



Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a life-threatening autoimmune disease characterized by adaptive immune system activation, formation of double-stranded DNA autoantibodies and organ inflammation. Five patients with SLE (four women and one man) with a median (range) age of 22 (6) years, median (range) disease duration of 4 (8) years and active disease (median (range) SLE disease activity index Systemic Lupus Erythematosus Disease Activity Index: 16 (8)) refractory to several immunosuppressive drug treatments were enrolled in a compassionate-use chimeric antigen receptor (CAR) T cell program. Autologous T cells from patients with SLE were transduced with a lentiviral anti-CD19 CAR vector, expanded and reinfused at a dose of 1×10^6 CAR T cells per kg body weight into the patients after lymphodepletion with fludarabine and cyclophosphamide. CAR T cells expanded in vivo, led to deep depletion of B cells, improvement of clinical symptoms and normalization of laboratory parameters including seroconversion of anti-double-stranded DNA antibodies. Remission of SLE according to DORIS criteria was achieved in all five patients after 3 months and the median (range) Systemic Lupus Erythematosus Disease Activity Index score after 3 months was 0 (2). Drug-free remission was maintained during longer follow-up (median (range) of 8 (12) months after CAR T cell administration) and even after the reappearance of B cells, which was observed after a mean (\pm s.d.) of 110 ± 32 d after CAR T cell treatment. Reappearing B cells were naïve and showed non-class-switched B cell receptors. CAR T cell treatment was well tolerated with only mild cytokine-release syndrome. These data suggest that CD19 CAR T cell transfer is feasible, tolerable and highly effective in SLE.

SLE is a prototypic systemic autoimmune disease with a prevalence of 0.1% in the general population predominantly affecting young women¹. In SLE, immune tolerance against nuclear antigens including double-stranded (ds) DNA and nuclear proteins is broken, leading to the emergence of autoantibodies against dsDNA, and other nuclear antigens, which subsequently trigger immune complex-induced inflammation in an array of different organs, such as the kidneys, the heart, the lungs and the skin. Mechanistically, SLE is triggered and amplified by enhanced cell death and immune systems' exposure to nuclear antigens, in the context of neutrophil extracellular trap (NET) formation, during infection or upon epithelial cell death following ultraviolet light exposure². Patients with SLE are considered to have deficits in the noninflammatory clearance of dead cells and of danger signals such as nucleic acids, which are in part genetically determined³. In patients with SLE, this results in the aberrant signaling of pattern recognition receptors, such as Toll-like receptors, the initiation of a type I interferon response and the activation of autoreactive B cells with autoantibody formation^{4–6}.

Despite substantial advances in the treatment of SLE, some patients do not respond to the current state-of-the art therapies and are at high risk for organ failure and even death⁷. Furthermore, there is currently no solid strategy for achieving drug-free remission or even cure of SLE, which would require a deep reset of the immune system. Therefore, patients with SLE usually require lifelong treatment. Because the B cell response against DNA and nuclear antigens precedes the onset of clinical symptoms, tackling SLE by B cell blockade is an attractive therapeutic strategy⁸. Monoclonal antibodies that interfere with B cell activation targeting BAFF/BLySS or deplete B cells targeting CD20 have been successfully used in the treatment of SLE^{9,10}. Nonetheless, such treatment approaches, although effective, only work for some patients and certain severe forms of SLE appear to be resilient. Biopsy studies have shown that the CD20-targeting monoclonal antibody rituximab does not thoroughly deplete B cells in the tissues^{11,12}. Thus, a considerable number, if not the majority of B cells escape depletion, hindering an effective reset of the (auto)immune response observed in SLE^{11,12}. The inaccessibility and persistence of autoreactive B cells, residing

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within lymphatic organs and inflamed tissues, could limit the efficacy of B cell depletion via CD20-targeting antibodies. In addition, CD20 is not expressed by plasmablasts and long-lived plasma cells, which are involved in autoantibody formation in systemic autoimmune diseases and escape their depletion by rituximab¹³.

Conceptually, a deep depletion of CD19⁺ B cells and plasmablasts in the tissues could trigger an immune reset in SLE that could allow the cessation of immunosuppressive treatment. T cells can be activated and kill B cells by expressing a vector construct that encodes for a CAR that binds a specific antigen on target cells¹⁴. Due to the highly effective and robust depletion of target cells, CAR T cells are of high interest in cancer therapy^{15,16}. At present, the most advanced approach of CAR T cell therapy is the targeting of B cells and their malignant descendants via their highly specific and ubiquitous surface antigen CD19. The first complete tumor responses to CD19 CAR T cell therapy were seen in patients with chronic lymphocytic leukemia and led to durable remission¹⁷. Subsequently, successful studies were carried out in acute lymphoblastic leukemia (ALL) and B cell non-Hodgkin lymphoma leading to approval of several CD19 CAR T cell products^{18–22}. Two preclinical studies in lupus-prone mice support the efficacy of CD19 CAR T cells in SLE^{23,24}. Both models rely on the development of systemic autoimmunity reflected by antinuclear and anti-dsDNA antibody formation and the development of nephritis and premature death²⁵. Upon administration of CD19-targeting CAR T cells, B cells were depleted, autoantibody production was suspended and glomerulonephritis and other organ manifestations were reversed.

Based on these preclinical data and stimulated by the principle feasibility of CAR T cell treatment in autoimmune disease shown in a single patient²⁶, we assessed tolerability and efficacy of CD19 CAR T cells in a small series of seriously ill and treatment-resistant patients with SLE.

Results

Patient characteristics. Detailed patient characteristics are shown in Table 1. Patients were consecutively enrolled between 14 February 2021 and 2 February 2022. Eligibility, screening and inclusion criteria are summarized in Extended Data Fig. 1. Briefly, all patients (four women and one man) were aged between 18 and 24 years. All patients had active SLE with Systemic Lupus Erythematosus Disease Activity Index-2000 (SLEDAI-2K)²⁷ scores of between 8 and 16 (patient 1, 16; patient 2, 16; patient 3, 10; patient 4, 8; patient 5, 9). All patients had multiorgan involvement with histology-proven glomerulonephritis, as well as involvement of the heart, lungs and joints, but not the central nervous system. Despite their young age, all patients had previously been exposed to several immunosuppressive drugs, such as pulsed glucocorticoids (5/5), hydroxychloroquine (5/5), mycophenolate mofetil (MMF; 5/5), belimumab (5/5), cyclophosphamide (3/5) and azathioprine (2/5). B cell counts at baseline were 19 (patient 1; previous rituximab exposure; 3.2% of lymphocytes), 85 (patient 2; 8.0%), 84 (patient 3; 6%), 280 (patient 4; 27.8%) and 234 (patient 5; 7.3%) cells per microliter.

Transduction efficacy and proliferation efficiency of chimeric antigen receptor T cells in vitro. A detailed treatment plan of patients with SLE is depicted in Fig. 1a. Briefly, leukapheresis was performed at day -13 and CAR T cells were generated from day -12 to day 0 (0 = day of infusion; Fig. 1a). After leukapheresis, 1×10^8 T cells were used as the starting population, activated (day -12) and transduced (day -11) with a lentiviral vector that contains the sequence for a single-chain variable fragment derived from an antihuman CD19 hybridoma clone (FMC63). Cells expanded more than 50-fold from day -11 to day 0 (Fig. 1b). The final product (MB-CART19.1) showed a transduction efficacy of between 20% and 40% (Fig. 1c), a high purity for T cells (>99%) with a preponderance of CD4⁺ T cells (Fig. 1d) and negligible lipopolysaccharide

concentrations (<0.05 EU ml⁻¹). While CD45RA⁺CD27⁺ naïve T cells and CD45RA⁻CD27⁺ central memory cells prevailed following initial cell collection, strong enrichment of CD27⁻CD45RA⁻ effector memory T cells (Fig. 1e,g) was observed within the final cell product. Furthermore, the expression of the T cell exhaustion markers CD57 and programmed cell death protein 1 (PD-1) decreased during the production process and was low in the CAR T cells (Fig. 1f,h).

To allow homeostatic CAR T cell expansion, all patients received a lymphodepleting chemotherapy with fludarabine 25 mg/m²/d intravenously (i.v.) from day -5 to day -3 and cyclophosphamide 1,000 mg/m²/d i.v. on day -3 before CAR T cell infusion (Fig. 1a). CAR T cells were administered as a single i.v. infusion on day 0 at a fixed dose of 1×10^6 CAR T cells per kg body weight. According to this dose specification, a total number of 44×10^6 (patient 1), 91×10^6 (patient 2), 76×10^6 (patient 3), 68×10^6 (patient 4) and 70×10^6 (patient 5) CAR T cells were administered to the patients. After infusion (at day 0), all patients remained hospitalized for daily toxicity monitoring for 10 d.

Dynamics of CAR T cell expansion and B cell elimination in vivo. CAR T cells constituted only a small proportion of circulating T cells shortly (at day 1) after their administration (Fig. 2a). Rapid expansion of the cells was observed in all five patients peaking on average at day 9 with 11% to 59% of all circulating T cells being CARs. Thereafter, circulating CAR T cell counts rapidly declined. Details on quantitative CAR T cell dynamics in vivo are depicted in Fig. 2b. In contrast to T cells not expressing the CAR, the phenotype of CAR T cells shifted to central memory T cells in vivo, which indicates their circulation to lymphoid organs and other tissue sites (Extended Data Fig. 2). B cells fully disappeared from the patients' peripheral blood from the second day after CAR T cell administration onwards (Fig. 2c). In contrast, other cell lineages (CD4⁺/CD8⁺ T cells, monocytes and neutrophils) showed only temporary decreases, most likely due to the lymphodepleting chemotherapy. With the exception of B cells, white blood cell counts rapidly recovered (Fig. 2d).

