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Application of Engineered NK-92 Cell Extracellular Vesicles in the Treatment of Systemic Lupus Erythematosus

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eLife Assessment

This **useful** study presents the first application of engineered NK-92 cell-derived extracellular vesicles displaying CD19 scFv for the treatment of systemic lupus erythematosus (SLE). The concept of using targeted extracellular vesicles as a "cell-free" alternative to CAR-T/CAR-NK therapies is good. However, the current results are **incomplete** and do not provide strong support for the experimental hypothesis, particularly with respect to EV purification, characterization, mechanistic validation, and adherence to current EV field standards. Several major concerns should be addressed to strengthen the translational relevance, reproducibility, and biological interpretation of the study.

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Abstract

Introduction Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterized by loss of immune tolerance, autoantibody production, and multi-organ damage. Current therapies, including glucocorticoids and CAR-T/CAR-NK cell therapies, are limited by adverse effects, high cost, and safety concerns.

Objectives To develop engineered NK-92 cell-derived extracellular vesicles displaying CD19 single-chain variable fragment (V-CD19-Exo) and evaluate their therapeutic efficacy in an MRL/lpr mouse model of SLE.

Methods The CD19scFv-LAMP-2B fusion construct was stably expressed in NK-92 cells via lentiviral transduction. Extracellular vesicles were isolated by differential centrifugation and characterized by NTA, TEM, and Western Blot. In vivo efficacy was assessed in MRL/lpr mice through B cell depletion analysis, renal function monitoring, cytokine profiling, autoantibody detection, and survival observation.

Results V-CD19-Exo significantly reduced splenic $CD19^{+}CD20^{+}$ B cells from 10.53% to 1.51% ($p < 0.0001$). Treatment attenuated proteinuria, ameliorated lupus nephritis pathology, reversed splenomegaly, and downregulated serum IgE, IL-17A, IFN-γ, anti-dsDNA, and ANA levels. Notably, V-CD19-Exo improved survival to approximately 80% compared to 25% in untreated controls.

Conclusion Engineered NK-92 cell-derived extracellular vesicles represent a novel, safe, and effective cell-free therapeutic strategy for SLE, offering advantages over conventional cell therapies including lower immunogenicity, scalable production, and no requirement for lymphodepletion.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a systemic inflammatory disease characterized by autoimmune reactions, primarily featuring loss of immune tolerance, massive production of autoantibodies, and deposition of immune complexes in multiple organs. The global prevalence is approximately 43.7 cases per 100,000 people, with higher incidence rates in women of childbearing age and Asian populations, showing significant gender and regional disparities [1, 2]. SLE manifests with diverse clinical symptoms, potentially affecting multiple organs including the skin, joints, kidneys, and nervous system. Among these, Lupus Nephritis (LN), as one of the most common end-organ damages, is often the main cause of renal failure and death, with approximately 40%–60% of SLE patients showing renal involvement at the early stage of the disease, significantly impacting prognosis [3–5]. The pathogenesis of SLE remains unclear, involving complex interactions among genetics, epigenetics, environmental factors, and immune system dysregulation. The core mechanism involves the abnormal activation of B cells; autoreactive B cells not only produce large amounts of autoantibodies but also promote T cell activation through antigen presentation and cytokine secretion, forming an autoimmune loop [6–8]. B Lymphocyte Stimulator (BLyS) and A PRoliferation-Inducing Ligand (APRIL) promote the survival and differentiation of autoreactive B cells by binding to BR3, TACI, and BCMA receptors on the B cell surface [9]. CD19, as a specific surface marker for B cells, is widely expressed from pro-B cells to mature B cells and plays a crucial role in B cell activation, proliferation, and differentiation. Therefore, B cell-related targets (CD20, CD19, CD40L, BAFF, etc.) have become focal points in drug development in recent years.

Current treatment for SLE primarily relies on glucocorticoids and immunosuppressants. Although these can control the disease, long-term use may be accompanied by severe adverse effects such as infections, metabolic disorders, and organ toxicity [4]. In recent years, targeted therapeutic strategies have made some progress. For instance, monoclonal antibodies targeting B-cell activating factor (BAFF/BLyS) or CD20 have shown significant efficacy in alleviating symptoms for some patients. However, clinical practice and studies indicate that their overall efficacy is limited by factors such as uneven depletion [10–12].

Cell therapy, mainly CAR-T cell therapy, is not only applied in treating hematological malignancies, particularly B-cell leukemia and lymphoma [13, 14], but also offers a highly promising potential technology for treating autoimmune diseases. Multiple clinical studies have confirmed that CD19-targeted CAR-T cell therapy can efficiently eliminate mature B cells and precursor B cells in vivo, leading to significant improvement in clinical symptoms and decreased autoantibody levels in patients with refractory SLE and psoriasis [15–17].

Gao et al. successfully applied allogeneic CD19-targeted CAR-NK technology in a First-in-Human clinical study for SLE. Among 9 patients followed for over 1 year, 6 patients (67%) achieved complete DORIS remission and reached a lupus low disease activity state, with no recurrence observed in any patient [18]. Wang et al. reported on iPSC-derived CAR-NK cell therapy for systemic sclerosis [19].

