

Forum

Autologous, allogeneic, *in vivo* CAR for autoimmune diseases

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Chimeric antigen receptor (CAR)-based therapies are emerging for autoimmune diseases (ADs). Early-phase clinical studies in systemic lupus erythematosus (SLE) show encouraging results using autologous, allogeneic, and *in vivo* CAR T-cell (CAR-T) strategies. This forum compares these three strategies, highlighting clinical design, safety, and efficacy, and explores the translational challenges of *in vivo* CAR engineering in ADs.

CAR-T cell therapy enters the clinic for ADs

Building on the successful clinical application of CAR-T cell therapy in CD19⁺ B-cell malignancies and BCMA⁺ multiple myeloma, CAR-T cells have now advanced into clinical evaluation for ADs, including multiple sclerosis (MS), SLE, systemic sclerosis (SSc), and lupus nephritis (LN) [1–4] (Box 1). Three CAR-T cell approaches are currently under investigation: autologous CAR-T cells, in which patient-derived T cells are engineered *ex vivo* and reinfused; allogeneic CAR-T cells, generated from healthy donors to provide an off-the-shelf therapeutic option; and *in vivo* CAR engineering, where genetic material is delivered directly into patients to reprogram T cells within the body [5]. Each strategy has shown encouraging results in preclinical models and early-phase clinical trials. Here, we present a side-by-side comparison of three recent CD19-targeting CAR-T (CAR19-T) cell therapy strategies

evaluated in patients with refractory SLE. This comparative analysis of manufacturing processes, clinical trial design, safety, and efficacy offers key insights for guiding the next generation of CAR-T therapies in ADs (Table 1).

Comparing autologous, allogeneic, and *in vivo* CAR therapies for treating patients with SLE

Recent clinical studies have begun to explore the application of different CAR-T modalities for refractory SLE. These efforts reflect a rapidly expanding field, where innovative strategies are being tested to balance safety, feasibility, and therapeutic efficacy. The first approach utilized autologous CAR-T cells and was tested in five patients [6]. The second involved allogeneic CAR-T cells derived from healthy donor peripheral blood mononuclear cells (PBMCs), engineered with multiplex gene editing to disrupt *TRAC*, *HLA-A*, *HLA-B*, *CIITA*, and *PD-1* loci, thereby mitigating the risks of graft-versus-host disease (GvHD) and host-mediated allojection; this strategy was evaluated in four patients [7]. The third employed a novel *in vivo* approach in which CD8 T cells were targeted with lipid nanoparticles (LNPs) encapsulating CD19 CAR mRNA, tested in five patients [8]. While the cohort sizes are small and results from single trials cannot fully capture the therapeutic potential, these studies represent the first clinical explorations of three distinct CAR-T modalities for SLE.

Comparing the therapeutic product manufacturing

Autologous CAR19-T cells were generated using a clinically established CAR-T cell manufacturing process. Following leukapheresis, approximately 1×10^8 T cells were collected, activated, and transduced with a lentiviral vector encoding a single-chain variable fragment (scFv) derived from the antihuman CD19 hybridoma clone FMC63. The cells were expanded in culture for 12 days, achieving a transduction efficiency of 20–40% [6].

The allogeneic CAR19-T cells were produced from healthy donor PBMCs. Isolated T cells were first transduced with a lentiviral vector encoding the FMC63-based CAR construct and subsequently subjected to CRISPR-Cas9-mediated multiplex gene editing to disrupt *HLA-A*, *HLA-B*, *CIITA*, *TRAC*, and *PD-1* loci [7]. The multiplex genetic engineering minimized the risks of GvHD and host-mediated allojection. The edited cells were expanded *in vitro* for 12 days, with each manufacturing run yielding sufficient product to treat over 100 patients (1×10^6 CAR-positive cells/kg per infusion). The same allogeneic CAR-T cell products were also applied in another early-phase trial for severe myositis and SSc, underscoring their versatility, scalability, and readiness for broad clinical application [7,9].

