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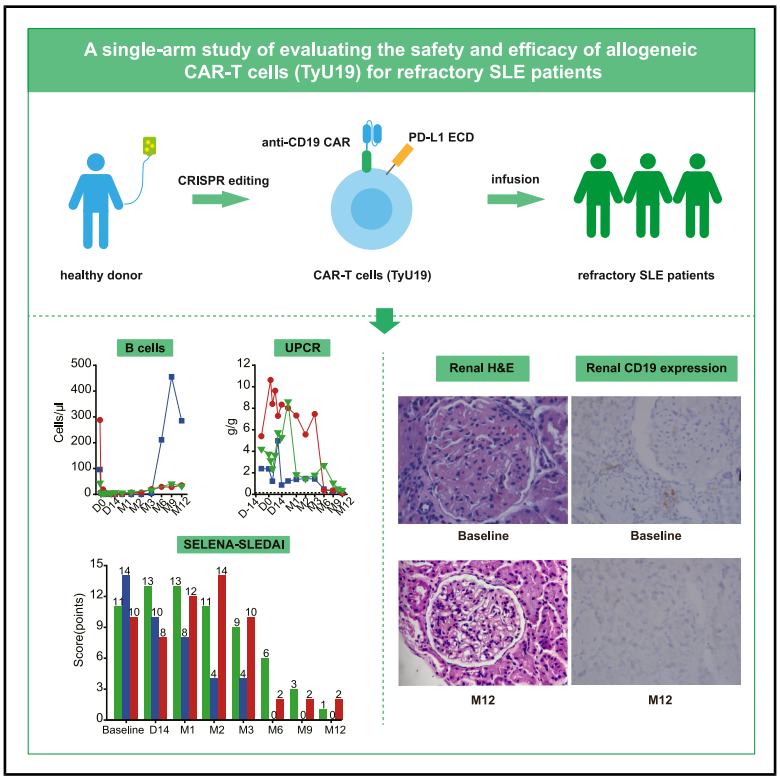
Allogeneic CD19-targeted CAR-T therapy in refractory systemic lupus erythematosus achieved durable remission

Graphical abstract



Highlights

- Allogeneic CAR-T cells expanded *in vivo* and efficiently eliminated B cells in SLE
- Patients with SLE received clinical SRI-4 response with allogeneic CAR-T cell therapy
- Allogeneic CAR-T cell therapy was well tolerated and promising for refractory SLE

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In brief

Wang et al. demonstrate that allogeneic CD19-targeted CAR-T cell therapy is well tolerated in all enrolled patients with SLE, resulting in significant depletion of peripheral and renal CD19^{+} B cells and achieving clinical remission as assessed by SRI-4 criteria.

Translation to Patients

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Allogeneic CD19-targeted CAR-T therapy in refractory systemic lupus erythematosus achieved durable remission

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CONTEXT AND SIGNIFICANCE Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with limited effective treatments, especially for relapsed or refractory cases. This study highlights a novel therapeutic approach, investigating the safety and efficacy of allogeneic CD19-targeted chimeric antigen receptor (CAR)-T cells in three patients with severe SLE. Results showed that the treatment was well tolerated, with no graft-versus-host disease (GvHD), cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), or immune cell-associated hemophagocytic syndrome (ICAHS) observed. The infused CAR-T cells proliferated robustly and effectively eliminated B cells, leading to a marked decrease in serum autoantibodies. Notably, all three patients achieved clinical remission as defined by SLE responder index-4 (SRI-4) criteria by the final assessment. These promising findings indicate that allogeneic CAR-T cell therapy represents a significant potential advancement in the treatment of SLE, warranting further investigation and potentially improving clinical outcomes.

SUMMARY

Background: Autoreactive B cells play a key role in the pathogenesis of systemic lupus erythematosus (SLE). The aim of this study is to assess the safety of allogeneic chimeric antigen receptor (CAR)-T cells for patients with lupus. This study was registered at ClinicalTrials.gov (NCT05859997).

Methods: In this study, 3 patients with refractory and severe SLE with multi-organ involvement were enrolled. Genetically engineered healthy-donor-derived, CD19-targeting CAR-T cells were infused intravenously at a dose of 1 million cells per kilogram of body weight. The safety indices, including the occurrence of graft-versus-host disease (GvHD), cytokine release syndrome (CRS), and immune effector cell-associated neurotoxicity syndrome (ICANS), were evaluated. The proliferation of CAR+ T cells and the number of peripheral B cells were assessed. The clinical efficacy was also assessed based on the SELENA-SLEDAI, SLEDAI-2K, BILAG, clinical SLE responder index-4 (SRI-4), and DORIS remission index.

Findings: Between August 2023 and October 2023, 3 patients with SLE were enrolled and completed a 12-month follow-up. No patient underwent GvHD, CRS, or ICANS, and no severe adverse events were recorded. CAR+ T cells expanded *in vivo*, peaking at day 14, and then declined. The percentage of B cells in lymphocytes and the absolute circulating B cell counts were profoundly decreased. Patient 1 withdrew

from the study at month 1 due to unresolved and severe thrombocytopenia and the need for the addition of an immunosuppressive drug. SELENA-SLEDAI and SLEDAI-2K scores declined, and all the patients reached SRI-4 remission at the last visit.

Conclusions: In patients with severe and refractory SLE, allogeneic CAR-T cell therapy showed profound safety and clinical efficacy for disease remission.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by an abundance of class-switched autoantibodies primarily to nuclear proteins, lymphoproliferation, immune complex deposition, and inflammation in multiple organs. Lupus nephritis (LN) is the most common complication of SLE, resulting from immune complex deposition in the kidneys.[1] Given that B cells are the source of pathogenic anti-nuclear antibodies and their generation precedes the clinical onset of SLE, therapeutic strategies have been focused on B cell blockade.[2] However, monoclonal antibodies that deplete B cells or blockade of the activation and proliferation of B cells have only achieved incomplete responses or remission in a small number of patients, largely attributed to insufficient depletion or failure to block the activation of autoreactive B cells.[2–4] Therefore, achieving deep and durable depletion of autoantibody-producing B cells poses a significant challenge in SLE.

In the last 2 years, autologous chimeric antigen receptor (CAR)-T cell therapy targeting the CD19 antigen has begun to be explored in autoimmune diseases.[5–12] CD19 CAR-T cells have shown promising results in inducing rapid and sustained depletion of circulating B cells, leading to complete clinical and serological remission of refractory SLE, dermatomyositis, and systemic sclerosis. However, the application of personalized autologous CAR-T cells has been limited by significant clinical, logistical, and economic barriers, such as the costs, variability, and risks of disease aggravation during the manufacturing of autologous products. Recently, new approaches, such as T cells to express a muscle-specific tyrosine kinase (MuSK) chimeric autoantibody receptor with CD137-CD3ζsignaling domains (MuSK-CAART)[13] and regulatory T cells (Tregs) overexpressing FoxP3 and harboring anti-CD19 CAR (Fox19CAR-Tregs),[14] showed promising efficacy in the depletion of antigen-specific B cells and restoration of human immune system composition in lymphoid organs, which indicated promising prospect for treating patients with autoimmune disease. However, both are in preclinical stages. Therefore, an "off-the-shelf" allogeneic CAR-T therapy may offer an attractive option to overcome the above issues and improve the accessibility of CAR-T cell therapy for all patients.[15,16] Thus, exploring the potential of applying allogeneic CAR-T products for treating refractory SLE is warranted.

In this study, we have developed a healthy-donor-derived, multiplex genome-edited allogeneic CD19-targeted CAR-T product for the treatment of 3 patients with refractory and severe SLE. The treatment achieved deep B cell depletion and significant clinical response in all patients that persisted throughout the 12-month monitoring period, with a highly desirable safety profile. This study shows the potential of an off-the-shelf, allogeneic CAR-T product for the treatment of refractory SLE.

RESULTS

Patient characteristics

All three patients were consecutively enrolled between August 8, 2023, and October 8, 2023, after giving signed informed consent. Detailed patient characteristics are shown in Table 1.

