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JOURNAL CLUB

Journal Club: Anti-CD19 Chimeric Antigen Receptor T Cell Therapy for Refractory Systemic Lupus Erythematosus

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Objective. Despite substantial advances in the treatment of systemic lupus erythematosus (SLE), some patients do not respond to the current state-of-the art therapies. This study assessed the tolerability and efficacy of CD19 chimeric antigen receptor (CAR) T cells in a small series of seriously ill and treatment-resistant patients with SLE.

Methods. Five patients with SLE (four female patients and one male patient) with a median age of 22 (range 18–24) years, a median disease duration of 4 (range 1–9) years, and active disease (median Systemic Lupus Erythematosus Disease Activity Index score of 16 [range 8–16]) refractory to several immunosuppressive drug treatments were enrolled in a compassionate-use 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 kilogram of body weight into the patients after lymphodepletion with fludarabine and cyclophosphamide.

Results. CAR T cells expanded in vivo and 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 definition of remission in SLE criteria was achieved in all five patients after 3 months, and the median Systemic Lupus Erythematosus Disease Activity Index score after 3 months was 0 (range 2). Drug-free remission was maintained during longer follow-up (median of 8 [range 12] months after CAR-T cell administration) and even after the reappearance of B cells, which was observed after a mean (±SD) of 110 ± 32 days after CAR-T cell treatment. Reappearing B cells were naive and showed non–class-switched B cell receptors. CAR-T cell treatment was well tolerated, with only mild cytokine release syndrome.

Conclusion. These data suggest that CD19 CAR-T cell therapy was feasible, tolerable, and effective in this small case series of refractory SLE. Nevertheless, larger placebo-controlled trials are warranted.

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by loss of immune tolerance that leads to aberrant inflammation and ultimately damage to multiple organs (1). Both innate and adaptive immune systems have been shown to play a critical role in the pathogenesis of SLE, with B cell–derived pathogenic autoantibodies being central to disease pathogenesis (2).

An enormous amount of scientific effort over the past decades has been dedicated to developing new therapies for SLE. However, severe lupus is still treated primarily with glucocorticoids and cytotoxic and immunosuppressive drugs, and patients with refractory disease face high morbidity and mortality rates (3). Although previous trials of type I anti-CD20 antibodies failed to show benefit in SLE, more recently, monoclonal antibodies that interfere with B cell activation targeting BAFF/B lymphocyte stimulator or B cell depleting type II anti-CD20 monoclonal antibodies have been successfully used in the treatment of SLE (4). However, CD20-targeting monoclonal antibodies may not thoroughly deplete B cells in the tissues, and they do not target plasmablasts and long-lived plasma cells (5,6). This might be the reason why certain severe forms of SLE are resistant to these therapies.

Injection of CD19-targeting chimeric antigen receptor (CAR) T cells in lupus-prone mice led to B cell depletion, a decrease in autoantibody production, and amelioration of glomerulonephritis

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and other manifestations. Moreover, CAR-T cell treatment was found to be effective in one patient with refractory SLE (7–9). These results led the authors to evaluate the tolerability and efficacy of CD19 CAR T cells in a small case series of treatment-resistant patients with SLE.

Methods

Patients. This is a small case series of five patients with treatment-refractory multiorgan SLE who were recruited at the Department of Internal Medicine 3 (Rheumatology and Immunology) of the Friedrich Alexander University Erlangen-Nurnberg. Inclusion criteria were 1) a diagnosis of SLE according to the European Alliance of Associations for Rheumatology (formally European League Against Rheumatism)/American College of Rheumatology 2019 criteria (10); 2) signs of active organ involvement; 3) failure to respond to multiple immunomodulatory therapies, including repeated pulsed glucocorticoids, hydroxychloroquine, belimumab, and mycophenolate mofetil; and 4) an understanding of the procedure of CAR-T cell therapy. Patients with neuropsychiatric involvement were excluded. More information regarding the consent process and the legal perspective of the compassionate use of this treatment is provided in the original article (11).