Clinical efficacy. Disease activity of SLE was continuously assessed after administration of CD19 CAR T cells using the SLEDAI-2K scale. All five patients had active SLE with multiorgan involvement at baseline with SLEDAI-2K scores between 8 and 16 (Fig. 3a). Signs and symptoms of SLE continuously improved in all five patients to SLEDAI-2K scores equal to zero in four of five patients and a SLEDAI-2K score of 2 in patient 2 at 3 months after CAR T cell administration. Patient no. 2 had residual low-level proteinuria after 3 months, most likely due to previously accrued damage in glomerular filter function. No active sediment (protein casts, cellular casts and dimorphic erythrocytes) was found in urine analysis. Furthermore, residual proteinuria (0.3 g/g creatinine) successively improved with dose adaptation of angiotensin-converting enzyme (ACE) inhibitors after 3 months. Nephritis ceased in all five patients upon CAR T cell treatment (Fig. 3b). Also, complement factor levels normalized in all five patients (Fig. 3c) and anti-dsDNA antibody levels, as measured by radioimmunoassay dropped below the cutoff (Fig. 3d). In addition, other severe manifestations of SLE such as arthritis (patient 4), fatigue (all patients; baseline numeric rating scale-based intensity, 8.2 ± 1.4 ; 3-months numeric rating scale-based intensity, 2.4 ± 1.6 ; $P = 0.007$; Fig. 3e), fibrosis of cardiac valves (patient 1) and lung involvement (restriction and diffusion impairment, patients 1 and 3) disappeared after the administration of CAR T cells. Interferon-alpha was detectable in the serum of three patients at baseline but was undetectable in all five patients at follow-up (Extended Data Fig. 3). DORIS remission criteria²⁸ and the Lupus Low Disease Activity State²⁹ definition were fulfilled by all five patients 3 months after treatment. Of note, all immunomodulatory

Table 1 | Patient characteristics at baseline

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 |
|--|---------------|---------------|--------------|-----------------|-----------------|
| Demographics | | | | | |
| Age (years) | 22 | 23 | 22 | 24 | 18 |
| Sex (female/male) | F | M | F | F | F |
| Disease duration (years) | 4 | 1 | 6 | 9 | 3 |
| Disease activity SLEDAI-2K (score) | 16 | 16 | 10 | 8 | 9 |
| Laboratory values | | | | | |
| Baseline hemoglobin (g dl ⁻¹) | 10.0 | 14.60 | 9.60 | 13.10 | 12.20 |
| Baseline white blood cells (N μl ⁻¹) | 8.69 | 5.36 | 5.85 | 3.88 | 7.25 |
| Baseline lymphocytes (N μl ⁻¹) | 0.7 | 1.2 | 1.4 | 1.4 | 1.4 |
| Baseline platelets (N μl ⁻¹) | 279 | 188 | 198 | 398 | 278 |
| Baseline C3 (mg dl ⁻¹) | 49 | 43 | 56 | 88 | 68 |
| Baseline anti-dsDNA (U ml ⁻¹) | 5,600 | 2,060 | 479 | 4 | 52 |
| Baseline ANA (titer) | 1:10,000 | 1:3,200 | 1:10,000 | 1:3,200 | 1:1,000 |
| Proteinuria (mg per 24 h) | 2,015 | 3,080 | 6,539 | 8,096 | 88 |
| Other autoantibodies | NUC, Sm | NUC, Sm Ro60 | NUC, PCNA | NUC, Sm, Ro60 | NUC, Sm, Ku |
| Organ involvement | | | | | |
| Skin (presence/absence) | + | + | + | + | + |
| Kidney (presence/absence) | + (stage III) | + (stage III) | + (stage IV) | + (stage III/V) | + (stage III/V) |
| Joints (presence/absence) | – | + | + | + | + |
| Lungs (presence/absence) | + | – | + | +/- | – |
| Heart (presence/absence) | + | – | – | + | – |
| Other (presence/absence) | HEM | – | SER | MYO | HEM |
| Treatments | | | | | |
| Glucocorticoid pulses (yes/no) | + | + | + | + | + |
| Hydroxychloroquine (yes/no) | + | + | + | + | + |
| MMF (yes/no) | + | + | + | + | + |
| Azathioprine (yes/no) | – | – | – | + | + |
| Cyclophosphamide (yes/no) | + | + | + | – | – |
| Rituximab (yes/no) | + | – | – | – | – |
| Belimumab (yes/no) | + | + | + | + | + |
| Other (yes/no) | TAC | – | – | MTX, LEF | – |

ANA, antinuclear antibody; C3, complement factor C3; HEM, hematologic abnormalities of SLE; LEF, leflunomide; MTX, methotrexate; MYO, myositis; NUC, anti-nucleosome antibodies; PCNA, proliferating cell nuclear antigen; SER, serositis; TAC, tacrolimus.

and immunosuppressive drugs, including glucocorticoids and hydroxychloroquine could be discontinued, achieving drug-free remission in all five patients.

Immune effects and preliminary long-term effects. In-depth analyses of the autoantibody spectrum of the patients at baseline and at 3-month follow-up not only confirmed the disappearance of anti-dsDNA antibodies (as measured by radioimmune and ELISA assays) but also showed a more comprehensive decrease in autoantibodies. The levels of antibodies against nucleosomes, secondary necrotic cells (SNECs), single-stranded (ss) DNA, Smith (Sm) antigen and Ro60 also decreased, while no antibodies against histones, Ro52 and SS-B/La were detected in any of the patients (Fig. 3f).

With respect to long-term effects, we observed that all five patients showed B cell reconstitution after an average time of 110 ± 32 d (median 110 d; range 63–142 d; Fig. 4a). Despite B cell reconstitution, no relapse of SLE was observed in the long-term follow-up of the patients while still being off any SLE-associated medication. Respective follow-up times of the patients are 17 (patient 1), 12 (patient 2), 8 (patient 3), 7 (patient 4) and 5 (patient 5)

months. Patient 1 was reconstituted with B cells over 12 months with no relapse of SLE and patient 2 was immune competent over 6 months, also with no relapse of SLE (Fig. 4b). Immune reconstitution of patients 3, 4 and 5 was shorter (1–3 months). Patient 3 developed a short phase of proteinuria without signs of nephritic sediment in the urine analysis 4 months after CAR T cell therapy. Kidney biopsy was carried out and histologic analysis revealed podocyte damage typical for minimal change in disease but no signs of immune complex deposition, complement activation or lupus nephritis. Complement levels also did not decrease. Proteinuria ceased after ACE inhibitor treatment and a pulse of glucocorticoids. Overall, no relapse of SLE was observed despite reappearance of B cells.

Immune phenotyping of B cells before CAR T cell treatment and of recurring B cells after CAR T cell therapy showed that reconstituted B cells are mostly CD21⁺CD27[–] naive cells, with CD21⁺CD27⁺, memory B cells and CD38⁺CD20[–] plasmablasts being low to absent (Fig. 4c). In addition, CD11c⁺CD21^{lo} activated memory B cells, which are expanded in SLE^{30,31}, were absent among recurring B cells. Furthermore, comparative data from B cell receptor (BCR)

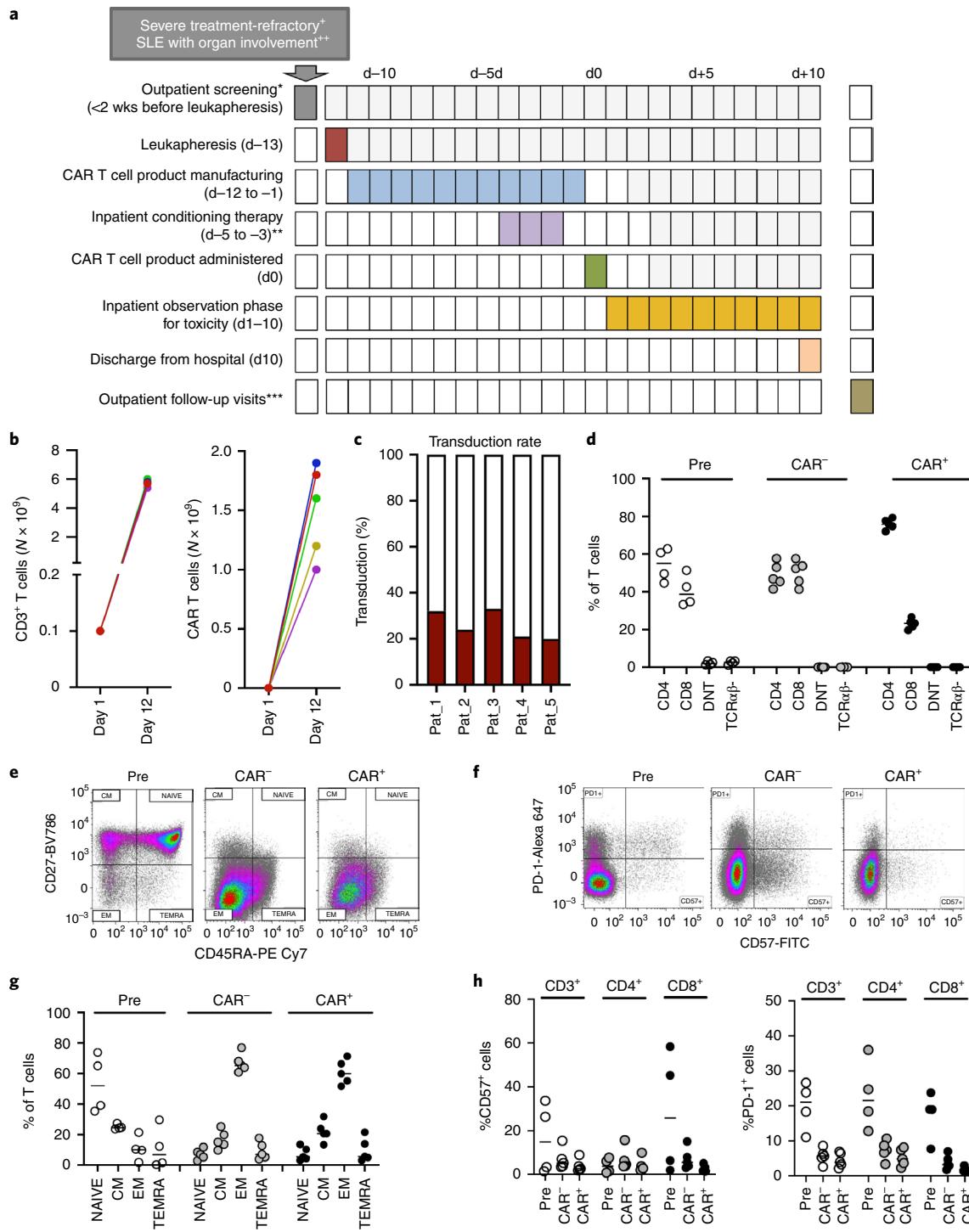


Fig. 1 | Description of process and CAR T cell generation. **a**, Timelines of CD19 CAR T cell treatment in patients with SLE. **b**, In vitro expansion of CD3⁺ T cells and CAR T cells between the beginning and the end of the production process ($N=5$). **c**, Transduction rate (percentage of transfected cells of all CD3⁺ T cells) and purity of T cells (percentage of CD3⁺ T cells of all CAR-positive cells) at the end of the production process ($N=5$). **d**, Fraction of CD4⁺ T cells, CD8⁺ T cells, double-negative T (DNT) cells and alpha/beta T cell receptor negative (TCR $\alpha\beta$) cells before (Pre) and after (CAR⁻ and CAR⁺) transduction and expansion of the CAR T cell product. Analyses were done in CAR-transfected (CAR⁺) and non-transfected (CAR⁻) cells ($N=5$). **e**, Representative dot plot of FACS staining of T cells for CD27 and CD45RA distinguishing central memory T cells (CM, upper left), effector memory T cells (EM, lower left), naïve T cells (upper right) and effector memory T cells reexpressing CD45RA (TEMRA, lower right) before (Pre) and after (CAR⁻ and CAR⁺) transduction and expansion of the CAR T cell product. **f**, Representative dot plot of FACS staining of T cells for CD57 and PD-1 as T cell exhaustion markers before (Pre) and after (CAR⁻ and CAR⁺) transduction and expansion of the CAR T cell product. **g,h**, Fractions of naïve, CM, EM and TEMRA cells (**g**) and exhausted CD57-expressing and PD-1-expressing T cells before (Pre) and after (CAR⁻ and CAR⁺) transduction and expansion of the CAR T cell product ($N=5$).