The first patient (S0301) recruited was an 18-year-old female with severe refractory SLE. She was first diagnosed with SLE in 2014 with severe edema in her face and both legs and was tested for proteinuria 3+, positive anti-nuclear antibody (ANA; 1:1,000), and low complements (complement 3 [C3]: 0.347 g/L, C4: 0.069 g/L). The diagnosis of SLE and LN was confirmed by renal biopsy, which revealed class IV LN according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) standards[17] (see Figure S1A). The patient had been taking 75 mg prednisone and 1.5 g mycophenolate mofetil (MMF) daily, and then the dose of prednisone was tapered with negative proteinuria in the following 1 year. In May 2015, the patient had a major flare of LN with a 24-h urine protein of 1.8 g, with thrombocytopenia (platelet count: 34×10^9/L), arthralgia, and finger vasculitis. She responded well with pulse glucocorticoid (GC) followed by 3 mg tacrolimus (TAC) daily. Then, she received prednisone (5–15 mg daily) and TAC (2–4 mg daily) for maintenance therapy in the following 6 years. In March 2022, the patient was prescribed 2 g MMF daily combined with 4 mg TAC daily due to a disease flare (platelet count: 59×10^9/L and 24-h urine protein: 3.13 g). However, her platelet count continued to decline to 8×10^9/L, and she was unresponsive to another round of pulse GC and intravenous immunoglobulin (IVIG). After confirmation of active proliferation by bone marrow puncture, she was given intravenous cyclophosphamide (CYC) and eltrombopag olamine. However, her platelet count continued to decline to $2–6 \times 10^9$/L. Eltrombopag olamine was withdrawn, and she received two rounds of umbilical cord-derived mesenchymal stem cell therapy (MSCT) and 160 mg telitacicept per week for 4 months. Several times the patient suffered vaginal bleeding with a platelet count varying from 2 to 29×10^9/L and a 24-h urine protein ranging from 8.27 to 15.39 g (Figure S2). The patient was recruited to be treated with allogeneic CAR-T cells (TyU19) in the current trial. Informed consent in accordance with the Declaration of Helsinki was obtained in writing from the patient. She started the preconditioning treatment on August 11, 2023.

Patient 2 (S0302), a 37-year-old woman, was diagnosed with SLE in 2012 and presented with malar rash, arthritis, leukopenia,

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Table 1. Baseline characteristics of the patients

	S0301	S0302	S0303
Demographics			
Age (years)	18	37	54
Sex (female/male)	female	female	female
Disease duration (years)	9	11	20
Disease progressing (yes/no)	yes	yes	yes
Laboratory values			
24-h proteinuria (mg/24 h)	8,271	2,872	7,851.4
Baseline UPCR (g/g)	4.2	2.39	5.4
Baseline ANA (a.u./mL)	446	16,850	170
Baseline dsDNA (IU/mL)	41	2,560	92
Baseline anti-SM (a.u./mL)	6.36	133	<3.50
Baseline C3 (g/L)	1.07	0.62	0.94
Baseline C4 (g/L)	0.2	0.07	0.27
SELENA-SLEDAI	11	14	10
BILAG-2004	2A	1A	1A
PGA	1.7	1.4	1.6
Previous treatment history			
Glucocorticoid	+	+	+
Hydroxychloroquine	+	+	+
Immunosuppressant drug			
Cyclophosphamide	+	+	+
Mycophenolate mofetil	+	+	+
Tacrolimus	+	+	+
Leflunomide	+	–	+
Tripterygium	–	–	+
B cell-targeted treatment			
Telitacicept	+	+	–
Belimumab	–	–	+
Cell therapy			
Mesenchymal stem cell treatment	+	+	+

and proteinuria. Initially, she was treated with GC (30 mg prednisone per day, gradually tapered to 5 mg per day), hydroxychloroquine sulfate (HCQ; 400 mg per day), and 0.4 g CYC every 2 weeks for 6 months, resulting in the resolution of proteinuria and the disappearance of rash and arthritis. Subsequently, CYC was replaced with azathioprine (AZA; 100 mg per day) for maintenance therapy. However, when the patient experienced recurrent proteinuria, the treatment was switched to TAC at a daily dose of 3 mg, which still failed to alleviate the proteinuria. As a result, the regimen was changed to MMF at a daily dosage of 1.5 g. Since then, the patient has maintained this regimen, which has effectively controlled the proteinuria. In January 2021, she developed severe lower limb edema and arthralgia in her wrists and ankle joints. Laboratory tests revealed increased proteinuria with a 24-h urine protein measured at 3.49 g. The patient's treatment was adjusted to include increased prednisone (40 mg per day) and MMF (2 g per day). After 3 months, prednisone was gradually tapered down to 10 mg per day, yet the patient experienced recurrence of arthralgia and unsatisfactory improvement in proteinuria (24-h urine protein: 2.09 g). Therefore, 2 g MMF per day was switched to 2 mg TAC per day plus 1 g MMF per day, which prevented further worsening of the condition. In December 2021, 160 mg telitacicept per week was added to the existing regimen of TAC, MMF, and GC, which led to a slight improvement in arthralgia, but proteinuria fluctuated between 1.32 and 8.54 g per day over the following year. In January 2023, intravenous CYC induction therapy (0.4 g every 2 weeks) was reintroduced alongside TAC (2 mg per day), but the patient responded poorly. Subsequently, on March 21, 2023, the patient received an infusion of MSCT, which did not significantly improve the condition. The patient continued to experience active LN, as confirmed by renal biopsy (ISN/RPS class III+V), with an active index of 4 and a chronic index of 2. Informed consent was obtained from the patient, and she started the preconditioning for treatment on August 24, 2023.

The third patient (S0303), a 54-year-old woman, received a diagnosis of SLE in 2004 and presented with reversible proteinuria and rash. Previous treatments with high-dose GC, HCQ, CYC, TAC, MMF, leflunomide (LEF), and tripterygium glycosides (TGs), and she suffered various disease flares. In March 2023, the patient reported severe abdominal pain, vomiting, oliguria, and edema. Cholecystitis and mesenteric vasculitis were further confirmed by computed tomography (CT) scan, and the symptom markedly improved by increased dose of GC and another round of CYC. However, sustained proteinuria and aggravated renal function were found (24-h urine protein was 17.74 g and urinary protein creatinine ratio (UPCR) was 12.45, with an albumin of 16.7 g/L and an estimated glomerular filtration rate (eGFR) minimum of 41.7 mL/min/1.73 m^2). Renal biopsy confirmed LN of ISN/RPS class V, with an active index of 2 and a chronic index of 3 (see Figure S1B). Having exhausted all available treatment options, she was ultimately enrolled in the trial and began preconditioning on October 11, 2023.

Allogeneic CD19-targeted CAR-T cell (TyU19) production and infusion

The allogeneic CD19-targeted CAR-T cells (code name: TyU19) were produced according to a previous study.[16] Briefly, T cells isolated from healthy peripheral blood mononuclear cells (PBMCs) were first transduced with lentivirus coding an anti-CD19 CAR construct and then were knocked out for human leukocyte antigen (HLA)-A, HLA-B, class II major histocompatibility complex transactivator (CIITA), T cell receptor alpha constant (TRAC), and PD-1 by electroporation-based CRISPR-Cas9 gene editing using Cas9 protein in complex with single guide RNAs (sgRNAs) for each target. Afterward, the T cells were expanded *in vitro* for 12 days. Finally, magnetic-activated cell sorting for purification of CD3− cells was performed to avoid graft-versus-host disease (GvHD) by the allogeneic T cells. 1×10^8 CAR+ TyU19 cells were cryopreserved to be used as an off-the-shelf product.