Tapering of immunosuppressive treatment before leukapheresis. The generation of sufficient amounts of CAR T cells was expected to be challenging given that patients with SLE are usually lymphopenic and treated with T cell–toxic drugs. Therefore, MMF and cyclophosphamide were discontinued 3 weeks before leukapheresis, and the prednisolone dose was reduced to less than 10 mg/day. Only one patient experienced worsening nephritis during this lead-in period, necessitating glucocorticoid pulse and cyclophosphamide treatment. The subsequent clinical course was not different from that of the other patients.

Screening procedures. Patients underwent screening for comorbidities with chest radiography, electrocardiography, echocardiography, and brain magnetic resonance imaging prior to initiation of CAR-T cell treatment. Female participants received a gonadotropin-releasing hormone analog for ovarian protection before lymphodepleting chemotherapy.

Lymphodepleting chemotherapy. Patients received fludarabine (25 mg/m^2/day intravenously [IV]) on days –5, –4, and –3 and cyclophosphamide (1000 mg/m^2/day IV) on day –3 before CAR-T cell transfer.

CAR-T cell treatment. CAR T cells were infused on day 0, followed by a period of inpatient monitoring for cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS).

For the detailed description of 1) CAR product and vector, 2) immunologic monitoring, 3) quantification of autoantibodies against nuclear antigens, 4) secondary necrotic cell preparation and anti–secondary necrotic cell enzyme-linked immunosorbent assay, 5) vaccination responses, 6) measurement of interferon-α levels, and 7) characterization of B cells and B cell receptor sequencing, please refer to the original article (11).

Statistical analyses. Descriptive statistics were reported at baseline and at 3 months' follow-up. Individual values are presented throughout because the sample size of this case series is small. Analyses were conducted using R v.4.1.1.

Results

Patient characteristics. Patients (four female patients and one male patient) were aged between 18 and 24 years. All patients had Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) scores between 8 and 16 and multiorgan involvement. All patients had previously received several immunosuppressive drugs, including pulsed glucocorticoids (five of five), hydroxychloroquine (five of five), MMF (five of five), belimumab (five of five), cyclophosphamide (three of five), azathioprine (two of five), and rituximab (one of five). Several patients had also been exposed to cyclophosphamide, azathioprine, rituximab, tacrolimus, methotrexate, and leflunomide. B cell counts at baseline were 19 (patient 1, previous rituximab exposure, 3.2% of lymphocytes), 85 (patient 2, 8.0% of lymphocytes), 84 (patient 3, 6% of lymphocytes), 280 (patient 4, 27.8% of lymphocytes), and 234 (patient 5, 7.3% of lymphocytes) cells/μl.

Transduction efficacy and proliferation efficiency of CAR T cells in vitro. Leukapheresis was performed at day –13, and CAR T cells were generated from day –12 to day 0 (0 = day of infusion). 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 contained the sequence for a single-chain variable fragment derived from an anti-human CD19 hybridoma clone (FMC63). Cells expanded more than 50-fold from day –11 to day 0. The final product (MB-CART19.1) showed a transduction efficacy between 20% and 40%, a high purity for T cells (>99%) with a preponderance of CD4+ T cells, and negligible lipopolysaccharide concentrations (<0.05 EU/ml). Although CD45RA+CD27+ naive T cells and CD45RA–CD27+ central memory cells prevailed following initial cell collection, strong enrichment of CD27–CD45RA– effector memory T cells was observed within the final cell product.

Dynamics of CAR-T cell expansion and B cell elimination in vivo. After lymphodepletion, CAR T cells were infused at a fixed dose of 1×10^6 CAR T cells per kilogram of body weight, which constituted only a small proportion of circulating T cells at day 1 after their administration. Rapid expansion of the cells was observed in all five patients, peaking on average at day 9 with 11% to 59% of circulating T cells being CARs, which thereafter, rapidly declined. The phenotype of CAR T cells shifted to central memory T cells in vivo, which could indicate their circulation to lymphoid organs and other tissue sites. B cells fully disappeared from the patients' peripheral blood from the second day after administration. In contrast, CD4+ and CD8+ T cells, monocytes, and neutrophils showed only temporary decreases. With the exception of B cells, white blood cell counts rapidly recovered.