For *in vivo* CAR engineering, LNPs were developed to target CD8⁺ T cells directly. The CD8-targeted LNPs were formulated with an ionizable lipid (ILB-3132) and encapsulated anti-CD19 CAR mRNA encoding an FMC63 scFv linked to a CD28 hinge, CD28 transmembrane and co-stimulatory domains, and CD3 ζ signaling domain. To achieve selective delivery, the LNP surface was conjugated with an anti-CD8 single-domain antibody, corresponding to the variable domain of a heavy-chain-only antibody (VHH fragment). This approach enables transient *in vivo* reprogramming of CD8⁺ T cells into CAR19-T cells without *ex vivo* manipulation [8]. Notably, selectively targeting CD8⁺ T cells may be advantageous for indications where CD4⁺ CAR expression and activation could induce adverse effects, such as ADs, especially given that CD4⁺ CAR-T cells have been identified as major drivers of cytokine release syndrome (CRS) in patients [10]. In preclinical studies using nonhuman primates, CD8-targeted LNPs achieved efficient *in vivo* transfection of CD19 CAR into CD8⁺ T cells, resulting in effective B-cell depletion without apparent toxicity [10].

Box 1. Clinical landscape of CAR-T cell therapy for ADs

To date, 197 CAR-T cell clinical trials targeting ADs are registered on [ClinicalTrials.gov](https://clinicaltrials.gov) (searched using ‘CAR-T’ and ‘autoimmune disease’; <https://clinicaltrials.gov/search?cond=autoimmune%20disease&term=CAR-T&viewType=Card>) (Figure 1). Geographically, the majority are in Asia (127 trials, including 117 in China), followed by North America (45 trials), Europe (24 trials), and other regions (9 trials). Some trials involve collaborations across multiple regions, which may lead to overlapping counts in geographical reporting. Most trials (191) focus on targeting autoreactive B cells using CARs directed against CD19, BCMA, CD22, or dual CD19/BCMA constructs, while a smaller number (4) target autoreactive T cells via CD7 or CD70. B cell-targeting CAR-T cell therapies are being investigated across a broad spectrum of ADs, including systemic lupus erythematosus, inflammatory myopathies, systemic sclerosis, Sjögren’s syndrome, multiple sclerosis, and idiopathic inflammatory myopathy. T cell-targeting CAR-T therapies are being explored in Crohn’s disease, ulcerative colitis, and dermatomyositis. Additionally, mixed CAR-T cell approaches targeting both BCMA and CD70 are under evaluation for juvenile dermatomyositis (JDM), polyarticular juvenile idiopathic arthritis, and systemic sclerosis (2 trials). Notably, since many ADs are driven by alloreactive B and T cells that express shared antigens (e.g., CD19 and CD7), a single CAR-T cell product can potentially target multiple disorders, and several clinical trials have been designed to evaluate such therapies across different disease indications. Currently, most trials are in early phases and recruiting. Seven trials employ allogeneic/universal CAR-T cells, while the remainder utilize autologous CAR-T cell therapy. Bulk industrial scale-up of CAR-T therapies for ADs is currently limited, as most trials rely on individualized autologous cells, with large-scale, off-the-shelf allogeneic approaches still in early development.

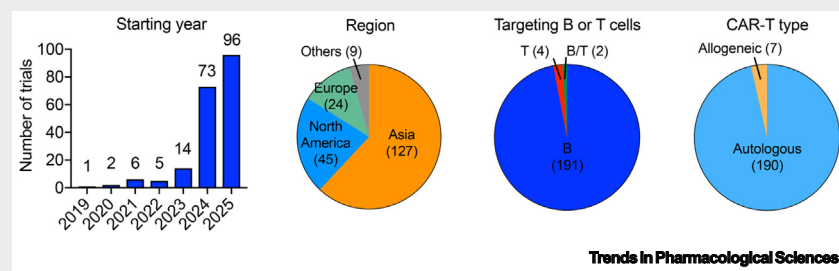


Figure 1. Landscape of clinical trials evaluating chimeric antigen receptor (CAR-T) cell therapy for autoimmune diseases (ADs), including trial initiation years, geographic regions, target cell types (B, T, or both cells), and CAR-T types (autologous or allogeneic).