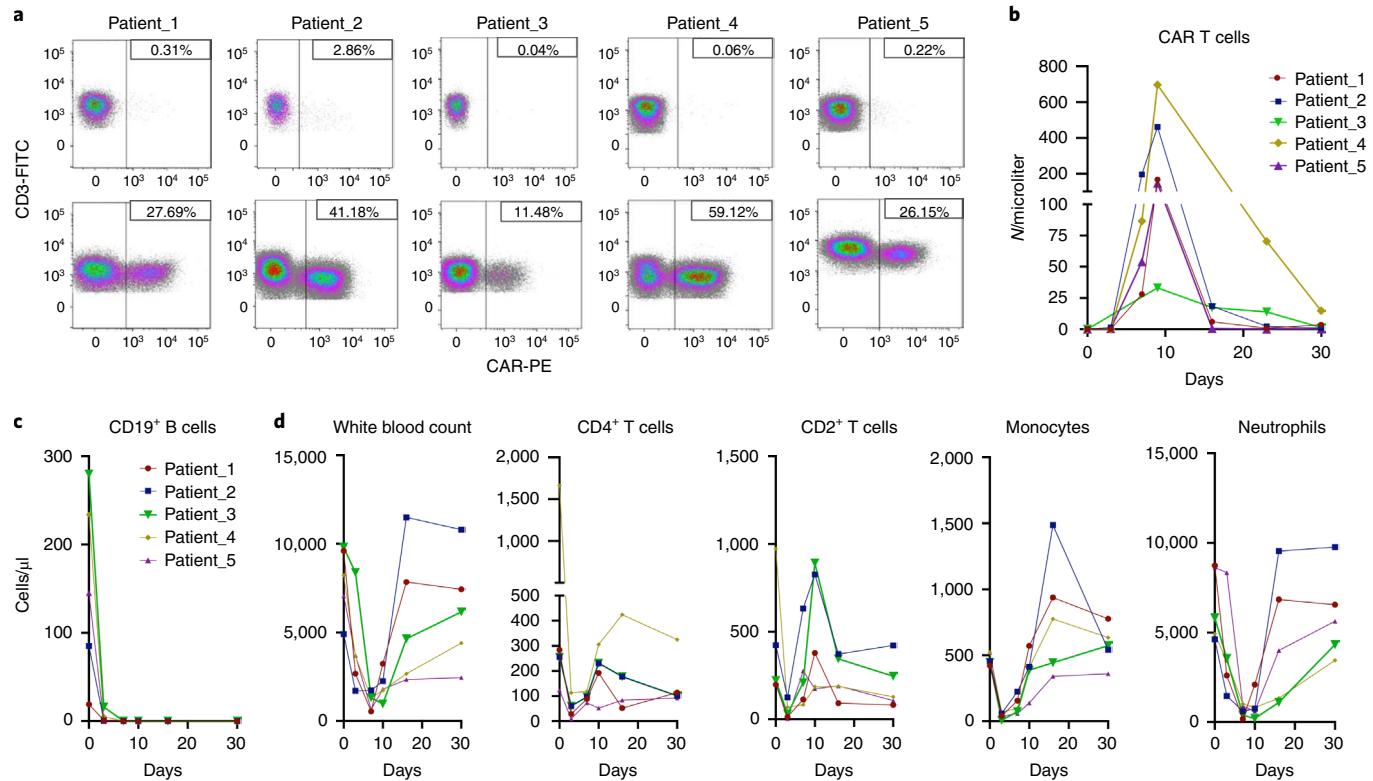


Fig. 2 | CAR T cell expansion in vivo and depletion of B cells. **a**, Dot plots showing CD3⁺ T cells (y axis) and CAR T cells (x axis) at day +1 and day +9 after CAR T cell administration. CAR T cells expanded in all five patients with SLE from day 1 to day 9. **b**, Circulating CAR T cell numbers in the five patients with SLE within the first 30 d after treatment ($N=5$). **c**, Circulating B cell numbers in the five patients with SLE within the first 30 d after treatment ($N=5$). **d**, Numbers of circulating total white blood cells, CD4⁺ T cells, CD8⁺ T cells, monocytes and neutrophils in the five patients with SLE within the first 30 d after treatment ($N=5$).

sequencing of B cells before as compared to after CAR T cell therapy showed a shift from class-switched IgG and IgA heavy chains at baseline to non-class-switched IgM and IgD heavy chains in the newly emerging B cells (Fig. 4d). In the two patients with the longest follow-up, seroconversion of antinuclear antibodies was observed, indicating that abrogation of autoimmune B cell clones may lead to a more widespread correction of autoimmunity.

Effects of vaccination antibody levels. We not only targeted autoimmune B cells directed against dsDNA but also assessed its impact on antibody titers of vaccinations carried out before lymphodepletion and CAR T cell therapy. We assessed viral vaccination responses against measles, rubella, mumps, varicella zoster virus and hepatitis B as well as bacterial vaccination responses against tetanus, diphtheria and pneumococci at baseline before CAR T cell therapy and 3 months after therapy. Overall, we did not find a substantial decline of vaccination responses indicating that the effects of CAR T cell therapy are primarily directed against autoantibody-producing cells rather than all immunoglobulin-producing cells (Extended Data Fig. 4).

Safety and tolerability. Cytokine-release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are frequently observed toxicities after CD19 CAR T cell therapy in lymphoma and leukemia³². In contrast, the five patients with SLE had no or only mild CRS. Fever (CRS grade 1) occurred in three of five patients, which was successfully treated by administration of metamizole (Fig. 5a). Fever lasted for 2 d in two patients and 3 d in one patient (patient 4), who additionally received a single infusion of 8 mg per kg body weight tocilizumab with immediate cessation

of the symptoms. No relevant hemodynamic changes were observed (Fig. 5b–d). C-reactive protein and interleukin (IL)-6 levels were increased in four of five patients between day 1 and day 5 (Fig. 5e,f). None of the patients with SLE developed ICANS suggesting that also this toxicity of CAR T cell treatment may be less pronounced in patients with SLE. Finally, no infection occurred in the short-term follow-up (during conditioning and immediately after CAR T cell administration) and during the phase of B cell aplasia.

Discussion

Our data show that CD19 CAR T cell treatment does not only effectively deplete B cells in patients with SLE but also leads to drug-free remission of this systemic autoimmune disease. Therefore, the clinical effect of CAR T cell treatment is associated with abrogation of autoimmunity in patients with SLE and remains present even after the patients reconstitute their B cells.

Our data reveal unexpected insights for a role of CAR T cells in nonmalignant diseases that could provide new opportunities for the treatment of autoimmune disease. First, our data show that the generation and administration of CAR T cells in patients with autoimmune disease is feasible and safe. This finding is important because patients with autoimmune diseases show an intrinsically activated T cell compartment and are therefore often exposed to drugs that deplete T cells and/or modulate their function. Hence, on the one hand, use of T cell-targeted drugs could impair production of sufficient numbers of functional CAR T cells, while on the other hand an intrinsically activated T cell compartment could potentially trigger systemic inflammatory responses upon reinfusion of cells due to the expansion of autoimmune T cell clones. However, our data show that sufficient numbers of functional CAR T cells could be