Clinical efficacy. Signs and symptoms of SLE improved in all five patients to an SLEDAI-2K score of 0 in four of five patients and a score of 2 in patient 2 (low-level proteinuria, no active sediment, which improved with angiotensin-converting enzyme [ACE] inhibitors) at 3 months after CAR-T cell administration. Nephritis remitted in all five patients upon CAR-T cell treatment, complement levels normalized, and anti–double-stranded DNA (anti-dsDNA) antibody levels dropped below the cutoff. Other severe manifestations of SLE, such as arthritis (patient 4), fatigue (all patients), 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-α was detectable in the serum samples of three patients at baseline but was undetectable at follow-up. Definition of remission in SLE criteria and the Lupus Low Disease Activity State definition were fulfilled by all five patients 3 months after treatment. Drug-free remission was achieved in all patients with successful discontinuation of all immunomodulatory and immunosuppressive drugs.

Immune effects and preliminary long-term effects. Analyses of the autoantibody spectrum at 3 months' follow-up confirmed not only the disappearance of anti-dsDNA antibodies but also the decrease or disappearance of autoantibodies against nucleosomes, secondary necrotic cells, single-stranded DNA, Sm antigen and Ro 60, histones, and Ro 52 and SSB/La. The average time to B cell reconstitution was 110 ± 32 days (median 110, range 63–142 days). Despite B cell reconstitution, no relapse of SLE was observed in the long-term follow-up while patients remained off any SLE-associated medication. Patient 3 developed a short phase of proteinuria without signs of nephritic sediment in the urine analysis 4 months after CAR-T cell therapy. A kidney biopsy showed only minimal change in disease, and complement levels did not decrease. Proteinuria ceased after ACE inhibitor treatment and pulse glucocorticoids.

Immune phenotyping of B cells before and after CAR-T cell therapy showed that reconstituted B cells were mostly CD21+CD27– naive cells, with CD21+CD27+, memory B cells, and CD38+CD20– plasmablasts being low to absent. CD11c +$CD21^{low}$ activated memory B cells, which are typically expanded in SLE, were absent among recurring B cells. Comparative data from B cell receptor sequencing of B cells before versus after CAR-T cell therapy showed a shift from class-switched immunoglobulin G (IgG) and IgA heavy chains to non–class-switched IgM and IgD heavy chains. Interestingly, the two patients with the longest follow-up became antinuclear antibody negative (seroconverted).

Effects of vaccination antibody levels. 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 did not show a substantial decline.

Safety and tolerability. Although CRS and ICANS are frequently observed toxicities after CD19 CAR-T cell therapy in lymphoma and leukemia, the five patients had no or only mild CRS, and no patients experienced ICANS. C-reactive protein and interleukin-6 levels were increased in four of five patients between day 1 and day 5. Three patients experienced fever (CRS grade 1), which was treated with metamizole, and one patient received an 8-mg/kg single infusion of tocilizumab with immediate resolution of symptoms. No infection occurred during conditioning and immediately after CAR-T cell administration or during the phase of B cell aplasia.

Discussion

In this small case series study, the authors hypothesized that a deep depletion of CD19+ B cells could trigger an immune reset in SLE that could allow the cessation of immunosuppressive treatment and potentially cure lupus. They espoused the idea of CD19+ B cell depletion through CAR-T cell therapy, which has been successfully used in lymphoproliferative malignancies, and it had previously shown promising data in preclinical studies in SLE as well as in one clinical case of refractory SLE. Five patients with SLE with multiorgan involvement and refractory disease to several immunosuppressive drug treatments were enrolled in a compassionate-use CAR-T cell program. Autologous T cells from patients with SLE were transduced with a lentiviral anti-CD19 CAR vector in vitro, expanded, and reinfused into the patients after lymphodepletion with fludarabine and cyclophosphamide. CAR T cells expanded in vivo, led to deep depletion of B cells, and improved clinical symptoms and serological parameters. Importantly, drug-free remission was maintained during a follow-up period of a median of 8 months, despite the reappearance of B cells. CAR-T cell treatment was well tolerated, with only mild CRS.