Overall, both the allogeneic CAR-T platform and *in vivo* LNP-based CAR engineering provide ‘off-the-shelf’ strategies that allow for treatment of multiple patients and potentially repeated dosing. Nevertheless, host immune rejection and immunogenicity remain important considerations for repeated administration. To minimize immune rejection, strategies such as human leukocyte antigen (HLA) molecule knockout, overexpression of inhibitory natural killer ligands (HLA-E or HLA-G), and more recently, cloaked gene engineering with eight immunomodulatory transgenes (*PD-L1*, *CD200*, *CD47*, *HLA-G*, *FASLG*, *SERPINB9*, *CCL21*, and *MFGE8*) have been developed to enable immune evasion [11,12].

Comparing the clinical trial designs

For autologous CAR19-T cells, all patients first received a standard lymphodepleting conditioning regimen to facilitate CAR-T cell engraftment and expansion. Fludarabine (25 mg/m²/day, intravenously) was administered on days –5 to –3, together with cyclophosphamide (1,000 mg/m²/day, intravenously) on day –3. On day 0, patients were infused with autologous CAR19-T cells at a fixed dose of 1 × 10⁶ CAR⁺ T cells per kg of body weight [6].

For allogeneic CAR19-T cells, patients S01–S03 received lymphodepletion with fludarabine (25 mg/m²/day, intravenously, on days –5 to –3) and cyclophosphamide (300 mg/m²/day, intravenously, on days

–5 and –4). Allogeneic CAR19-T cells were infused on day 0 at a dose of 1 × 10⁶ CAR⁺ T cells/kg. Patient S04 had previously been treated with telitacicept and required a 6-week washout before enrollment. However, during this period, disease progression occurred with worsening arthralgia, chest tightness, and low-grade fever, necessitating cyclophosphamide (600 mg) for disease control. After a subsequent 2-week washout, CAR19-T cells were infused without additional lymphodepleting chemotherapy [7].

For *in vivo* CAR-T cell generation using CD8-targeted LNP/mRNA, immunosuppressive medications were discontinued within 1 week prior to the first infusion. Patients received between one and three doses of LNP/mRNA, at either 2 mg or 4 mg per dose [8].

In summary, both autologous and allogeneic CAR19-T cell therapies required preconditioning to support CAR-T cell engraftment and expansion, with both approaches utilizing the same dosing strategy adapted from prior successful clinical trial experience. By contrast, the *in vivo* CAR approach represents a novel regimen still under investigation, with optimal dosing and scheduling yet to be defined. Notably, because this strategy relies on direct *in vivo* engineering of T cells, lymphodepleting preconditioning is not required.

Evaluating the safety outcomes

Autologous CAR19-T cell therapy was well tolerated, with only mild CRS observed. Among the five SLE patients, three developed grade 1 CRS characterized by fever, which was effectively managed with metamizole. No hemodynamic changes or cases of immune effector cell-associated neurotoxicity syndrome (ICANS) were reported [6]. Importantly, the safety profile was more favorable than that observed when the same CAR19-T product was used to treat lymphoma or leukemia, likely reflecting the substantially

Table 1. Comparison of the three CAR-T strategies in treating refractory SLE

Feature	Autologous CAR19-T [6]	Allogeneic CAR19-T [7]	<i>In vivo</i> LNP/mRNA CAR [8]
Source	Patient-derived T cells	Healthy donor-derived T cells	Patient CD8 ⁺ T cells reprogrammed <i>in vivo</i>
Manufacturing	Clinically established <i>ex vivo</i> culture; lentiviral transduction; 12-day expansion; 20–40% transduction	Lentiviral transduction + CRISPR-Cas9 multiplex gene editing (<i>TRAC</i> , <i>HLA-A/B</i> , <i>CIITA</i> , <i>PD-1</i>); 12-day expansion; scalable to >100 patients per batch	CD8-targeted LNP encapsulating CAR mRNA; anti-CD8 VHH for selective delivery; transient <i>in vivo</i> CAR expression
Preconditioning	Lymphodepletion with fludarabine + cyclophosphamide	Lymphodepletion (most patients); one patient infused without preconditioning	No preconditioning required
Dosing	1 × 10 ⁶ CAR ⁺ T cells/kg, single infusion	1 × 10 ⁶ CAR ⁺ T cells/kg, single infusion	1–3 doses of 2–4 mg LNP/mRNA
Safety	Mild CRS (grade 1); no ICANS	Mild CRS (grade 1); no ICANS; no GvHD	Mild CRS; no ICANS; transient lymphopenia; manageable cytokine elevations
Efficacy	Deep, durable B-cell depletion; SLE remission in all patients; drug-free long-term remission; B-cell reconstitution at ~110 days	Robust B-cell depletion; sustained clinical improvement; B-cell reconstitution at ~3 months; naive B-cell predominance	Rapid but transient B-cell depletion; partial clinical improvement; B-cell and anti-dsDNA rebound within 2 months
Advantages	High efficacy; long-term immune reset	Off-the-shelf availability; scalable; sustained efficacy; low risk of GvHD	Minimally invasive; repeatable dosing; off-the-shelf potential; avoids <i>ex vivo</i> manufacturing
Limitations	Requires individualized manufacturing; resource-intensive	Preconditioning required; potential immunogenicity on repeat dosing	Short-lived efficacy; optimal dosing and regimen still under investigation