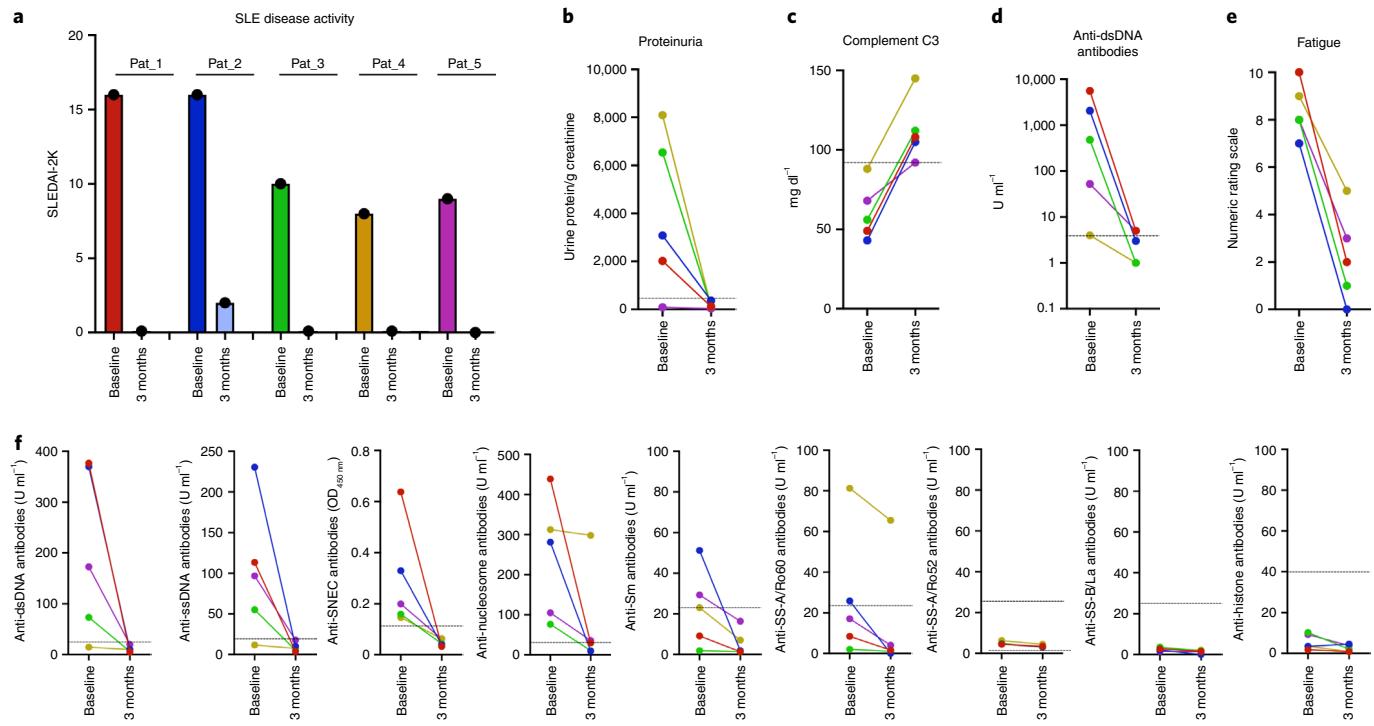


Fig. 3 | Effects of CAR T cell treatment on the activity of systemic lupus erythematosus. **a**, SLEDAI-2K scores at baseline and 3 months after CAR T cell administration ($N=5$). **b**, Proteinuria at baseline and 3 months after CAR T cell administration ($N=5$). **c**, Complement factor C3 levels at baseline and 3 months after CAR T cell administration ($N=5$). **d**, Anti-dsDNA antibodies assessed by radioimmunoassay at baseline and 3 months after CAR T cell administration ($N=5$). **e**, Fatigue measured by numerical rating scale (0-10) at baseline and 3 months after CAR T cell administration ($N=5$). **f**, ELISA-based quantification of antibodies against double stranded (ds) DNA, single stranded (ss) DNA, secondary necrotic cells (SNECs), nucleosomes, Smith (Sm) antigen, Sjogren's syndrome (SS)-A/Ro60, SS-A/Ro52 and SS-B/La antigens and histones at baseline and 3 months after CAR T cell administration ($N=5$).

generated from patients with autoimmune disease. Furthermore, the CAR T cell product was well tolerated in all patients, supporting the feasibility of this approach.

A second finding is the rapid and sustained breakdown of the B cell-mediated autoimmune response in SLE after CD19 CAR T cell treatment. While autoantibodies against dsDNA usually also decrease by conventional therapy, seroconversion is infrequent³³. All patients in our study seroconverted, lost dsDNA antibodies and showed decreases of levels of other SLE-associated antibodies. Considering that SLE in these patients was highly resistant to several previous immunosuppressive treatments, these findings indicate that CD19 CAR T cells could abrogate the underlying autoimmune processes of SLE. Deep depletion of memory B cells and plasmablasts upon CD19 CAR T cell treatment could be one explanation: CAR T cells bring effector function directly to the B cell niches in the tissues, such as the lymph nodes and the bone marrow. This feature is considered to be an important advantage of CAR T cells during the antitumor immune response, as they migrate and stay in the tissues, including immunologic niches, where they can kill their target cells². In contrast, antibody-mediated B cell depletion, which is also used in autoimmune disorders, seems to achieve a much more incomplete B cell depletion. Despite their elimination from circulation, tissue B cells are still present after CD20-targeted B cell-depleting antibody treatment^{23,24}. Hence, deep depletion of B cells in the tissues paired with the approach of targeting CD19, which, in contrast to CD20, is also expressed by plasmablasts, may be a key advantage of this CAR-based approach to treat autoimmune diseases. This concept may also explain why CD19 CAR T cell therapy can also work on patients with SLE that previously failed on anti-CD20 therapy, as was observed in one of the five patients with

SLE. The observations that overall IgG levels did not decrease substantially after CAR T cell therapy, that no regular immunoglobulin substitution was required and that anti-vaccination responses remained stable suggest that the majority of long-lived plasma cells are not targeted by this approach and that the eradication of activated B cells and plasmablasts can suffice to achieve clinical and immunologic remission in at least some of the patients with SLE. The extensive fatigue associated with SLE, which was observed in all five patients, resolved upon CAR T cell therapy.

The third insight from these treatments is that SLE remains absent, even though B cell reconstitution occurred about 100 d after CAR T cell treatment. Despite the return of B cells, none of the patients developed a flare of SLE and none of them required any kind of immunosuppressive medication since the initiation of CAR T cell treatment, indicating drug-free remission despite B cell reconstitution. While a definitive answer as to whether CAR T cell treatment corrects the underlying immune dysfunction in SLE cannot be made at this stage, the observation of disappearance of enriched BCR clonotypes and the exclusive expression of IgD and IgM in the newly emerging B cells support the notion of a rebooted immune system without signs of humoral autoimmunity³⁴.

While these data indicate a potential new treatment option for patients with severe SLE, there are limitations to be considered at this stage. Of note, the data supporting favorable toxicity and efficacy of CAR T cell treatment are based on a small series of patients with SLE and therefore need to be seen with caution. Currently, the ideal patient profile for CAR T cell treatment in SLE is not established and not every patient may respond. In our study, patients with multiorgan involvement were resistant to conventional treatments and had limited organ damage. It remains unknown if there

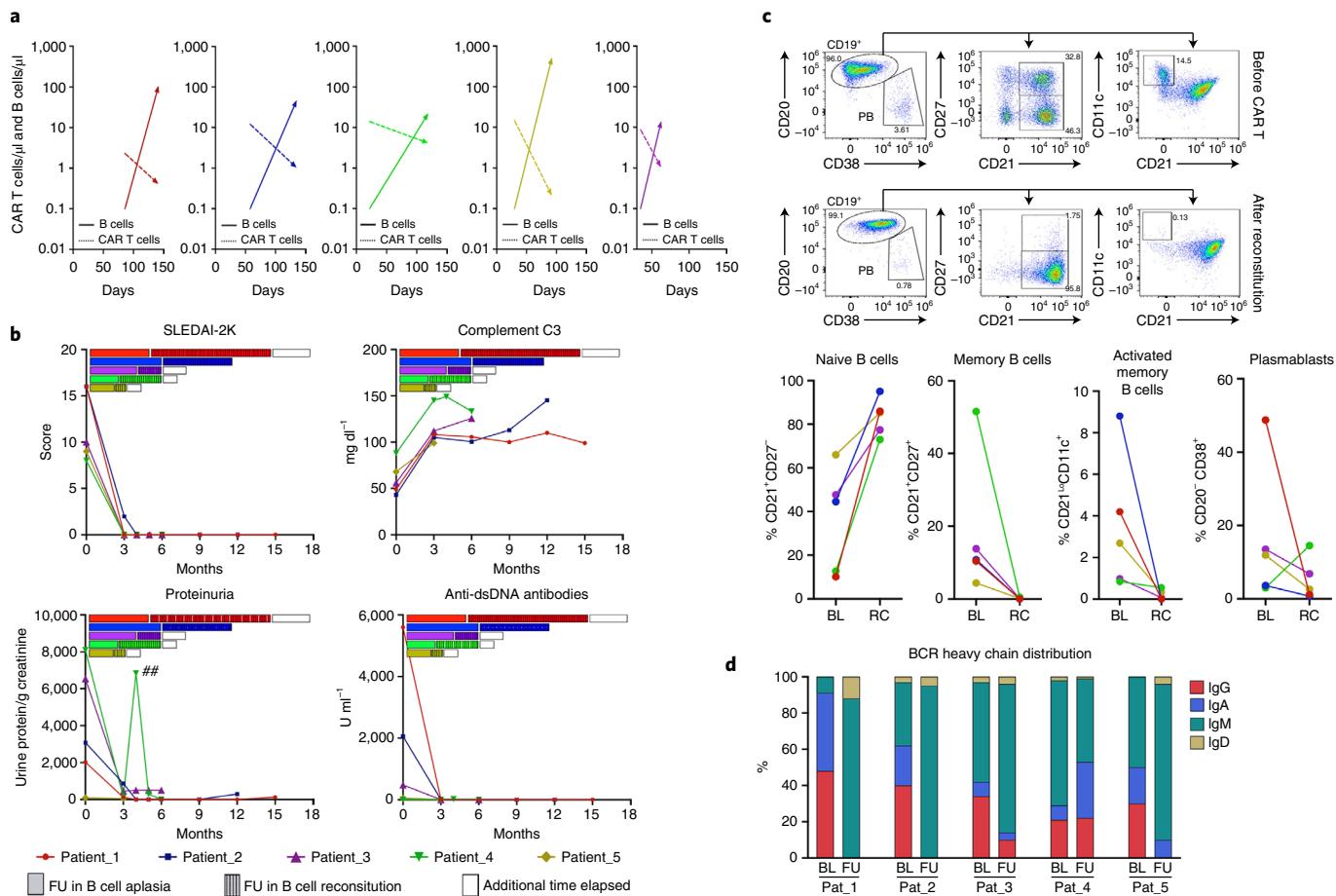


Fig. 4 | Long-term follow-up and analysis of recurrent B cells. a, Time of recurrence of B cells after CAR T cell therapy (indicated by days in the x axis) with changes in CAR T cell numbers and B cell numbers. The last time point with an absence of circulating B cells and the first time points with a new appearance of circulating B cells are indicated ($N=5$). **b**, Long-term follow-up (FU) of the patients: SLEDAI-2K, serum complement factor C3 levels, proteinuria and anti-dsDNA antibodies in the five patients with SLE treated with CAR T cells. Bars indicate the respective follow-up periods. A hash symbol indicates the phase of proteinuria in patient 4, in which SLE relapse was excluded by kidney biopsy (therefore not calculated in SLEDAI-2K score). **c**, Exemplary dot plots showing FACS analysis of B cells before CAR T cell therapy (upper row) and of reappearing B cells (lower row) stained for naïve B cells ($CD21^+CD27^-$), memory B cells ($CD21^+CD27^+$), plasmablasts ($CD20^+CD38^+$) and activated memory B cells ($CD11c^+CD21^{\circ}$). Changes in the numbers of naïve B cells, memory B cells, plasmablasts and activated memory B cells between baseline (before CAR T cell therapy) and B cell reconstitution in the five patients; BL, baseline; RC, reconstitution. **d**, Distribution of heavy chain in the BCRs at screening ('original' B cells at baseline, BL) and after CAR T cell treatment ('reappearing' B cells at follow-up, FU) by mRNA sequencing of B cells.

are specific patient characteristics that would influence the response to CAR T cell therapies in SLE. Furthermore, generalizability of the CD19-targeted CAR T cell approach to other autoimmune diseases is unclear. This approach requires that the disease is driven by B cell activation and plasmablast generation rather than on long-lived plasma cells, which are usually CD19 negative³⁵. Hence, patients in whom autoimmunity is driven by autoantibody production by long-lived plasma cells, for example, those with antibodies against RNA-binding proteins, may respond less well to the approach presented here³⁶.