Upon enrollment, each patient was hospitalized in a single room, and all immunosuppressants were stopped before lymphodepletion except for GCs, which were gradually tapered down. All the patients received a standard preconditioning regimen involving 25 mg/day/m^2 fludarabine from day 5 (5 days before infusion) to day 3 and 300 mg/day/m^2 CYC on

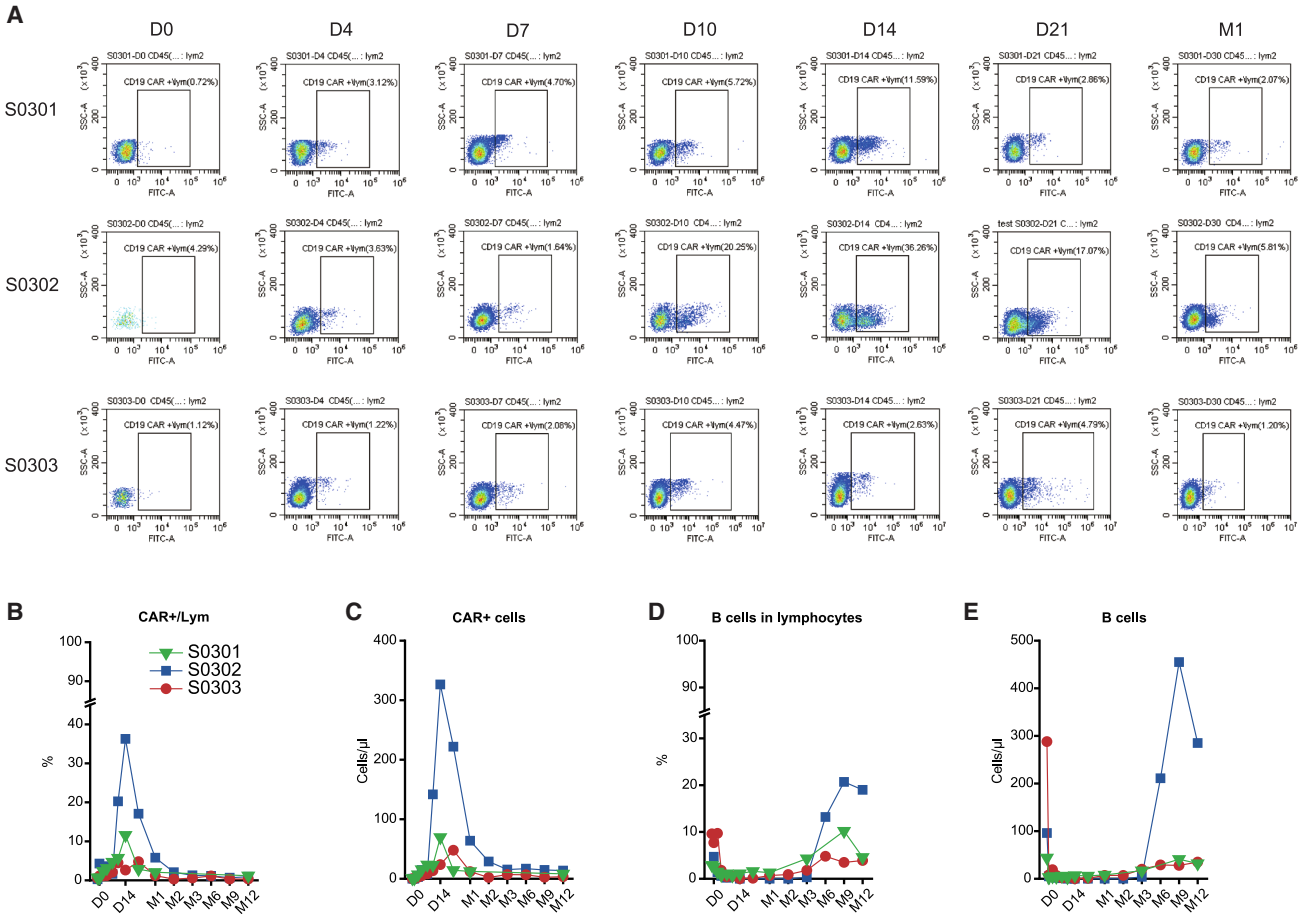

Figure 1. Allogeneic CD19-targeted CAR-T cell expansion and B cell depletion

(A) Flow cytometry analysis of CAR+ T cells for 3 patients at different time points.

(B) The percentage of circulating TyU19 cells among total lymphocytes at different time points after infusion.

(C) The absolute number of circulating TyU19 cells at different time points after infusion.

(D) The percentage of circulating B cells at different time points after TyU19 cell infusion.

(E) The absolute number of circulating B cells at different time points after TyU19 cell infusion.

days 5 and 4 for lymphodepletion. Then, 1×10^6 CAR+ TyU19 cells/kg were infused intravenously. The patient was discharged from the hospital until the 14th day after TyU19 cell infusion under the condition that no severe adverse event (AE) was observed. Each patient underwent systematic follow-up for safety and efficacy on the 14th day (day 14) and at 1-, 2-, 3-, 6-, and 12-month intervals after TyU19 infusion. A medical team, including rheumatologists and a hematologist, will undertake the visits.

Allogeneic CD19-targeted CAR-T cell expansion and B cell eradication

After infusion, the percentage of anti-CD19 CAR+ cells among CD45+ lymphocytes in patients' peripheral lymphocytes was monitored using flow cytometry and staining CAR with CD19-fluorescein isothiocyanate (FITC). For subject S0301, the percentage of CAR+ cells significantly increased after infusion, peaked at 11.59% on day 14, and dropped to 2.86% and 2.07% on day 21 and month 1, respectively (Figures 1A and 1B). This patient withdrew from the study at month 1 because of severe thrombocytopenia. She was then on the rescue treatment. For subject S0302, the CAR+ cells significantly increased within the first 2 weeks after infusion, peaking at 36.26% on day 14, and then gradually dropped to 5.81% at month 1, 1.29% at month 3, and 1.13% at month 6 (Figures 1A and 1B). Consistently, the absolute numbers of CAR+ cells peaked at 326.34 cells/μL on day 14 and at 15.48 and 16.95 cells/μL at month-3 and -6 follow-ups (Figure 1C). For subject S0303, there was a marked increase in both the percentage and absolute number of CAR+ cells. The percentage of CAR+ cells significantly increased within the first week after infusion, peaked at 4.47% on day 10, and dropped to 2.63% on day 14. On day 21, there was another increase in the percentage of CAR+ cells (4.79%; Figures 1A and 1B), indicating successful *in vivo* expansion of implanted cells. The absolute number of CAR+ cells declined to 7.08 cells/μL at month 3, 6.72 cells/μL at month 6, 2.56 cells/μL at month 9, and 3.50 cells/μL at month 12 (Figure 1C).

In line with the expansion of CAR+ cells, the percentage of B cells in lymphocytes and the absolute circulating B cell counts