lower B-cell burden in SLE compared with B-cell malignancies [13].

For allogeneic CAR19-T cells, all patients experienced only grade 1 CRS, presenting as transient fever lasting 2–3 days. No ICANS, GvHD, or infections occurred during treatment, underscoring the safety of *TRAC* knockout as a strategy to mitigate alloreactivity [7].

In the *in vivo* CAR approach, no grade 3 or 4 CRS or ICANS was observed. Clinical results showed transient increases in C-reactive protein and IL-6, with three patients requiring a single dose of tocilizumab for grade 1–2 CRS. No significant hepatotoxicity or cytopenias were reported, aside from a short-lived lymphopenia [8].

Overall, all three CAR strategies demonstrated favorable safety profiles, likely attributable to the lower B-cell burden in SLE compared with malignancies. The absence of GvHD in the allogeneic setting highlights the safety of multiplex gene editing [7], while the *in vivo* LNP/mRNA

approach showed encouraging tolerability, supporting its potential as a safe and scalable therapeutic strategy.

Evaluating the efficacy results

Autologous CAR19-T cells exhibited robust *in vivo* expansion, reaching peak levels around day 10, during which they comprised approximately 11–59% of circulating T cells, followed by a gradual decline thereafter. This expansion induced profound B-cell depletion, with no detectable circulating B cells by day 30 post-infusion. Clinical responses were striking: all five patients achieved remission of SLE according to Definition Of Remission In SLE (DORIS) criteria within 3 months, with normalization of laboratory markers including proteinuria and anti-double-stranded DNA (dsDNA) antibody levels. Median Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores dropped to zero, and drug-free remission was maintained during long-term follow-up, even after B-cell reconstitution at ~110 ± 32 days. Notably, reconstituted B cells were naive and non-class-switched,

suggesting effective elimination of autoreactive clones and durable immune reset [6].

Allogeneic CAR19-T cells also demonstrated strong efficacy, with peak expansion between days 7 and 14 and significant B-cell depletion within 1 week of infusion. Reconstitution occurred by ~3 months, with a predominance of naive B cells and reduced memory B cells. Clinically, all four patients showed sustained improvement, meeting Systemic Lupus Erythematosus Responder Index-4 (SRI-4) response criteria and achieving SLEDAI scores of zero and Physician's Global Assessment (PGA) <1 within 3–6 months [7].

The *in vivo* LNP/mRNA CAR approach achieved rapid but transient efficacy. CD8⁺ CAR-T cells were detectable within 6 h post-infusion, with CAR expression and mRNA peaking at 6 h and returning to baseline within 2–3 days. B-cell depletion was dose-dependent: patients receiving 2 mg showed partial reduction, whereas those receiving 4 mg achieved

near-complete depletion for 7–10 days. However, B-cell levels and anti-dsDNA antibodies rebounded within 2 months, limiting long-term disease control [8]. This transient efficacy may be attributed to the inherently short-lived nature of mRNA expression, rapid CAR-T cell turnover, and the lack of sustained *in vivo* expansion or persistence, highlighting a challenge for achieving durable responses with mRNA-based CAR therapies.