With respect to toxicity of CAR T cell therapy observed in hematologic malignancies, CRS is a known side effect due to expanding, activated CAR T cells, which induce systemic inflammation via the recruitment of myeloid cells that abundantly release pro-inflammatory cytokines, such as IL-6 and tumor necrosis factor. Rapid expansion of CAR T cells together with high tumor burden correlates with the risk for severe toxicities. Grade 3–4 CRS with hemodynamic instability and organ dysfunctions is observed in about 10% of the patients treated with CAR T cells for B cell malignancies^{4–8}. Those patients require tight monitoring and, frequently,

intensive care treatment. This is in line with data suggesting that CRS is associated with B cell burden. Of note, higher-grade CRS was not observed in the patients with SLE treated with CAR T cells, which is most likely based on the substantially lower B cell burden in patients with SLE than in patients with active B cell malignancies. Also, no ICANS occurred in patients with SLE, all of whom received anticonvulsive prophylaxis according to institutional guidelines. The frequency of ICANS in CD19 CAR T cells that are based on a 4-1BB co-stimulatory domain, as the product used herein, is comparatively low. However, 20% of patients with B cell malignancies receiving CAR T cells develop mostly low-grade ICANS^{1–8,37}. The pathophysiology of this toxicity is not fully clear but spillover of proinflammatory cytokines into the brain as well as off-target effects through CD19-expressing mural cells in the brain have been considered³⁸. Furthermore, while toxicity data are appealing, higher-grade CRS and ICANS could occur in the treatment of autoimmune disease and their response to treatment is not clear to date. Nonetheless, treatment protocols for higher-grade CRS and ICANS have been successfully established in oncology and can be applied in patients with autoimmune disease. For the moment, however, tight

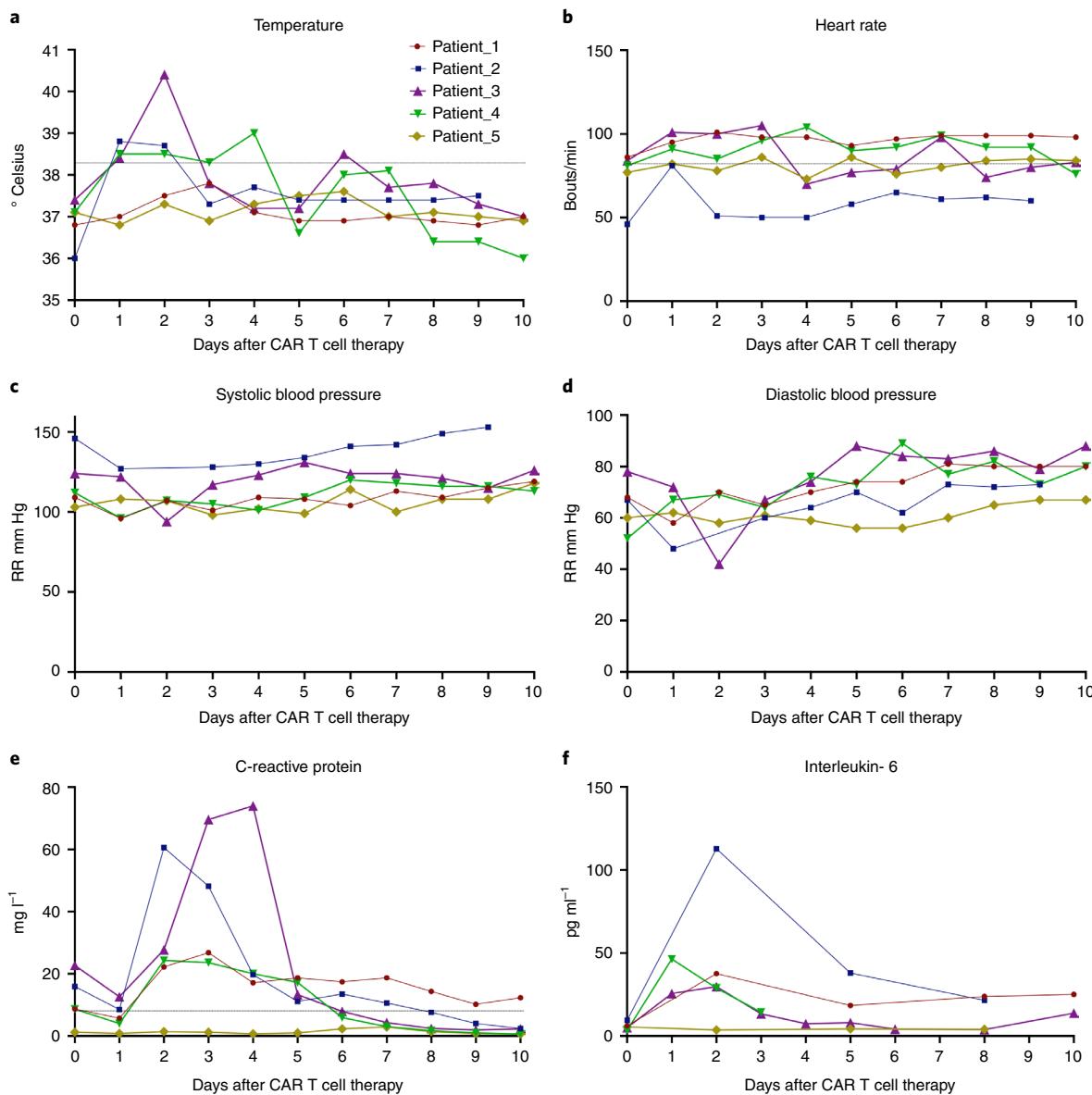


Fig. 5 | Safety of CAR T cell treatment in systemic lupus erythematosus. **a-f**, Body temperature (**a**), heart rate (**b**), systolic blood pressure (**c**), diastolic blood pressure (**d**), serum levels of C-reactive protein (**e**) and IL-6 (**f**) during the first 10 d after CAR T cell administration (all $N=5$); no IL-6 measurements are depicted in patient 4 after receiving anti-IL-6R infusion (tocilizumab) as the measurement of IL-6 is not reliable (false high) during detection of neutralizing antibodies.

monitoring of CAR T cell-treated autoimmune patients is of utmost importance and treatment should be performed in a collaboration of experienced CAR T cell centers in an inpatient setting³⁹.

It cannot be completely excluded that the conditioning regimen contributed to the short-term effects of treatment. We believe that such an effect is likely small and short-lived because several patients had received higher doses of cyclophosphamide before and responded only transiently. Whether fludarabine influences the activity of SLE is unknown and would need to be tested. Because the effects on immune cell subset were short-lived and all subsets studied including CD4⁺ T cells reached adequate numbers shortly after CD19 CAR T cell therapy, the long-standing remission of SLE without any further drug therapy cannot be explained by the lymphodepleting therapy. On the other hand, the composition of lymphodepleting therapy is crucial to achieve CAR T cell expansion and activity in B cell malignancies, which might be similarly true for CAR T cells used to treat autoim-

une diseases⁴⁰. Fast reconstitution of white blood cells (except the targeted B cells) also suggests a good bone marrow reserve in patients with SLE, in contrast to the findings in some patients with B cell malignancies that show delayed reconstitution of white blood cells after lymphodepleting chemotherapy followed by CD19 CAR T cell transfer⁴¹.

Finally, to date, no long-term data on the efficacy of CAR T cell in autoimmune diseases exist. However, such data could inform us about the length of drug-free remission in the patients who are reconstituted with B cells. The long disease-free state observed in the first two patients despite B cell reconstitution, the continuous absence of any necessity to reinstall immunosuppression, the persisting absence of any SLE flares and the repopulation with naïve B cells with loss of BCR clonotype enrichment support the notion that rebooting of the immune system may occur after CAR T cell treatment. Nonetheless, sustained drug-free remission of SLE needs to be confirmed over time.

In conclusion, these data provide new therapeutic possibilities to control SLE disease activity through engineered autologous cells. However, longer follow-ups in larger cohorts of patients will be necessary to confirm sustained absence of autoimmunity and resolution of inflammation in patients with SLE who have received CAR T cell therapy.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-022-02017-5>.

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Methods

Patients. All five patients with treatment-refractory SLE, who underwent CAR T cell therapy, were recruited at the Department of Internal Medicine 3 (Rheumatology and Immunology) of the Friedrich Alexander University Erlangen-Nürnberg. Patients screened for this compassionate-use program had to have (i) a diagnosis of SLE according to the EULAR/ACR 2019 criteria⁴², (ii) signs of active organ involvement, (iii) failure to respond to multiple immunomodulatory therapies including repeated pulsed glucocorticoids, hydroxychloroquine, belimumab and MMF and (iv) an understanding of the procedure of CAR T cell therapy. Seven patients with severe refractory SLE were identified as eligible for the program and received additional evaluation by an Interdisciplinary Specialist Board consisting of rheumatologists and hematopoietic oncologists. One patient was excluded as there was concomitant severe psoriasis, for which treatment with ustekinumab was initiated. The remaining six patients were selected for CAR T cell therapy and received detailed information on the procedure. One patient declined informed consent, while the other five patients accepted to be included into the compassionate-use program. From the legal aspect, CAR T cell therapy can be 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 have severe life-threatening disease such as SLE, (ii) patients have failed on previous treatments and (iii) a scientific rationale exists that potential efficacy of the respective treatment exists 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 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 sex were identical in all five patients (four females and one male). All patients provided written informed consent according to CARE guidelines and in compliance with the Declaration of Helsinki principles. No commercial sponsor was involved.

Tapering of immunosuppressive treatment before leukapheresis. Patients with SLE are usually lymphopenic and additionally treated with T cell-toxic drugs, that is, glucocorticoids and MMF. Therefore, in theory, the generation of sufficient amounts of CAR T cells was expected to be challenging. Based on these considerations, intensity of T cell-targeted therapy was tapered with cessation of MMF and cyclophosphamide 3 weeks before leukapheresis and reduction of prednisolone dose to less than 10 mg per day. This approach worked in 4 of 5 patients; however, patient 3 experienced worsening of nephritis with elevation of creatinine levels just before leukapheresis and had to receive a glucocorticoid pulse and cyclophosphamide. Nonetheless, enough T cells could be retrieved from this patient and later CAR T cell generation was not impaired and not different from the other patients.