Mackensen et al (11) provided evidence that CD19 CAR-T cell transfer can be feasible, tolerable, and effective in patients with

multiorgan SLE who had previously failed other immunosuppressive agents. Interestingly, not all patients in the study had the same level of serologically active disease, as evidenced by the complement level and the titer of dsDNA before CAR-T cell therapy. In terms of the dynamics of CAR-T cell expansion and viability, the percentages of the circulating T cells post expansion were not as high as expected based on the studies previously performed in lymphoproliferative diseases, and neither was their life span, which, in cases of malignancies, has been shown to be many years post treatment (12,13).

Important to note is that all patients received cytotoxic therapy for lymphdepletion prior to the CAR-T cell treatment with fludarabine and cyclophosphamide. Cyclophosphamide is commonly used in the treatment of severe lupus (3). Also, a small pilot study has previously shown that a 6-month course of fludarabine led to improvement in proteinuria and filtration in membranous nephritis, whereas a phase I/II study in lupus nephritis showed that fludarabine in combination with IV cyclophosphamide for at least 3 monthly cycles can lead to significant improvement in renal outcomes, which in some cases lasted for more than 1 year (14,15). However, this combination caused severe bone marrow toxicity (15). Also, lymphodepletion with rituximab, cyclophosphamide, and fludarabine followed by CD34-selected autologous hematopoietic cell transplant in refractory SLE, can lead to greater than 50% complete remission, which can last for more than a decade (16). This leaves open the question of whether it was the conditioning treatment that contributed, at least to some extent, to the improvement of the patients. A placebo-controlled study with the conditioning regimen without CAR T cells should be undertaken.

Given the pivotal role of B cells in the pathogenesis of SLE, the idea of tackling the disease by B cell blockade remains an attractive therapeutic approach (4). However, the challenges previously faced with the use of anti-CD20 antibodies have raised the question of what would be the best target to efficiently deplete autoreactive B cells not only in the periphery but also in the tissues while maintaining the protective/regulatory B cells (17). In this study, the authors showed that the dynamic expansion and transformation of CAR T cells post infusion led to a shift to central memory T cells in vivo. Although this is an interesting finding and could indicate their circulation to lymphoid organs and other tissue sites, it does not prove that this led to depletion of tissue B cells. Moreover, the role of the long-lived plasma cells in the bone marrow and the tissues cannot be underestimated, especially in the long term.

The advantage of the depletion of both CD19+ B cells and plasmablasts through CD19 CAR-T cell therapy compared to previously used anti-CD20 therapies was emphasized by the authors. Interestingly, though, only one of the five patients included in the study was previously treated with and failed IV rituximab. Considering that IV rituximab is a widely available and cost-effective treatment, it would be interesting to see whether patients who fail IV rituximab would be good candidates for CAR-T cell treatment.

A definitive answer as to whether CAR-T cell treatment corrects the underlying immune dysfunction in SLE cannot be given at this stage given the small sample size and relatively short-term follow-up. Nevertheless, it is encouraging that patients achieved a disease-free state despite B cell reconstitution.

Conclusions

This work provides new therapeutic possibilities to control SLE disease activity through engineered autologous cells. To better understand the role of these therapies, we will need, besides larger placebo-controlled trials in carefully chosen individuals, ways to improve CAR-T cell expansion and viability.

AUTHOR CONTRIBUTIONS

All authors drafted the article, revised it critically for important intellectual content, and approved the final version to be published.

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