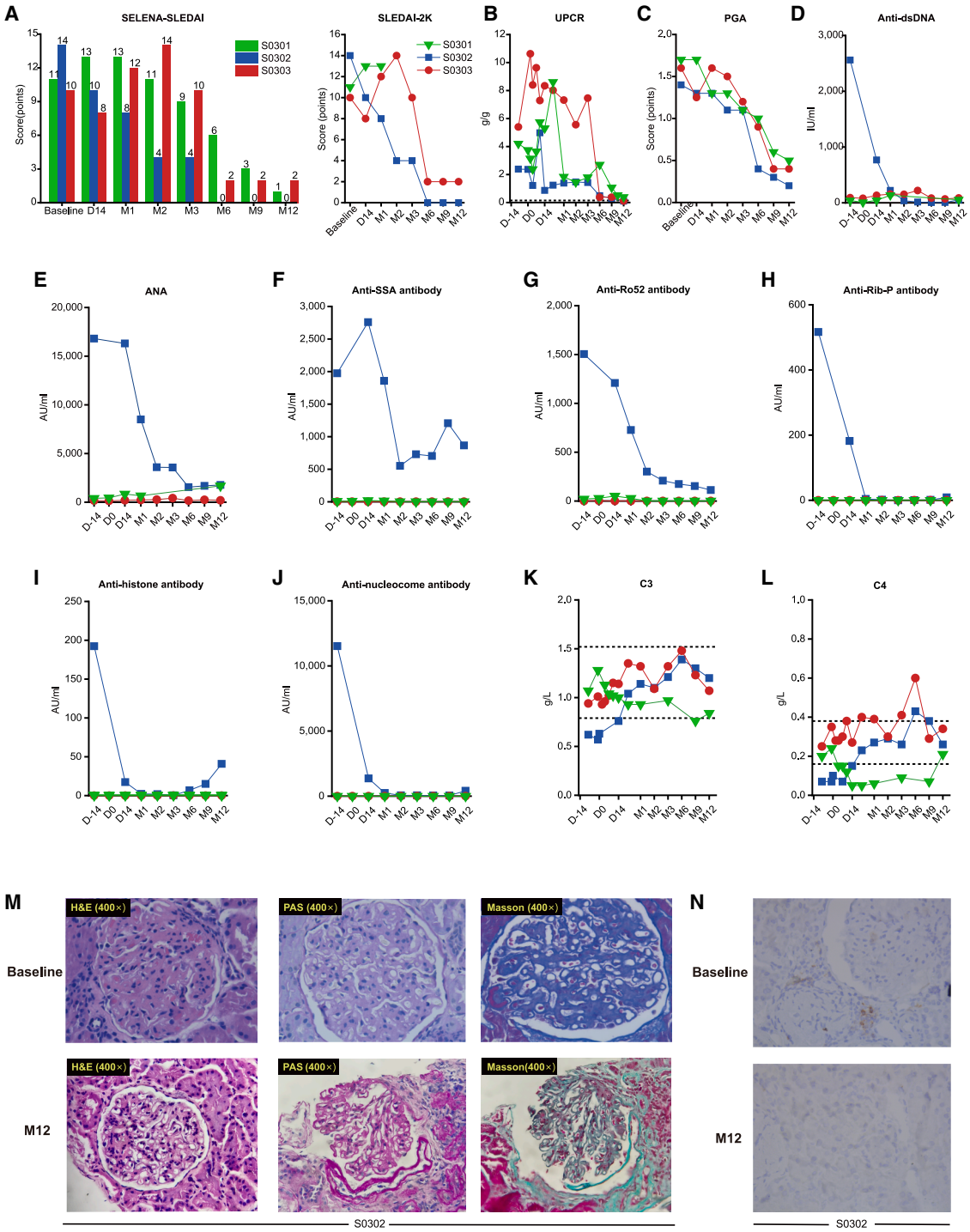

Figure 2. Clinical efficacy following allogeneic CD19-targeted CAR-T cell treatment

(A) SELENA-SLEDAI as well as SLEDAI-2K scores before and after TyU19 cell therapy.

(B) The change of UPCR before and after TyU19 cell therapy.

(C) PGA scores before and after TyU19 cell therapy.

(D) Serum anti-dsDNA antibody before and after TyU19 cell therapy.

(E) Serum ANA before and after TyU19 cell therapy.

(F) Serum anti-SSA antibody before and after TyU19 cell therapy.

(G) Serum anti-Ro52 antibody before and after TyU19 cell therapy.

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were profoundly decreased (Figure 1D). The absolute number of B cells for subject S0301 dropped to 3 cells/μL on day 10, when CAR+ cells proliferated at peak level. The B cell counts were 7, 6, and 8 cells/μL on day 14, on day 21, and at month 1, respectively, and remained consistently low throughout the entire monitoring period, although she withdrew from the trial (Figure 1E).

For subject S0302, B cells disappeared beginning on day 10 and remained at zero by month 2, then slightly increased at month 3, when the absolute number of B cells is 4 cells/μL. B cells recovered to 211, 455, and 285 cells/μL at the month-6, -9, and -12 visits, respectively (Figure 1E). For subject S0303, the absolute number of B cells dropped to 0 on day 14 and then 1 cell/μL on day 21. B cell count increased to 20 cells/μL at month 3 and was sustained at 29 and 35 cells/μL at months 6 and 12, respectively (Figure 1E).

Clinical efficacy following allogeneic CD19-targeted CAR-T cell treatment

Disease activity of SLE was continuously assessed after administration of TyU19 cells using both the SELENA-SLEDAI and SLEDAI-2K scale. All 3 patients had active SLE with multi-organ involvement at baseline, with SELENA-SLEDAI scores between 10 and 14. For subject S0301, the SELENA-SLEDAI score increased from 11 at baseline to 13 on day 14 due to the new onset of hypocomplementemia (Figure 2A). Additionally, the platelet count remained at a very low level (7–9 $\times$ 10^9/L) during the first month, which could have led to unstoppable vaginal bleeding. However, during this period, the UPCR had decreased from 4.20 to 1.84 g/g (Figure 2B). Despite this, the patient voluntarily withdrew from the study in month 1 because of the uncontrollable vaginal bleeding. At that point, she started taking 2 mg TAC daily, and a platelet transfusion was administered due to a potential life-threatening condition. Her platelet count increased to 54 $\times$ 10^9/L by month 6 and 96 $\times$ 10^9/L by month 12 (Figure S2). Although she withdrew from the trial, she continued to be monitored. As shown in Figure 2A, her UPCR was 2.70 g/g at month 6, 1.07 g/g at month 9, and 0.39 g/g at month 12. Her SELENA-SLEDAI score was 6 at month 6, 9 at month 9, and 1 (due only to leukopenia) at month 12. At her last follow-up, she was treated with 7.5 mg prednisone and 2 mg TAC daily and had achieved a clinical SRI-4 response.

For subject S0302, arthrodynia and alopecia disappeared beginning at the month-1 visit. UPCR dropped from 2.39 g/g at baseline to 1.39 g/g at month 1, 1.43 g/g at month 3, 0.49 g/g at month 6, 0.30 g/g at month 9, and 0.10 g/g at month 12 (Figure 2B). SELENA-SLEDAI score decreased from 14 at the baseline to 0 at month 6 and remained at score 0 by the month-12 follow-up, regardless of the recovery of normal levels of B cells (Figure 2A). At the month-6, -9, and -12 follow-ups, the patient achieved clinical SRI-4 and DORIS remission, with the BILAG-2004 score improving from 1A to 1D. Additionally, there was a progressive reduction in the Physician's Global Assessment (PGA) (Figure 2C). Prednisone was withdrawn at month 6, and now she does not use any SLE-associated medication.

For subject S0303, her UPCR was 5.4 g/g at baseline, which was not ameliorated at the first 3 monthly visits (UPCR was 7.32 g/g at month 1, 5.56 g/g at month 2, and 7.46 g/g at month 3). However, significant improvement of proteinuria was found after the month-3 visit. At month-6, -9, and -12 visits, her UPCR dropped to 0.38, 0.38, and 0.11 g/g, respectively (Figure 2B). No active sediment (protein casts, cellular casts, or dimorphic erythrocytes) was found in urine analysis. The patient reached clinical SRI-4 response at the month-6 visit. Now, she has maintained 2.5 mg prednisone per day without any other immunosuppressants.

Serologic improvement by allogeneic CD19-targeted CAR-T cell treatment

All 3 patients showed positive serum ANA at baseline. Patient S0301 showed negative anti-double-strand DNA (dsDNA) antibody and anti-Smith antibody at baseline. Serum ANA demonstrated a slight increase from 446 to 662 a.u./mL at month 1, when she withdrew from the study. Moreover, ANA continued to increase to 1,681 a.u./mL, and a positive anti-Smith antibody (227 a.u./mL) was found at the month-12 visit.

Along with remarkable B cell depletion, patient S0302 experienced deep serological remission with significantly decreased ANA and anti-dsDNA antibody after CAR+ cell infusion (Figures 2D and 2E). Similarly, the serum level of anti-Sjögren's syndrome A antibody (SSA), anti-Ro52, anti-Rib-P, anti-histone, and anti-nucleosome antibodies also dropped to a normal range by month 1 and remained at low levels at month 12 (Figures 2F–2J).

Patient S0303 showed a slight positive level of ANA (170 a.u./mL) and anti-dsDNA antibody (92 IU/mL) at baseline, and the concentration of these two antibodies was maintained at a low level at the 12-month visit (195 a.u./mL for ANA and 87.3 IU/mL for anti-dsDNA antibody; Figures 2D and 2E). She maintained negative anti-SSA and anti-Ro52 at the other visits (Figures 2F and 2G).

