibodies against several nuclear antigens were assessed by commercial ELISAs (Orgentec, Mainz, Germany), including those against double stranded (ds) DNA (#ORG 604; 20 U/mL), single stranded (ss) DNA (#ORG 605; 20 U/mL), nucleosomes (#ORG 528; 20 U/mL), Sm (510; 25 U/mL), and SS-A/Ro52 (#ORG 652; 25 U/mL). Antibodies against secondary necrotic cells were analyzed as described previously (4). Autoantibody profiles for IIM and SSc were measured by immunoblots from Euroimmune (Lübeck, Germany). All measurements were taken from distinct samples.

Quantification of vaccination responses
IgG antibodies against measles (cutoff 150 mIU/mL) and mumps (cutoff 70 U/mL) were analyzed by ELISAs from Virion/Serion (Würzburg, Germany). IgG antibodies against rubella (cutoff 5 IU/mL) were analyzed by Bioline- ELISA from Abbott (Chicago, IL), IgG antibodies against pneumococcal polysaccharide vaccine (PPV) 23 by ELISA from The Binding Site (Birmingham, UK; cutoff 3 mg/L). IgG responses against tetanus (cutoff 0.15 U/mL) were assessed by ELISA from VaccZyme (The Binding Site; Birmingham, UK) and antibodies against severe acute respiratory syndrome coronavirus (SARS)-2 (cutoff 0.8 optical density 450nm) by Euroimmune (Lübeck, Germany). All measurements were taken from distinct samples.

Characterization of B cells
To investigate the B cell compartment, isolated PBMCs were stained with following surface marker antibodies at baseline, after short-term reconstitution (3-6 months) and after long term follow up (12-24 months). The following antibodies were used: CD3-SparkBlue 550 (SK7, BioLegend), CD19-BV421(HIB19, BioLegend), CD20-AF700 (2H7, BioLegend), CD27–PE-Cy7 (M-T271, BioLegend), CD38–PerCP-Cy5.5 (HIT2, BD Biosciences). Additionally CD21-PE (Bu32, BioLegend), IgD-BV785 (IA6-2, BioLegend) were used for baseline and short term reconstitution time points and CD21-PE (Bu32, BioLegend), IgD-PE (IA6-2, BioLegend) for long term follow up samples. Zombie NIR (BioLegend) and Fixable viability stain 780 (Thermo Fischer) were used as viability dye, respectively. PBMCs were acquired on a Cytek Northern Lights spectral analyzer and analyzed using FlowJo. B cells were gated on live CD3-CD19+ cells. Subpopulations of B cells were identified as CD20+CD38+CD21-CD27- immature B cells, CD20+CD27- naïve B cells, CD20-CD27+ plasmablasts and CD20+CD27+ memory B cells. Memory B cells were further divided in CD27-IgD+ naïve B cells, CD27+IgD+ pre-class switched memory B cells, and CD27+IgD- class switched memory B cells. Furthermore, in SLE patients $CD11c^{+}$ $CD21^{low}$ activated memory B cells were determined

Single Cell Sequencing Library Preparation
B cells were enriched by the untouched B cells magnetic isolation kit (Milteny Biotec, B-CLL Cell Isolation Kit) and RNA was isolated as described (5). To prepare libraries for single-cell sequencing, we utilized the Chromium Next GEM Single Cell 5´Reagent Kits v2 from 10X Genomics. Isolated B cells were encapsulated into droplets, and a reverse transcription of RNA was conducted to generate barcoded cDNA molecules. Subsequently, these final libraries were sequenced on an Illumina NovaSeq sequencer, producing 150-base pair long paired-end reads. For B cell receptor (BCR) region amplification, polymerase chain reaction (PCR) was employed with locus-specific primers targeting constant regions of the respective BCR. High-throughput sequencing of the amplification products was carried out on an Illumina NovaSeq platform, aiming for an average of 15,000 mean reads per cell. To convert reads into FASTQ format, the mkfastq tool from Cell Ranger 7.0.0 (10x Genomics) was used.
Integration of the BCR data was performed using the Seurat R package (v.4.3). All single patient datasets were integrated into a single object for comprehensive analysis.

For VH repertoire analysis of re-emerging B cells, VDJ library preparation and Illumina MiSeq sequencing was performed as described (5) using human IgH-specific

primer sets that are available upon request to the authors. The pRESTO (6) and Change-O tools (7) were used for paired-end merging, quality filtering and VDJ annotation. The Argalaxy workflow (Circos) was done as described in the method paper https://immcantation.readthedocs.io/en/stable/index.html. All these tools are part of the Immcantation/suite Docker image.

Statistical analyses

Individual values are presented throughout the manuscript. In addition, descriptive statistics are used for reporting specific parameters at baseline and follow-up. Analyses were conducted using R v 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

References

1. Hehle V, Fraser LD, Tahir R, et al Immunoglobulin kappa variable region gene selection during early human B cell development in health and systemic lupus erythematosus. Molecular Immunology, 2015;65: 215-223.
2. Bashford-Rogers RJM, Bergamaschi L, McKinney EF *et al.* Analysis of the B cell receptor repertoire in six immune-mediated diseases. Nature 2019;574, 122–126.
3. Wang JJ, Colella AD, Beroukas D, Chataway TK, Gordon TP. Precipitating anti-dsDNA peptide repertoires in lupus. Clin Exp Immunol. 2018;194:273-282.
4. Biermann, M.H.C. et al. Autoantibodies Recognizing Secondary Necrotic Cells Promote Neutrophilic Phagocytosis and Identify Patients With Systemic Lupus Erythematosus. Front. Immunol. 2018; 9:989.
5. Werner A, et al. Determining immunoglobulin-specific B cell receptor repertoire of murine splenocytes by next-generation sequencing. Star Protoc. 2022;3:101277.
6. Vander Heiden JA, Yaari G, Uduman M, Stern JNH, O'Connor KC, Hafler DA, Vigneault F, Kleinstein SH. pRESTO: a toolkit for processing high-throughput sequencing raw reads of lymphocyte receptor repertoires. Bioinformatics 2014;30: 1930-2.
7. Gupta NT, Vander Heiden JA, Uduman M, Gadala-Maria D, Yaari G, Kleinstein SH. Change-O: a toolkit for analyzing large-scale B cell immunoglobulin repertoire sequencing data. Bioinformatics 2015,31, 3356-8.