Screening procedures. Before initiation of CAR T cell treatment, all patients were intensively screened for comorbidities using chest X-ray, electrocardiography, echocardiography and brain magnetic resonance imaging. In addition, female patients received gynecologic consultation with administration of GnRH analogon (3.6 mg goserelin subcutaneously) for protection of the ovaries and reproductive function before lymphodepleting chemotherapy.

Description of chimeric antigen receptor product and vector. The investigational medicinal product MB-CART19.1 consisted of autologous CD19 CAR transduced CD4⁺/CD8⁺-enriched T cells, derived from a leukapheresis product and processed by using the Clinimacs Prodigy device. CD4⁺ and CD8⁺CD3⁺ T cells were enriched from the patients' peripheral blood apheresis product and a total of 1×10^8 cells were used as the starting cell population. The cells were transfected 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, derived from the murine antihuman 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 d under cleanroom conditions at the Good Manufacturing Practice-certified laboratory of the Universitätsklinikum Erlangen (Department of Medicine 5, Hematology and Oncology) using the Clinimacs Prodigy system (Miltenyi Biotec) 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). T cells at the beginning of the manufacturing process as well as CAR-transfected and non-transfected T cells at the end of the production process were analyzed for the expression of CD45RA (clone HI100) and CD27 (clone L128; both BD Biosciences) by FACS to differentiate central memory T cells, effector memory T cells, naïve T cells and effector memory T cells reexpressing CD45RA

(TEMRA). In addition, cells were stained for T cell exhaustion markers CD55 (clone NK-1) and PD-1 (clone EH12.1; both BD Biosciences).

Lymphodepleting chemotherapy. patients received lymphodepleting chemotherapy with fludarabine (25 mg/m²/d i.v.) on days -5, -4 and -3 and cyclophosphamide (1,000 mg/m²/d i.v.) on day -3 before CAR T cell transfer to enhance the in vivo expansion of CAR T cells.

Chimeric antigen receptor T cell treatment. CAR T cells were given as a short infusion (at day 0) after prophylactic application of antihistamines and acetaminophen. Patients were monitored every day for signs of CRS and ICANS over a period of 10 d.

Immunomonitoring. The following antihuman 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), CD19 CAR Detection Reagent and Biotin antibody (clone REA746; both Miltenyi Biotec). Absolute cell counts were determined with BD Trucount tubes (BD Biosciences) according to the manufacturer's instructions. For monitoring of CAR T cells, peripheral blood mononuclear cells (PBMCs) 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. Gating strategies are depicted in Extended Data Fig. 5. Data were acquired on an LSRFortessa (BD Biosciences) and analyzed by FlowJo v10 software (Tree Star). All measurements were taken from distinct samples.

Quantification of autoantibodies against nuclear antigens. Antibodies against dsDNA were assessed by radioimmunoassay (Tecan IBL). In addition, antibodies against several nuclear antigens were assessed by commercial ELISAs (Orientec), including those against histones (ORG 509; cutoff 40 U ml⁻¹), dsDNA (ORG 604; 20 U ml⁻¹), ssDNA (ORG 605; 20 U ml⁻¹), nucleosomes (ORG 528; 20 U ml⁻¹), Sm (510; 25 U ml⁻¹), SS-A/Ro52 (ORG 652; 25 U ml⁻¹), SS-A/Ro60 (ORG 660; 25 U ml⁻¹) and SS-B/La (ORG; 25 U ml⁻¹). All measurements were taken from distinct samples.

Secondary necrotic cell preparation and anti-secondary necrotic cell ELISA. PBMCs were isolated by density gradient-based isolation using Lymphoflot (Bio-Rad) of heparinized whole blood from healthy donors. Isolated PBMCs were adjusted to a concentration of 5×10^6 cells per ml in PBS and irradiated using 240 mJ/cm² of ultraviolet B light for 90 s. After incubation for 24 h at 37°C and antigen retrieval at 56°C for 30 min, SNECs (2) were stored at -20°C in 10 mM Tris buffer containing 1 mM EDTA (pH 8.0) until coating of ELISA plates, as described previously⁴³. Ninety-six-well microtiter plates (Nunc-ImmunoMaxisorp) were coated overnight at 4°C with 50 µl of poly-L-lysine (20 µg ml⁻¹) in 10 mM Tris buffer containing 1 mM EDTA (pH 8.0). Plates were washed three times with PBS, 0.05% Tween 20, pH 7.4. SNECs were coated on poly-L-lysine-coated plates overnight at 4°C in 10 mM Tris buffer containing 1 mM EDTA (pH 8.0). The plates were then blocked for 1 h at room temperature (RT) with 100 µl blocking agent (2% BSA in PBS) per well. Fifty microliters of serum samples per well (1:200 dilution in PBS-T) was added and incubated for 1 h at RT. Next, 50 µl of goat antihuman IgG Fc horseradish peroxidase-conjugated (Southern Biotech) antibody (1:50,000 dilution in washing buffer) was added and incubated for 1 h at RT. Finally, plates were incubated with 50 µl of substrate solution (substrate buffer (0.1 M Na₂HPO₄, 0.05 M citrate acid, pH 5.0), 10% tetramethylbenzidine (TMB) and 0.02% H₂O₂ (30%)) for 5 to 10 min. The reaction was stopped with 50 µl of H₂SO₄ (25%) and absorbances were read at 450 and 620 nm reference wavelengths. All measurements were taken from distinct samples.

Vaccination responses. IgG antibodies against measles (cutoff 150 mIU ml⁻¹), mumps (cutoff 70 U ml⁻¹) and varicella zoster virus (cutoff 50 mIU ml⁻¹) were analyzed by ELISAs from Virion/Serion. IgG antibodies against rubella (cutoff 5 IU ml⁻¹) was analyzed by Bioline ELISA from Abbott, antibodies against hepatitis B surface antigen (cutoff 3 mIU ml⁻¹) by ELISA from Diasorin and anti-PPV23 by ELISA from The Binding Site (cutoff 3 mg l⁻¹). IgG responses against tetanus (cutoff 0.15 U ml⁻¹) and diphtheria toxin (cutoff 0.1 U ml⁻¹) were done by ELISAs from VaccZyme (The Binding Site). All measurements were taken from distinct samples.

Measurement of interferon-alpha levels. Serum concentrations of interferon-alpha were measured by commercial ELISA (IBL International).

Characterization of B cells. B cells from peripheral blood were characterized at baseline (leukapheresis) and after reconstitution of B cells using FACS. Isolated PBMCs were stained with optimal concentrations of directly fluorochrome-conjugated antibodies targeting the surface markers CD45-APC-Fire 750 (HI30, BioLegend), CD19-BV421 (HIB19, BioLegend), CD38-PerCP-Cy5.5 (HIT2, BD Biosciences), CD20-AF700 (2H7, BioLegend), CD21-PE

(Bu32, BioLegend), CD27–PE–Cy7 (M-T271, BioLegend), CD11c–BV650 (Bu15, BioLegend), IgG–BV510 (G18–145, BD Biosciences) and IgA–AF647 (polyclonal, Jackson ImmunoResearch). Zombie NIR (BioLegend) was used for exclusion of dead cells. PBMCs were acquired on a Cytek Northern Lights spectral analyzer (Fremont) and analyzed using FlowJo. B cells were gated as CD45⁺CD3⁺CD19⁺ cells. Frequencies of naïve B cells (CD21⁺CD27[−]), memory B cells (CD21⁺CD27⁺), immature B cells, CD11c⁺CD21^{lo} mature B cells and plasmablasts (CD38⁺CD20[−]) were measured. All measurements were taken from distinct samples.

B cell receptor sequencing. PBMCs were subjected to the 10x Genomics Chromium 5' Single Cell V(D)J immunoprofiling workflow with enrichment for B cells and T cells and the mRNA gene expression analysis workflow according to the manufacturer's instructions. Library sequencing was performed on an Illumina HiSeq 2500 sequencer to a mean depth of 339.4 million reads for expression, 69.9 million reads for T cells and 70.4 million reads for B cells; in all samples, the mean number of reads per cell was greater than 282.1, 55.4 and 49.5 million reads, respectively. Reads were converted to FASTQ format using mkfastq from Cell Ranger 7.0.0 (10x Genomics). Alignment, V(D)J-assembly and quantification were performed using the Cell Ranger multi pipeline with default parameters on the most recent prebuild human reference packages refdata-gex-GRCh38-2020-A for expression and refdata-cellranger-vdj-GRCh38-alts-ensembl-7.0.0 for repertoire analysis.

Statistical analyses. Individual values are presented throughout as the sample size of this case series is small. In addition, descriptive statistics are used for reporting specific parameters at baseline and at 3-months follow-up. Analyses were conducted using R v.4.1.1 (R Foundation for Statistical Computing).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All numeric data from this paper can be obtained at https://mega.nz/file/EXUFmIrT#dIj24lI99kfqQLr52_vz_-2mhJFQKncz9Dumz3qETfk. Patient data can only be shared in pseudonymized form. Otherwise, there are no restrictions to data access. All data graphs in the figures (Figs. 1b–h, 2d, 3a–f, 4a–d and 5a–f) show raw data and depict individual values. Source data are provided with this paper.

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Author contributions

A.M., D.M., G.K. and G.S. designed the treatments and analyses. F.M., S.B. and W.R. monitored the patients. D.S., A.K., S. Kretschmann and S.U. collected clinical data. A.M., L.M., H.R., H.B., M.H., A.B.E., C.B., K.M.H. and T.W. performed molecular analyses. M.A., S. Kharbouthli and R.G. produced CAR T cells. S.V. performed immune monitoring. G.S. and A.M. wrote the manuscript.

Competing interests

There are no competing interests related to this study. The study received no commercial funding. F.M., S.B., A.W., M.A., S.V., D.S., A.K., L.M., S. Kretschmann, S. Kharbouthli, R.G., H.R., W.R., S.U., M.H., A.B.E., C.B., K.M.H. and T.W. declare no competing interests. A.M. received speaker honoraria from BMS, Celgene, Gilead, Janssen, KITE, Miltenyi Biomedicine and Novartis, which were not related to this study. G.K. has received speaker honoraria from AbbVie, BMS, Eli Lilly, GSK, Janssen, Novartis and Sanofi. D.M. received speaker honoraria from AbbVie, Amgen, AstraZeneca, BMS, Celgene, Gilead, Hexal, Jazz Pharmaceuticals, Miltenyi Biomedicine, Novartis, Roche and Takeda, which were not related to this study. H.B. is employee of Orgentec and provided quantitative analyses for autoantibodies against nuclear antigens free of charge. G.S. received speaker honoraria from AbbVie, Janssen and Novartis, which were not related to this study.