Serum complements for subjects S0301 and S0303 were all normal at baseline and persisted stably to the month-12 visit for subject S0303. C4 for subject S0301 decreased to 0.06 g/L by month 1 after CAR+ T cell infusion and recovered to a normal level by the month-12 visit (0.23 g/L). For subject S0302, the significantly low levels of C3 and C4 at baseline markedly increased and reached normal levels at month 1 and were sustained satisfactorily at month 12 (Figures 2K and 2L). For subject S0303 with renal insufficiency, shown by a decreased eGFR of 64.3 mL/min/1.73 m^2 at baseline, this index increased to 90.8 mL/min/1.73 m^2 at month 9 and 81.5 mL/min/1.73 m^2 at month 12.

(H) Serum anti-Rib-P antibody before and after TyU19 cell therapy.
(I) Serum anti-histone antibody before and after TyU19 cell therapy.
(J) Serum anti-nucleosome antibody before and after TyU19 cell therapy.
(K) Serum complement 3 (C3) before and after TyU19 cell therapy.
(L) Serum C4 before and after TyU19 cell therapy.
(M) Histopathological changes of renal biopsy before and after TyU19 cell therapy.
(N) Renal CD19 expression by immunochemical staining for subject S0302 before and 12 months after TyU19 cell therapy.

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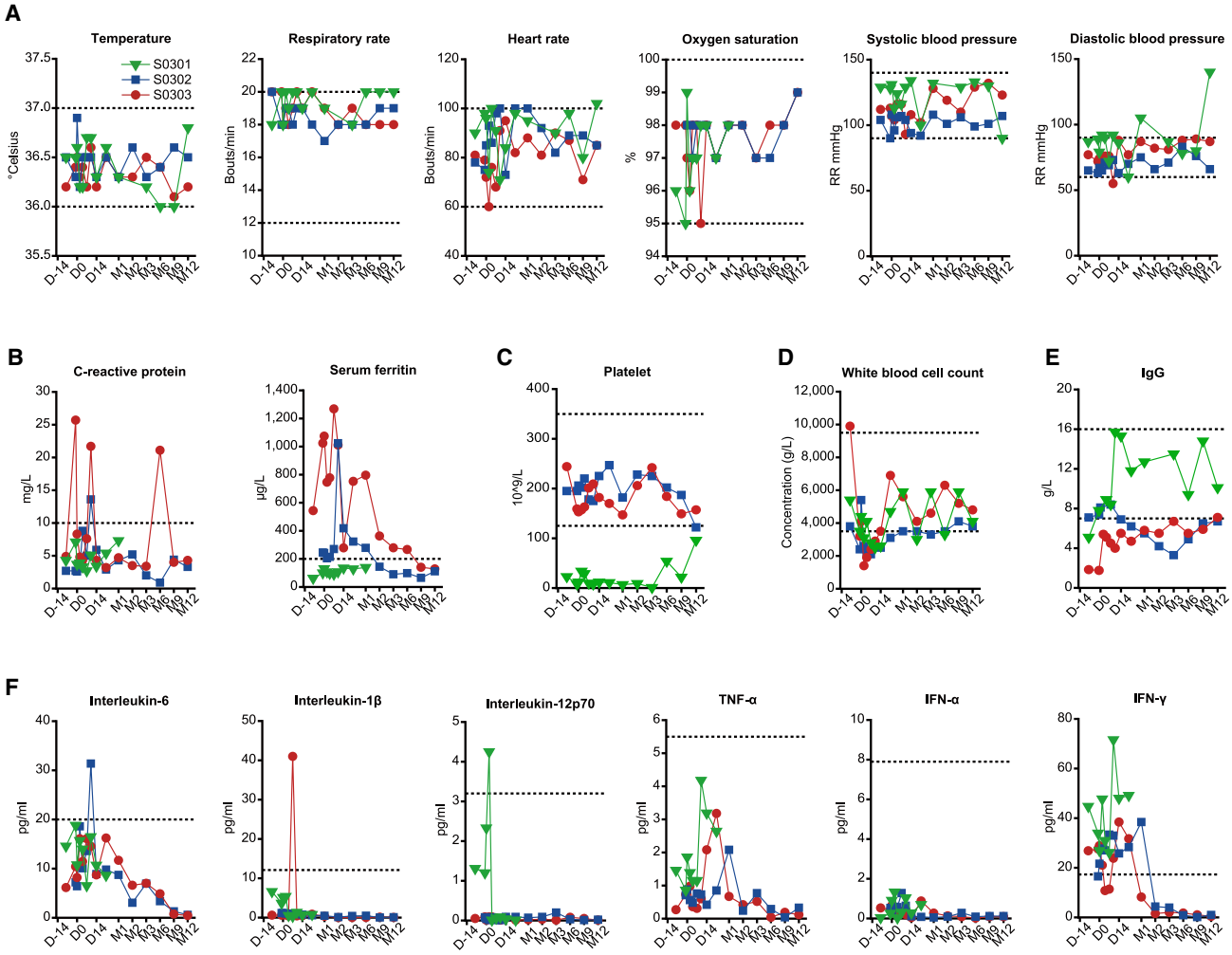

Figure 3. Safety profiling following allogeneic CD19-targeted CAR-T cell treatment

(A) Records of vital signs throughout the treatment and post-treatment follow-up periods.

(B) Changes in serum levels of C-reactive protein (CRP) and ferritin.

(C) Platelet count before and after TyU19 cell therapy.

(D) Changes of white blood cell count before and after TyU19 cell therapy.

(E) Changes in serum level of IgG.

(F) Changes in serum levels of CRS-related cytokines, IL-6, IL-1β, IL-12p70, TNF-α, IFN-α, and IFN-γ.

Repeated renal biopsy

Patient S0302 underwent a repeated renal biopsy at month 12, and the histopathological findings indicate significant improvement in LN. Notably, there is a reduction in cellular and mesangial proliferation, and the previously occluded capillary loops are now open (Figure 2M). Additionally, CD19 expression was stained in renal tissues before and 12 months after TyU19 cell infusion, and we found that renal CD19 expression markedly decreased after therapy, which indicated a deep B cell deletion in the targeted tissue (Figure 2N).

Safety profiling following allogeneic CD19-targeted CAR-T cell treatment

In all 3 patients, no GvHD, cytokine release syndrome (CRS), or immune effector cell-associated neurotoxicity syndrome (ICANS) was observed after cell infusion. No severe AEs were reported. According to the grading and management of immune effector cell-associated hematotoxicity (ICAHT) following CAR-T therapy,[18] neither early nor late ICAHT events were found for any of the 3 patients. Fever and myalgia (grade 1; did not reach the criterion for CRS) occurred in one of the three patients (S0302, day 10), which lasted for 3 days and disappeared without any symptomatic treatment. A whole-body rash with itching was found in patient S0301 on day 20, which was evaluated as an allergy to platelet infusion and was resolved by antihistamine therapy. Patient 1 suffered an upper respiratory tract infection at month 10, characterized by fever, chest pain, coughing white phlegm, and a C-reactive protein (CRP) that increased to 211.8 mg/L. She was successfully treated by the administration of imipenem.

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All patients exhibited stable vital signs, and no relevant hemodynamic changes were observed (Figure 3A). CRP and serum ferritin (SF) levels were slightly increased after about 2 weeks of CAR+ cell infusion and proliferated *in vivo*, then decreased to normal levels (Figure 3B). Platelet count remained stable for subjects S0302 and S0303 and increased for subject S0301 (Figure 3C). White blood count slightly decreased during the period of lymphodepletion and recovered after 14 days of TyU19 cell treatment (Figure 3D). Serum immunoglobulin G (IgG) decreased after TyU19 infusion and returned around the lower limit by month 12 (Figure 3E). Serum cytokines of interleukin (IL)-6, IL-1β, IL-12p70, tumor necrosis factor alpha (TNF-α), interferon (IFN)-α, and IFN-γ remained stable (Figure 3F).