In summary, both autologous and allogeneic CAR19-T cell therapies induced deep and durable B-cell depletion, translating into sustained clinical remission and long-term disease control. By contrast, the *in vivo* CAR approach, while less durable, offers a flexible, repeatable, and off-the-shelf strategy that could provide routine disease management with optimized dosing schedules.

Concluding remarks

Early clinical experiences with CAR-T cell therapy in refractory SLE highlight the transformative potential of cellular immunotherapies for ADs. Both autologous and allogeneic CAR19-T cell strategies demonstrated deep B-cell depletion, sustained clinical remission, and favorable safety profiles, underscoring their therapeutic promise. While autologous CAR-T cells currently provide the most durable efficacy, their individualized, labor-intensive manufacturing limits broad accessibility [6]. Allogeneic CAR-T cells, by contrast, offer scalability and consistency, with multiplex gene editing effectively mitigating the risks of GvHD and alloreactivity [7].

The *in vivo* CAR engineering approach represents a paradigm shift, enabling direct reprogramming of T cells within cancer and AD patients without *ex vivo* manipulation [10,14]. Although current results show only transient efficacy, this strategy holds significant advantages as a repeatable, off-the-shelf therapy that could simplify logistics, reduce costs, and expand global accessibility [8]. Future directions should focus on optimizing LNP formulations for greater persistence, refining cell-specific targeting strategies, and combining with immune-modulatory regimens to improve durability while minimizing host immune rejection.

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Declaration of interests

L.Y. is a scientific advisor to AlzChem and Amberstone Biosciences, and a cofounder, stockholder, and advisory board member of Appia Bio. None of the declared companies contributed to this study. Y.-R.L. declares no competing interests.

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References

- Li, Y.-R. *et al.* (2024) Frontiers in CAR-T cell therapy for autoimmune diseases. *Trends Pharmacol. Sci.* 45, 839–857
- Fischbach, F. *et al.* (2024) CD19-targeted chimeric antigen receptor T cell therapy in two patients with multiple sclerosis. *Med (New York, N.Y.)* 5, 550–558.e2
- Mougiakakos, D. *et al.* (2021) CD19-targeted CAR T cells in refractory systemic lupus erythematosus. *N. Engl. J. Med.* 385, 567–569
- Wang, W. *et al.* (2024) BCMA-CD19 compound CAR T cells for systemic lupus erythematosus: a phase 1 open-label clinical trial. *Ann. Rheum. Dis.* 83, 1304–1314
- Li, Y.-R. *et al.* (2025) *In vivo* CAR engineering for immunotherapy. *Nat. Rev. Immunol.* 25, 725–744
- Mackensen, A. *et al.* (2022) Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. *Nat. Med.* 28, 2124–2132
- Yang, C. *et al.* (2025) Allogeneic anti-CD19 CAR-T cells induce remission in refractory systemic lupus erythematosus. *Cell Res.* 35, 607–609
- Wang, Q. *et al.* (2025) *In vivo* CD19 CAR T-cell therapy for refractory systemic lupus erythematosus. *N. Engl. J. Med.* 393, 1542–1544
- Wang, X. *et al.* (2024) Allogeneic CD19-targeted CAR-T therapy in patients with severe myositis and systemic sclerosis. *Cell* 187, 4890–4904.e9
- Hunter, T.L. *et al.* (2025) *In vivo* CAR T cell generation to treat cancer and autoimmune disease. *Science* 388, 1311–1317
- Pavan, C. *et al.* (2025) A cloaked human stem-cell-derived neural graft capable of functional integration and immune evasion in rodent models. *Cell Stem Cell* 32, 710–726.e8
- Li, Y.-R. *et al.* (2024) Managing alloreactivity in off-the-shelf CAR-engineered cell therapies. *Mol. Ther.* 33, P2368–P2390
- Schubert, M.-L. *et al.* (2021) Side-effect management of chimeric antigen receptor (CAR) T-cell therapy. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* 32, 34–48
- Xu, J. *et al.* (2025) *In-vivo* B-cell maturation antigen CAR T-cell therapy for relapsed or refractory multiple myeloma. *Lancet* 90, 103623