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s41591-022-02017-5>.

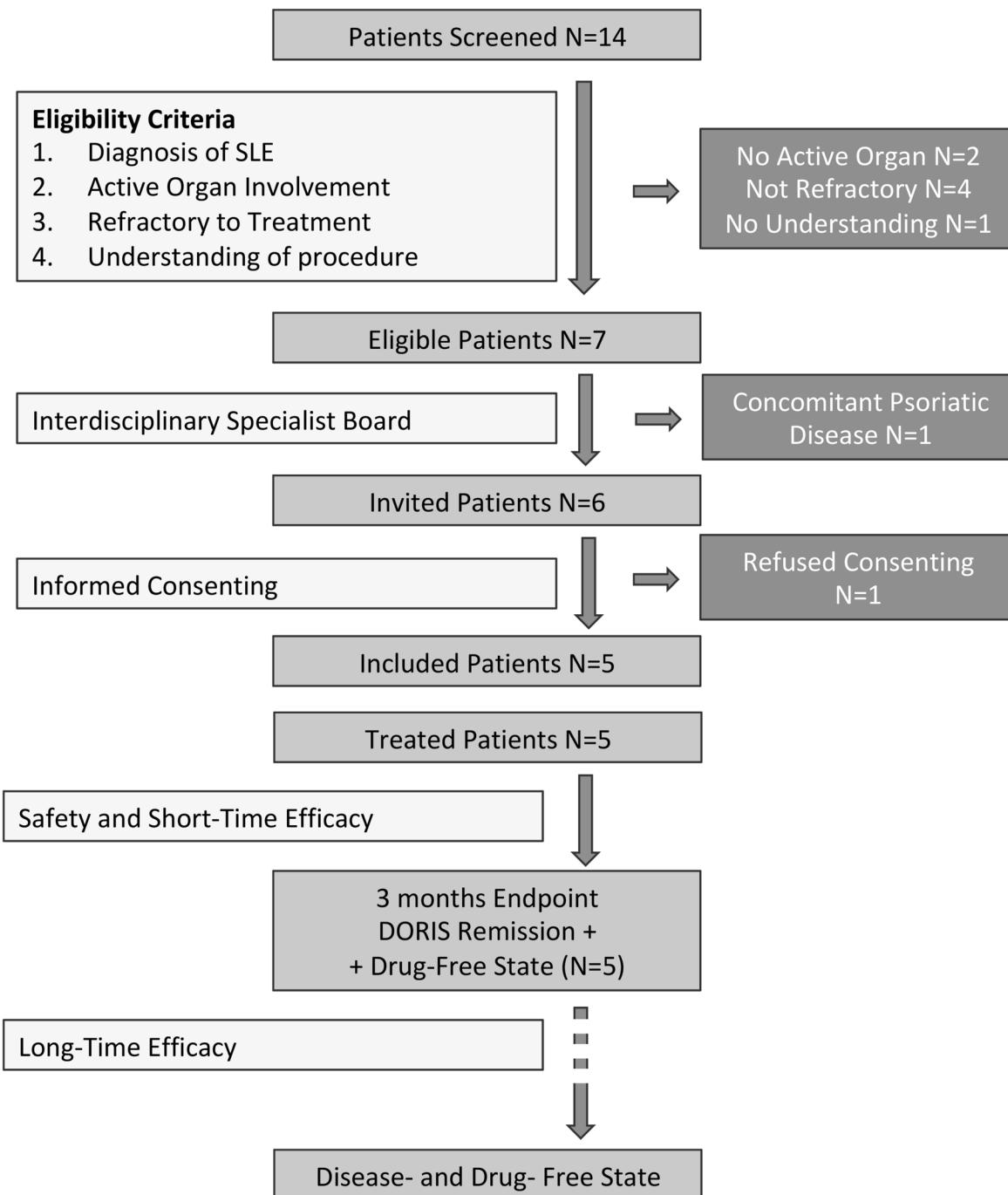
Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41591-022-02017-5>.

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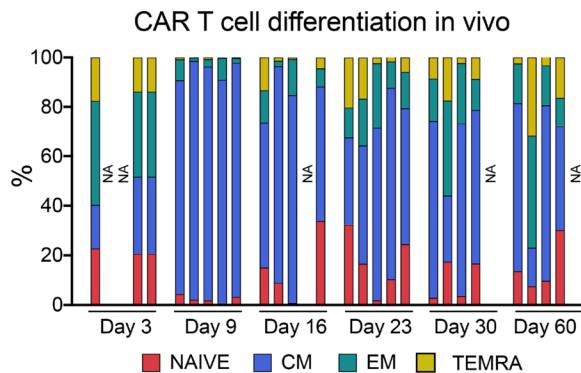
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CONSORT Flow Diagram

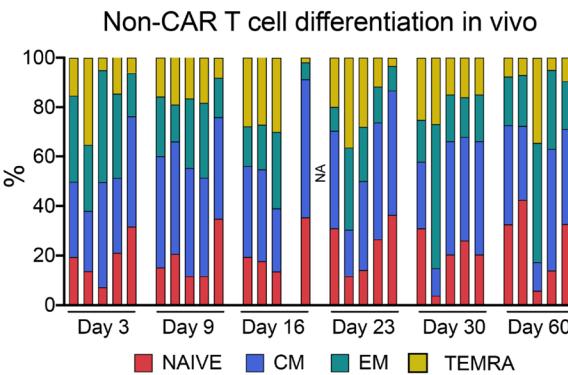


Extended Data Fig. 1 | Consort Flow Diagram. Flow diagram showing eligibility criteria for patients with systemic lupus erythematosus (SLE) to receive treatment with CD19 chimeric antigen receptor (CAR) T cells. Of the 14 SLE patients screened, 2 had no active involvement of the inner organs, 4 had not received all treatments approved for SLE and 1 patient did not understand the procedure. The remaining 7 SLE patients were reviewed by an interdisciplinary specialist board, refusing treatment in 1 patient with concomitant psoriatic disease. One more patient did not consent leaving 5 SLE patients starting CAR T cell therapy. Safety, short time efficacy and long term efficacy endpoints are also shown.

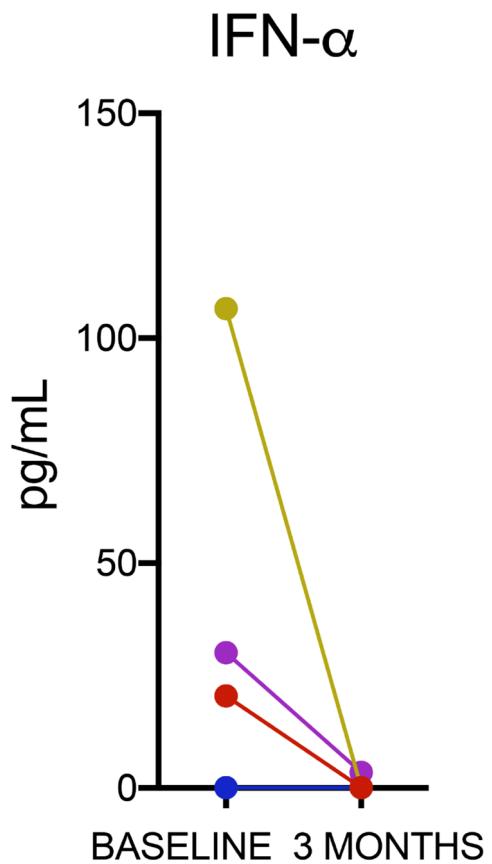
A.



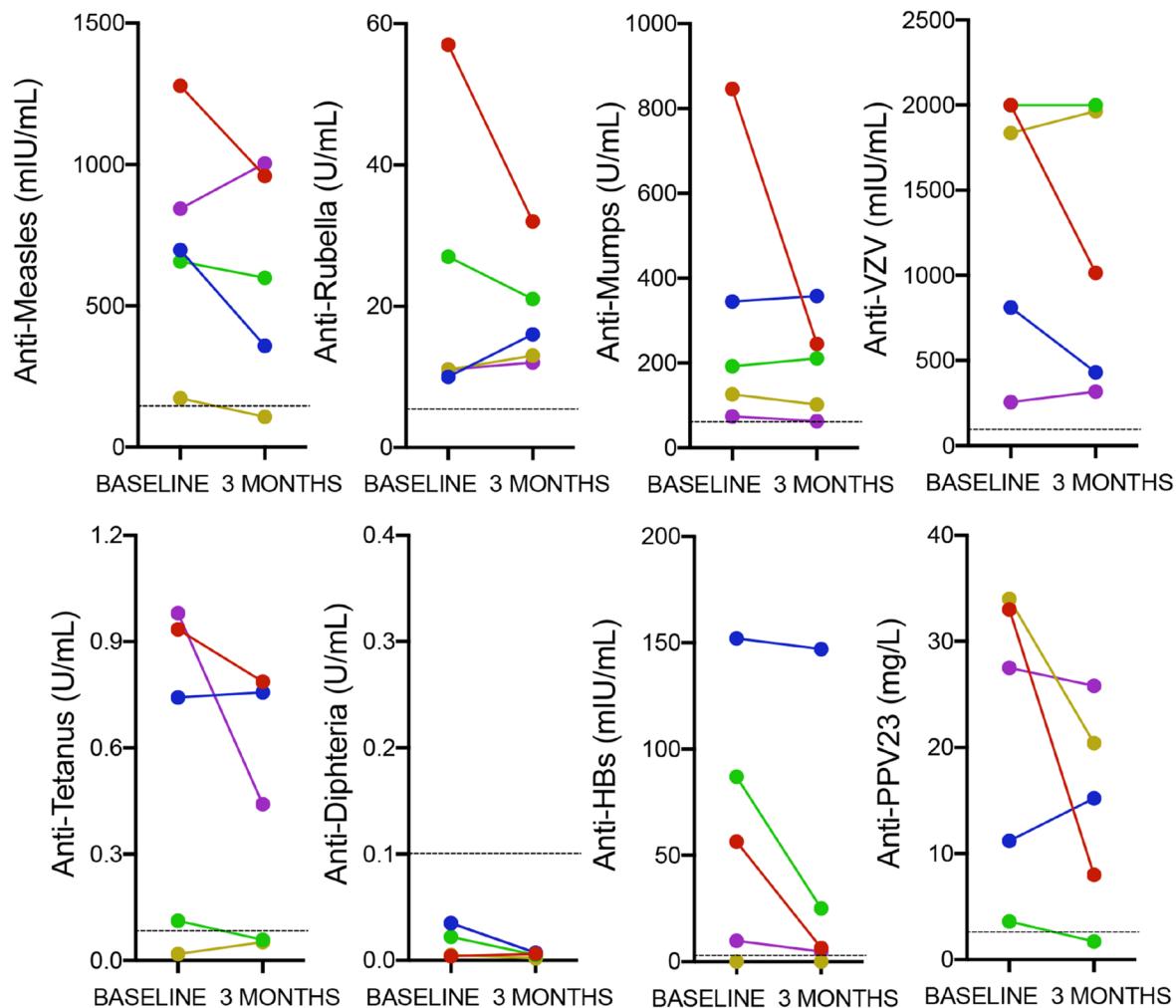
B.