DISCUSSION

Novel cellular therapies (mesenchymal stem cells, CAR-T cells, and Tregs) are currently evaluated as a therapeutic option for patients with severe and refractory autoimmune diseases.[19,20] This study represents the first use of an off-the-shelf, healthy-donor-derived anti-CD19 CAR-T cell product to treat patients with severe and relapsing SLE. All three patients achieved satisfactory clinical outcomes at different time points, including the resolution of arthralgia, myalgia, alopecia, mouth ulcers, and proteinuria, although subject S0301 voluntarily withdrew from the trial in month 1. They also exhibited significantly reduced autoimmune antibody levels, with one patient showing a normal platelet count after suffering from severe thrombocytopenia. The patients tolerated the infusion of allogeneic CAR-T cells well, with no serious AEs (SAEs) reported. This study highlights the efficacy and safety profile of allogeneic CAR-T therapy for refractory lupus, particularly in cases of LN and thrombocytopenia.

In a previous study conducted by another group using autologous CAR-T cell therapy, it was shown that patients experienced a more rapid clinical response (3 months) and maintained it for 29 months.[5,21] However, with allogeneic CAR-T cell therapy, it appeared that more time was needed for clinical remission, with remission observed at 6 months for subject S0302, 9 months for subject S0303, and 12 months for subject S0301. It is worth noticing that our patients in the trials were all refractory and severe cases who had failed to respond to all other standard treatments before. However, we observed a more sustained B cell deficiency in these patients, suggesting a longer-lasting efficacy in treating SLE with TyU19. For subjects S0302 and S0303, neither immunosuppressants nor increased GCs were added after the TyU19 cell infusion, and subject S0302 discontinued all lupus-related drugs. Therefore, the clinical efficacy was attributed to CAR-T cell therapy alone rather than the combined use of other drugs. In both autologous and allogeneic CAR-T cell therapies, lymphocyte clearance was achieved using fludarabine and CYC. Given the patients' previous relapses despite higher doses of CYC, the mild CYC-based preconditioning is unlikely to be the main factor responsible for the profound and lasting clinical remission. Importantly, all three patients achieved renal remission after the month-6 visit, by which time the effects of the transient fludarabine and CYC treatments had long since subsided.

Recently, Li et al. reported a patient with refractory lupus with severe thrombocytopenia who was successfully treated with autologous CD19 CAR-T cell therapy.[22] We also observed an impressive outcome of both negative proteinuria and nearly normal platelet count in patient S0301 at the 12-month visit. However, she experienced severe thrombocytopenia and uncontrolled vaginal bleeding and was unresponsive to platelet transfusion at the month-1 visit, putting her at high risk if she continued immunosuppressant-free therapy. At that time, peripheral B cell maturation antigen (BCMA)+ B cells were checked because we suspected that the allogeneic anti-CD19 CAR-T cells might not be eliminating BCMA+ plasma cells in this patient. This might also explain why her peripheral B cell count was not fully depleted after preparatory lymphodepletion. Indeed, we found a high level of peripheral BCMA+ B cells in this patient both before and 21 days after the TyU19 infusion (Figure S3). After discussion with the investigators, the patient withdrew from the trial, and TAC (2 mg per day) was added to her treatment regimen. The patient's platelet counts gradually increased at the month-5 and -6 visits, dropped at month 9 (when she developed pneumonia), and then rose again at month 11, reaching almost normal levels at month 12. Notably, significant improvement in proteinuria was also observed, with multiple tests confirming negative proteinuria at month 12, despite elevated levels of serum ANA and anti-dsDNA antibodies. Meanwhile, the percentage of BCMA+ CD19− plasma cells decreased from 36.47% (day 1) to 1.51% at 12 months, potentially indicating a favorable prognosis. We cannot rule out the effect of allogeneic CAR-T cell therapy for this patient, as she was unresponsive to both long-term, high-dose TAC and MSCT before participating in this trial. Nonetheless, more time is needed to assess the long-term impact of allogeneic CAR-T cell therapy in this patient. Moreover, we also found an obvious decline in the BCMA+ CD19− plasma cell percentage for patient 2 (Figure S3), who received a satisfactory clinical remission, so the percentage of BCMA+ CD19− cells may be irrelevant for clinical responses.

Regarding the toxicity of allogeneic CAR-T cell therapy observed in this study, the adverse effects of allogeneic anti-CD19 CAR-T cell treatment were minimal, with no case of CRS or ICANS and no prolonged bone marrow toxicity. Serum inflammatory cytokines rose slightly and briefly after the TyU19 infusion but returned to normal levels in subsequent visits. Most AEs, such as lymphopenia and leukopenia, were attributed to the lymphodepletion treatment. Additionally, the incidence of infections was low, and total Ig levels were only moderately decreased. Nevertheless, careful monitoring of these patients is warranted.

Overall, the study demonstrates that the allogeneic anti-CD19 CAR-T product (TyU19) can achieve clinical efficacy comparable to autologous anti-CD19 CAR-T products, albeit with a lower proliferation rate of allogeneic CAR-T cells *in vivo* and a longer period required for renal and hematologic recovery.

Limitations of the study

This study has several limitations. First, the sample size is relatively small. Second, the follow-up duration was not long enough, so the results and conclusions should be interpreted with caution. Long-term monitoring of the safety and efficacy profile of the TyU19 product in a larger cohort of patients will be necessary to further support its broader clinical application.

RESOURCE AVAILABILITY

Lead contact

Further information should be directed to the lead contact, Huji Xu (xuhuji@smmu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper, including individual deidentified participant data, will be shared by the lead contact upon request. The data will become available 2 months after the paper is published and last for 3 years. The study protocol and statistical analysis plan are provided in the supplemental information (Method S1).
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

H.X. and L.S. designed the clinical trial. B.T., B.D., B.Z., and M.L. contributed to product design. D.W., X.Z., C.W., and X.F. conducted the clinical study. X.C., L.G., Y.W., H.Z., X. Wen, X. Wu, and X.F. performed data curation. D.W. and X. Wang prepared the original manuscript draft. H.X., L.S., and B.D. reviewed and edited the manuscript. S.Y. and H.Z. conducted data visualization. H.X. and L.S. provided overall supervision. D.W. and H.X. had unrestricted access to all data. All authors have read and approved the final manuscript and take full responsibility for its content.

DECLARATION OF INTERESTS

This study was partially supported by BRL Medicine, Inc.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- METHOD DETAILS
  - Study population
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
FITC Mouse Anti-Human CD19	ACRObiosystems	Cat#CD9-HF2H2 and CD9-HF251; RRID: AB_3076267 and AB_3076268
APC Anti-Human CD3	Biolegend	Cat#300312 RRID: AB_314048
PE Mouse Monoclonal Anti-Human HLA-A	BD biosciences	Cat#567739; RRID: AB_2916718
PE-Cy7 Mouse Monoclonal Anti-Human HLA-DR	BD biosciences	Cat#560651; RRID: AB_1727528
BV421 Mouse Monoclonal Anti-Human CD279 (PD-1)	BD biosciences	Cat#562516; RRID: AB_11153482
Chemicals, peptides, and recombinant proteins		
CAR containing plasmid	BioVector NTCC	Cat#pELPs 19-BB-z
pELPs	Addgene	Cat#193253
pRSV-Rev	Addgene	Cat#12253
pMDLg/pRRE (Gag/Pol)	Addgene	Cat#12251
pMD2.G (VSVG envelope) packaging plasmid DNA	Addgene	Cat#12259
polyethyleneimine (PEI)	MCE	Cat#HY-K2014
X-VIVO 15 medium	LONZA	Cat#02-053Q
Sp.Cas9 protein	Thermofisher	Cat#A36499
sgRNA	GenScript	Customized
p3s-Cas9HC	Add gene	Cat#43945
Proteinase K	TransGen Biotech	Cat#GE201-01
RNase A	TransGen Biotech	Cat#GE101-01
Dynabeads MyOne Streptavidin T1 beads	Invitrogen	Cat#65601
Critical commercial assays		
TruSeq Nano DNA LT Sample Prepararion kit	Illumina	Cat#FC-121-4001
P3 Primary Cell 4D-Nucleofector X Kit S	LONZA	Cat#V4XP-3032
Cytokine Co-Detection Kit (Immunofluorescence)	CELLGENE BIO	Cat#P110100403
KAPA HTP Library Preparation Kit	KAPA BIOSYSTEMS	Cat#KR0426
Experimental models: Cell lines		
Human embryonic kidney 293T cells	ATCC	CRL-3216
Software and algorithms		
FACSCalibur system	BD Biosciences	https://www.bdbiosciences.com/zh-cn
Cas-OFFinder	CRISPR RGEN Tools	http://www.rgenome.net
Illumina HiSeq X Ten platform	Qingdao OE Biotech	https://www.illumina.com.cn/systems/sequencing-platforms.html
fastp	Chen et al.	https://github.com/OpenGene/fastp
BWA V0.7.12	Li and Durbin	https://bio-bwa.sourceforge.net/
SAMtools V1.4	Li et al.	http://www.htslib.org/download/
GATK V4.1.0.0	Broad Institute	https://gatk.broadinstitute.org/hc/en-us
Picard V4.1.0.0	Broad Institute	https://broadinstitute.github.io/picard/
CNVkit V 0.9.5	Talevich et al.	http://cnvkit.readthedocs.io/
Lumpy V0.2.13	Layer et al.	https://github.com/arq5x/lumpy-sv