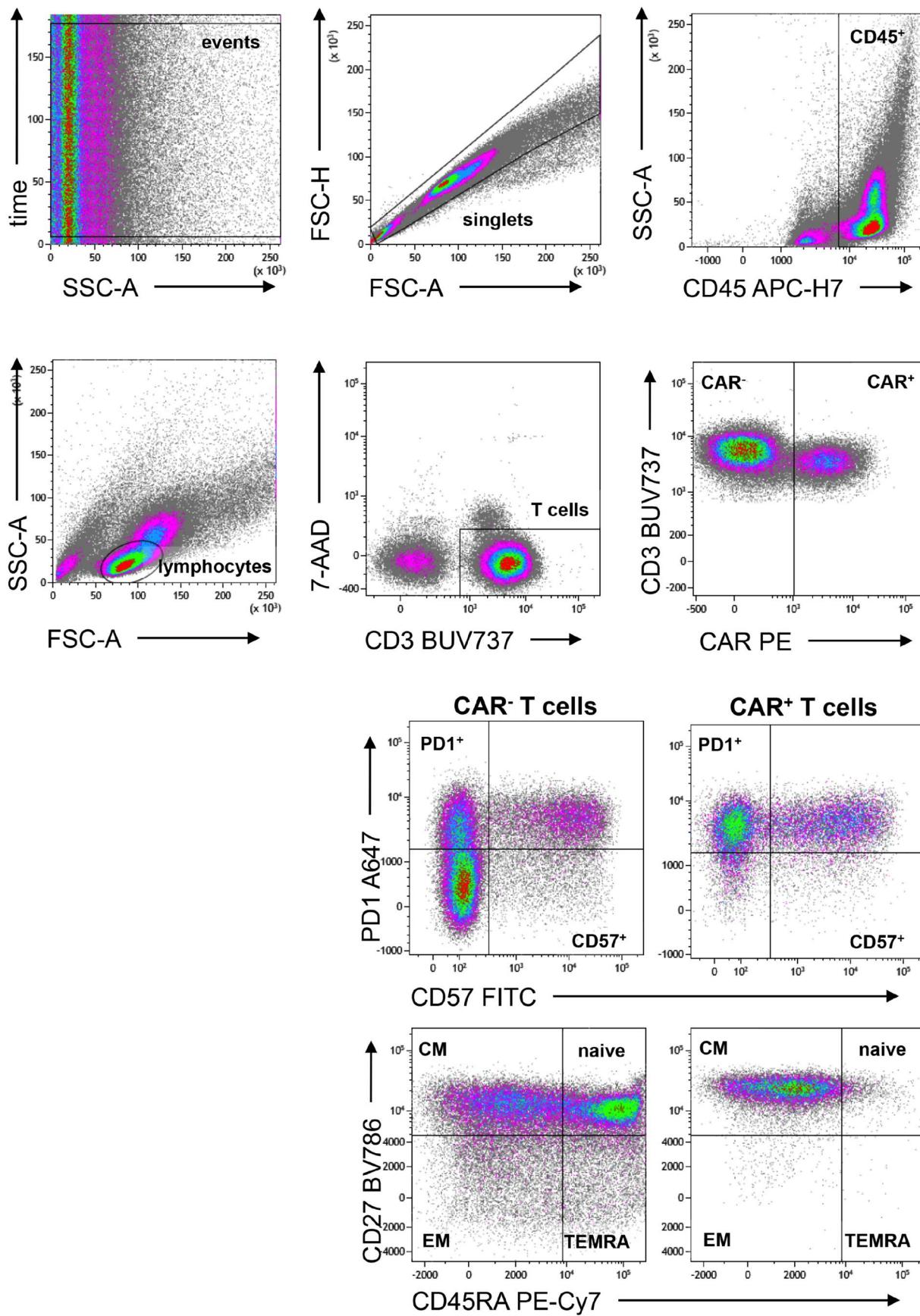
Extended Data Fig. 2 | CAR T cell differentiation in vivo. Immunophenotyping of T cells expressing the chimeric antigen receptor (CAR) (A) and those not expressing the CAR (non-CAR, B). Peripheral blood mononuclear cells from the 5 Systemic Lupus Erythematosus (SLE) patients that received CAR T cells were analyzed using FACS analysis. T cells were analyzed 3, 9, 16, 23, 30 and 60 days after CAR T cell treatment. Stainings distinguished naïve T cells (CD45RA+ CD27+), central memory cells (CD45RA-CD27+), effector memory T cells (CD27-CD45RA-) and effector memory T cells re-expressing CD45RA (TEMRA). Each bar represents data from one patient at one specific time point; left to right shows SLE patient 1 to 5. NA, not analyzed.



Extended Data Fig. 3 | Interferon-alpha levels. Serum levels of interferon (IFN)-alpha at baseline and 3 months after treatment with chimeric antigen receptor (CAR) T cells. IFN-alpha was measured by enzyme-linked immunosorbent assay and detectable in 3 out of 5 patients at baseline and in none of the patients 3 months after CAR T cell therapy.



Extended Data Fig. 4 | Antibody levels against infection agents and vaccinations. Antibody levels against measles, rubella, mumps, varicella zoster virus (VZV), hepatitis B (HBs, hepatitis B surface antigen), tetanus, diphtheria and pneumococci (PPV23, polyvalent pneumococcal vaccine -23) at baseline and 3 months after CAR T cell administration (N=5).



Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5 | Gating strategy for CAR detection. Relevant for data presented in Figs. 1 and 2; Time parameter was used to monitor instrument stability, doublets were excluded by FSC-H/FSC-A, CD45⁺ events were gated, lymphocytes were determined by FSC-A/SSC-A, viable T cells were gated by CD3⁺ and 7-AAD⁻, and further subdivided in CAR⁺ and CAR⁻ T cells. CAR⁺ and CAR⁻ T cells were analyzed for PD1, CD57, CD27, and CD45RA expression.

Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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Give P values as exact values whenever suitable.
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- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection FlowJo v10.6.1 (Becton Dickinson, Ashland, OR) and Kaluza v2.1 (Beckmann Coulter, Brea, CA)

Data analysis Rv4.1.1 (R Foundation for Statistical Computing; Vienna, Austria)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

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All numeric data of this manuscript can be obtained at https://mega.nz/file/EXUFmlrT#dlj24lI99kfqQLr52_vz_-2mhJFQKncz9Dumz3qETfk. Patient data can only be shared in pseudonymized form. Otherwise there are no restrictions to data access. All data graphs in the figures (figures 1B-H, 2-D, 3A-F, 4A-D, 5A-F) show raw data as and depict individual values.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|-----------------|--|
| Sample size | Case series of a compassionate use program. No sample size calculation is applicable |
| Data exclusions | No data were excluded. |
| Replication | N/A; this was not a study but a compassionate use program; there is no replication applicable |
| Randomization | N/A; this was not a study but a compassionate use program; there is no control arm, hence no randomization |
| Blinding | N/A; this was not a study but a compassionate use program; there is no control arm, hence no blinding; |

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | | Methods | |
|-------------------------------------|---|-------------------------------------|--|
| n/a | Involved in the study | n/a | Involved in the study |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Antibodies | <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines | <input type="checkbox"/> | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology | <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms | | |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Human research participants | | |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Clinical data | | |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern | | |

Antibodies

| | |
|-----------------|---|
| Antibodies used | 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). Fluorochrome-conjugated antibodies targeting the surface markers CD45–APC-Fire 750 (HI30, BioLegend), CD19–BV421 (HIB19, BioLegend), CD38–PerCP-Cy5.5 (HIT2, BD Biosciences), CD20–AF700 (2H7, BioLegend), CD21–PE (Bu32, BioLegend), CD27–PE-Cy7 (M-T271, BioLegend), CD11c–BV650 (Bu15, BioLegend), IgG–BV510 (G18-145, BD Biosciences) and IgA–AF647 (polyclonal, Jackson ImmunoResearch). |
| Validation | All antibodies are validated for specificity to their respective target on human cells as provided by the information by the manufacturer. |

Human research participants

Policy information about [studies involving human research participants](#)

| | |
|----------------------------|---|
| Population characteristics | Demographic and clinical characteristics of the patients are summarized in Table 1. |
| Recruitment | All 5 patients with treatment-refractory SLE, who underwent CAR T cell therapy were recruited at the Department of Internal Medicine 3 (Rheumatology and Immunology) of the Friedrich Alexander University Erlangen-Nürnberg. Patients were consecutively enrolled between February 2021 and February 2022. Patients screened for this compassionate use program had to have (i) a diagnosis of SLE according to the EULAR/ACR 2019 criteria, (ii) signs of active organ involvement, (iii) failure to multiple immunomodulatory therapies including repeated pulsed glucocorticoids, hydroxychloroquine, belimumab and mycophenolate mofetil and (iv) be able to understand the procedure of CAR T cell therapy. No bias in recruitment is expected as enrollment in the compassionate use program had clear criteria (see above). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

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All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration N/A

Study protocol N/A

Data collection All data were collected over a period of 3 months.

Outcomes DORIS remission of SLE plus no immunosuppressive treatment

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Absolute cell counts were determined with BD TruCount tubes according to the manufacturer's instruction. For CAR T cell monitoring, peripheral blood mononuclear cells (PBMC) were isolated from EDTA whole blood by gradient density centrifugation (Pancoll, PAN Biotech, Aidenbach, Germany) and directly analyzed or stored in liquid nitrogen until assayed. PBMC were washed in PBS and stained with the indicated antibodies according to the manufacturer's instructions.

Instrument

Samples were measured on a LSRFortessa (BD Biosciences, Heidelberg, Germany)

Software

Flow cytometry data were analyzed with FlowJo v10.6.1 and Kaluza v2.1.

Cell population abundance

1%-60%

Gating strategy

Gating strategy is provided in the supplementary information. SSC-A <100x103; FSC-A 50-150x103; FSC-H <100x103; CD45-APC-H7 >1x104; CD3-BUV737 >1x103; CAR-PE >1x103;

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.