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
FlowJo software (version 10.8.1)	Flowjo Software	www.flowjo.com
GraphPad Prism software (version 9.3.0)	GraphPad Software	www.graphpad.com
Other		
TyU19	BRL Medicine Inc. Shanghai	Drug Standard Code: BRL-301
Electroporation systems	MaxCyte	https://maxcyte.com/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

This was a single-arm study designed to evaluate the safety and efficacy of allogeneic CAR T cells for severe SLE patients. The trial protocols were reviewed and approved by the Institutional Review Board of The Affiliated Drum Tower Hospital of Nanjing University Medical School (Approval number: 2023-179), and this study was conducted according to the principles of the Declaration of Helsinki, and to Good Clinical Practice. All participants provided informed consent prior to evaluation for eligibility. All participants self-identified as belonging to Asian. Participants also provided self-selected information on sex, age and race. Information on ethnicity and socioeconomic status was not collected. The 3 participants included in the study had a median (range) age of 36 (18–54) years, and 100% were female.

METHOD DETAILS

Study population

This study involved three patients with recurrent refractory systemic lupus erythematosus. All patients were from southeastern China. These patients were diagnosed as SLE according to the 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria for SLE,[23] with SELENA-SLEDAI score>6[24] and organ involvements based on British Islet Lupus Assessment Group Index (BILAG-2004).[25] All patients have tried multiple therapeutic options but have not achieved remission. This study was approved by the Ethics Committee at The Affiliated Drum Tower Hospital of Nanjing University Medical School and all patients provided written informed consent in accordance with the 1995 Helsinki declaration before enrollment. This study was registered at ClinicalTrials.gov (NCT05859997).

The production, testing, and quality control of allogeneic CAR T cells

(1) Construction of CAR cassette.

Second-generation CD19 CAR were constructed using single chain antibody fragments derived from antibody clones FMC63, hinge and transmembrane regions from CD8A, intracellular domain from 4-1BB (CD137), and intracellular domain from CD3-zeta subunit (pELPs 19-BB-z). CD19 CAR and extracellular domain of PD-L1 (PD-L1 ECD) were cloned into a commercial lentiviral vector backbone pELPS downstream of an EF1a promoter and were flanked by lentiviral 5′ and 3′ long-terminal repeats (LTRs).

(2) Lentiviral package and transduction.

Third-generation, self-inactivating lentiviral supernatant was produced in the 293T cells. In brief, 70% confluent 293T 10cm plates were co-transfected with 10 μg pELPS vector plasmid, and 4 μg pRSV-Rev, 4 μg pMDLg/pRRE (Gag/Pol) and 6 μg pMD2.G (VSVG envelope) packaging plasmid DNA using polyethyleneimine (PEI). Medium was replaced at 24 h after transfection. The 72 h viral supernatants were collected, combined and concentrated by ultracentrifugation at 25,000 rpm for 2.5 h. Concentrated lentiviral stocks were frozen with X-VIVO 15 medium (LONZA) at −80°C for future use. For lentiviral infection, cells were co-cultured with CD19-CAR cassette containing lentivirus vector for 72 h (37°C, 5% CO_2) with the multiplicity of infection (MOI) of 8. After the infection, cells were washed twice with wash buffer, and then prepared for nucleofection with MaxCyte electroporation systems.

(3) CRISPR knockout.

CRISPR-Cas9 gene knockout was performed by transient Cas9/gRNA (RNP) complex electroporation using the MaxCyte electroporation systems (MaxCyte). On day 5 of culture, CAR T cells were counted, pelleted and resuspended in electroporation buffer at 1×10^8 cells per 1 mL reaction. 70ug Sp. Cas9 protein (Thermofisher) and 70ug chemically modified synthetic sgRNA (GenScript, China) (1:1 mass ratio gRNA:Cas9) per reaction was pre-complexed for 20 min at room temperature to create ribonucleoprotein complexes (RNP). A 1 mL cell suspension was mixed with RNP and electroporated using the "Expanded T cell 2" program in the electroporation cassette (CL-1.1). Cells were recovered at 37°C for 20 min in 1 mL T cell medium (X-VIVO15, LONZA) then expanded as usual. Knockout efficiency was determined using fluorescence-activated cell sorting (FACS) on day 5 after the electroporation.

(4) Off-target assessment.

We used Cas-OFFinder (http://www.rgenome.net) to find potential off-target sites that differed from on-target sequences by up to 8 nt mismatches. For whole-genome-sequencing, genomic DNA was extracted using standard protocols. TruSeq Nano DNA LT Sample Prepararion kit (Illumina, San Diego, CA, USA) was used for DNA libraries. The libraries were subjected to massively parallel sequencing in the Illumina HiSeq X Ten platform (Qingdao OE Biotech, China) to generate 150bp paried-end reads. Data analysis was also conducted by OE Biotech Co., Ltd (Qingdao, China). For WGS bioinformatics, raw reads were filtered using fastp (Version 0.19.5). Clean reads were aligned to the reference human genome (GRCh37) using BWA (version 0.7.12), after which the mapped reads were sorted and indexed with SAMtools (Version 1.4). To generate analysis-ready BAM files, recalibration of base quality scores, single nucleotide polymorphism (SNP) and insertion/deletion (INDEL) realignment were performed using GATK (Version 4.1.0.0), while duplicate reads were marked using Picard (Version 4.1.0.0). Variant calling was conducted using these final BAM files, with reference to several annotation databases such as Refseq, 1000 Genomes, the Catalog of Somatic Mutations in Cancer (COSMIC), OMIM, etc. ANNOVAR was used for annotation. Copy number variation (CNV) was inferred from sequencing data using CNVkit (Version 0.9.5), while structural variation (SV) was called using Lumpy software (Version 0.2.13). The resulting genomic variation information was visualized using a Circos diagram. In brief, for GUIDE-seq library construction, repaired, A-tailed and ligated to the annealed adaptor set 1. The adaptor-ligated DNA was then fragmented by NEBNext dsDNA Fragmentase (NEB) and then quenched with 0.5 M EDTA. The fragmented DNA was then ligated to the second adaptor by KAPA HTP library preparation kit (KAPA BIOSYSTEMS). Then, the dual adaptor-ligated DNA was enriched with Dynabeads MyOne Streptavidin T1 beads (Invitrogen). Finally, two rounds of nested PCR amplification to recovery DNA from streptavidin beads and to add index for sequencing. Completed libraries were quantified and sequenced on MGI 2000 platform with 150-bp paired-end reads.

(5) Flow cytometry for cell analysis.

CAR was labeled with FITC-conjugated human CD19 (20–291) protein (ACRO biosystems, CD9-HF2H2 and CD9-HF251). For T cell phenotyping the following antibodies were used: APC anti-human CD3 (300312) was from Biolegend; PE Mouse Anti-Human HLA-A (567739), BB700 Rat anti-human HLA-B (752626), PE Cy7 mouse anti-human HLA-DR (560651) and BV421 mouse anti-human CD279 (PD-1) (562516) were from BD biosciences.

Clinical trial information and design

This was an investigator-initiated study designed to evaluate the safety and efficacy of allogeneic anti-CD19 CAR-T cells in treating refractory autoimmune disease. The clinical protocol has been registered at ClinicalTrials.gov (NCT05859997).

(1) Common inclusion criteria:
   1) Age ranges from 18 to 65 years old (including threshold), regardless of gender.
   2) Positive expression of CD19 on peripheral blood B cells determined by flow cytometry.
   3) The functions of important organs meet the following requirements:
      A. Bone marrow function needs to meet a. White blood cell count $\geq$3 x 109/L; b. Neutrophil count $\geq$1 x 10^9/L (no colony-stimulating factor treatment within 2 weeks before examination); c. Hemoglobin$\geq$60 g/L.
      B. Liver function: ALT $\leq$3 x ULN, AST$\leq$ 3 x ULN, TBIL $\leq$1.5 x ULN (excluding Gilbert syndrome, total bilirubin $\leq$3.0 x ULN) (No requirements for conditions caused by the disease itself).
      C. Renal function: creatinine clearance rate (CrCl) $\geq$ 60 mL/min (Cockcroft/Fault formula).
      D. Coagulation function: International standardized ratio (INR) < 1.5 x ULN, prothrombin time (PT) < 1.5 x ULN.
      E. Cardiac function: Good hemodynamic stability.
   4) Female subjects with fertility and male subjects whose partners are women of childbearing age are required to use medically approved contraception or abstinence during the study treatment period and at least 6 months after the end of the study treatment period; Female subjects of childbearing age tested negative for serum HCG within 7 days before enrollment in the study and were not in lactation.
   5) Voluntarily participate in this clinical study, sign an informed consent form, have good compliance, and cooperate with follow-up.

(2) The specific inclusion criteria for systemic lupus erythematosus (SLE).
   1) Complies with the 2019 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria for SLE.
   2) In the stage of moderate to severe disease activity, SLEDAI-2000 score >6.
   3) At least one A (severe) score or two B (moderate) scores of British Islet Lupus Assessment Group Index (BILAG-2004), or both.
   4) Definition of refractory recurrence: If conventional treatment is ineffective or if the disease becomes active again after remission. The definition of conventional treatment: use of glucocorticoids (1 mg/kg/d or more) and cyclophosphamide, as well as any one or more of the following immunomodulatory drugs for more than 6 months: antimalarials, azathioprine, mycophenolate mofetil, methotrexate, leflunomide, tacrolimus, cyclosporine, and biological agents including rituximab, belimumab, and telitacicept.

(3) Common Exclusion criteria.
1) Individuals with a history of severe drug allergies or allergic constitution.
2) Existence or suspicion of uncontrollable or treatable fungal, bacterial, viral or other infections.
3) Individuals with relatively serious heart diseases, such as angina pectoris, myocardial infarction, heart failure, and arrhythmia.
4) Individuals with congenital immunoglobulin deficiency.
5) Individuals with other malignant tumors (excluding those with non-melanoma skin cancer, cervical cancer *in situ*, bladder cancer, and breast cancer who survived disease-free more than 5 years).
6) Individuals with end-stage renal failure.
7) Individuals with positive hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HBcAb) and HBV DNA titer in peripheral blood higher than the upper limit of detection; Individuals with positive hepatitis C virus (HCV) antibodies and positive peripheral blood HCV RNA; Individuals with positive human immunodeficiency virus (HIV) antibodies; Individuals who have tested positive for syphilis.
8) Individuals with mental illness and severe cognitive impairment.
9) Individuals who have participated in other clinical trials within the first 3 months of enrollment.
10) Women who are pregnant or planning to conceive.
11) The researchers believe that there are other reasons why subjects cannot be included in this study.

(4) Specific Exclusion criteria for SLE.
1) Individuals with neuropsychiatric lupus.
2) Individuals with thrombotic thrombocytopenic purpura (TTP) or thrombotic microangiopathy (TMA).

Therapy, and follow-up

(1) Preconditioning strategy: Prior to the administration of the TyU19 infusion, all immunosuppressive medications were discontinued, except for glucocorticoids, which were tapered to a dose deemed appropriate by the treating physicians.
(2) Conditioning regimen: Patients received 25 mg/m2/day Fludarabine (FLU) from day −5 to day −3, and 300 mg/m2/day Cyclophosphamide (CTX) on days −5 and −4.
(3) Patients received an intravenous infusion of CAR T cells at a dose of 1×10^6 cells/kg on D0.
(4) Post-therapy cell proliferation was examined on D0, D2, D4, D7±1day, D10 ± 2days, D14 ± 2days, D21 ± 2days, D30 ± 3days, M2±3days, M3±3days, M6±7days, M9±7days, M12 ± 7d.
(5) Clinical outcomes were methodically evaluated at specific intervals: baseline, Day 14 ± 2 days, Day 21 ± 2 days, Day 30 ± 3 days, Month 2 ± 3 days, Month 3 ± 3 days, Month 6 ± 7 days, Month 9 ± 7 days, and Month 12 ± 7 days, accompanied by thorough monitoring for potential adverse events.

Efficacy evaluation

(1) SLEDAI-2K score.
(2) SELENA-SLEDAI score.
(3) PGA score.
(4) BILAG score.
(5) Renal pathological changes. The pathological kidney specimens were routinely fixed in 10% neutral buffered formalin solution and embedded in paraffin, H&E staining was performed for morphological observation. Immunohistochemistry (IHC) staining for renal CD19 expression, from patient S0302 before and 12 months after TyU19 cell infusion, was performed. Representative 4 mm serial sections of the renal biopsy were prepared from 10% FFPE tissue blocks for IHC. Briefly, all slides were exposed to 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. CD19(Dako Denmark A/S, Denmark) incubated with kidney sections in a humidified chamber at 4°C overnight, followed by the secondary anti-rabbit peroxidase-conjugated secondary antibody (EnVisionTM Detection Kit, Dako, Glostrup, Denmark) at 37°C for 30 min. Both negative (without the primary antibody) and positive controls were carried out in each run. Cytoplasmic staining intensity was considered positive.

Safety monitoring

(1) The patient's temperature and vital signs were recorded at each time point under the same circumstances.
(2) Routine examinations of blood cell count, lymphocyte subsets; and biochemical indices of liver, kidney, and heart function were conducted during regular follow-ups. These tests were conducted by the professional technical staff at the Medical Laboratory Center of Nanjing Drum Tower Hospital, following the instructions provided in the test kit manuals.
(3) C-reactive protein (CRP) was quantified by immunoturbidimetry. The test was conducted by the professional technical staff at the Medical Laboratory Center of Nanjing Drum Tower Hospital, following the instructions provided in the test kit manuals.
(4) The concentrations of various cytokines in the supernatants of peripheral blood mononuclear cells were precisely quantified. This measurement was performed using immunofluorescence analysis on a BD FACS Calibur system. The analysis was conducted at the Medical Laboratory Center of Nanjing Drum Tower Hospital, following the instructions provided in the test kit manuals.

(5) The patient was closely monitored throughout the entire process for potential adverse events previously reported in other UCART studies, including CRS, ICANS, and GVHD.
   1) The safety evaluation was conducted using the NCI-CTCAE 5.0 standards.
   2) For CRS, the ASTCT Consensus Grading Standards were employed.
   3) The assessment of ICANS was based on the Adult ASTCT ICANS Consensus Grading Standards.
   4) Acute GVHD was graded according to the 2016 Mount Sinai Acute GVHD International Consortium criteria, while chronic GVHD was evaluated using the standards set forth in the 2020 NCCN Guidelines for Hematopoietic Stem Cell Transplantation, 1st Edition.

QUANTIFICATION AND STATISTICAL ANALYSIS

Descriptive statistics are used for reporting specific parameters of all 3 patients at baseline and at each follow-up time-point. The flow cytometry data were analyzed by Flowjo software (version 10.8.1). The column or bar graph was prepared by GraphPad Prism software (version 9.3.0).