However, CAR-T and CAR-NK cell therapies face limitations including high cost, lengthy preparation time, requirement for lymphodepletion before treatment, and risks such as cytokine release syndrome (CRS). Allogeneic CAR-NK cell therapy also encounters challenges like low expansion efficiency, insufficient persistence in vivo, and high costs [15, 20], restricting the application of CAR-T/CAR-NK in treating autoimmune diseases. Therefore, continuing to explore safer, more precise, and more accessible next-generation therapies has become a crucial research direction.

Extracellular vesicles are nanoscale vesicles (approximately 30–150 nm in diameter) secreted by various cells and present in body fluids. Naturally carrying bioactive molecules such as proteins, nucleic acids, and lipids, they are important mediators of intercellular communication, possessing low immunogenicity, high stability, excellent nanomaterial properties, and tissue penetration capability [21–30]. Numerous studies have confirmed that extracellular vesicles inherit the functions of their parent cells. For example, mesenchymal stem cell-derived exosomes (MSC-exos)

show significant potential in regulating immune responses and promoting tissue repair. By delivering functional molecules like miRNAs, they regulate macrophage polarization, promote regulatory T cell (Treg) proliferation, and inhibit aberrant immune responses [28, 31]. Exosomes derived from Natural Killer cells or engineered CAR-NK cells have demonstrated therapeutic effects in melanoma and breast cancer therapy, retaining tumor cell killing functions. More importantly, they offer higher safety, a wide source, and are easy to preserve and transport [32–35].

However, naturally produced extracellular vesicles have limitations such as low yield and poor targeting. Engineered modification strategies, including parental cell gene editing, membrane surface modification, and cargo loading, can address these challenges [36–40]. NK-92 cells, as a natural immune cell line with strong cytotoxicity and easy expandability, have been successfully used in numerous clinical trials for cancer treatment (e.g., patients with recurrent HER2-positive glioblastoma receiving intracranial injection of CAR-NK-92 cells; patients with refractory/relapsed AML receiving NK-92 cell infusions) [41, 42]. Extracellular vesicles derived from NK-92 cells also inherit the killing activity and immunomodulatory capacity of NK cells, showing promise for cancer therapy with good clinical translation potential [43–46]. NK-92 exosomes have demonstrated cytotoxicity against leukemia HL-60 cells [27].

Based on the above background, we utilized NK-92 cell-derived extracellular vesicles to construct an engineered nanovesicle platform combining targeting ability and immunomodulatory functions. We fused the CD19 single-chain variable fragment (CD19scFv) with the N-terminus of Lysosome-Associated Membrane Protein 2B (LAMP-2B), constructed a recombinant plasmid, and transfected it into NK-92 cells, thereby displaying the CD19 targeting structure on the surface of secreted extracellular vesicles. We systematically evaluated the therapeutic efficacy and safety of these vesicles in an MRL/lpr mouse model, aiming to provide a novel “cell-free therapy” strategy for SLE and other autoimmune diseases.

2. Materials and Methods

2.1. Cell Culture and Reagents

The human NK cell line NK-92 was generously provided by Professor Li Jing from Ocean University of China. NK-92 cells were cultured in α-MEM (Genscript, Nanjing, China) supplemented with 12.5% fetal bovine serum, 12.5% horse serum, 1% penicillin (100 U/mL) and streptomycin (100 μg/mL) (P/S), and 200 U/mL recombinant human interleukin-2 (IL-2). Human embryonic kidney (HEK293T) cells were kindly provided by Professor Gu Yuchao from Qingdao University of Science and Technology and cultured in high-glucose Dulbecco’s Modified Eagle Medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin (100 U/mL) and streptomycin (100 μg/mL) (P/S) (Beyotime Biotechnology). All cell lines were cultured at 37°C in a 5% CO incubator. All FBS mentioned above was purchased from ExCell Bio (Grand Island). Antibodies were sourced as specified in the flow cytometry section.

2.2. Plasmid Construction, Preparation of CD19scFv-LAMP-2B Viral Particles, and Cell Transfection

First, gene sequences for Lysosome-Associated Membrane Glycoprotein 2 (LAMP-2B) and the anti-CD19 protein recognition region (CD19 scFv) were obtained from the NCBI GenBank. The target DNA fragment was synthesized and cloned into the pLVX-IRES vector backbone carrying a GFP tag using XhoI and SpeI restriction enzymes to generate the CD19-scFv expression construct; the pLVX-IRES plasmid without CD19-scFv served as a control to verify the specific therapeutic effect of CD19-scFv. HEK-293T cells in the logarithmic growth phase were seeded in 10 cm culture dishes and cultured in DMEM with 10% FBS until reaching 70%–80% confluence. The target vector plasmid was co-transfected with the helper packaging plasmid psPAX2 and the envelope plasmid pMD.2G into cells using Lipofectamine 3000 (Thermo Fisher, USA) to produce viral particles. Subsequently, NK-92 cells were transfected with the lentiviral particles via polybrene-mediated transduction to integrate CD19scFv-LAMP-2B into the cellular genome and establish the CD19scFv-

NK92 cell line. Green fluorescence was observable under a fluorescence microscope 48 hours post-transfection. Cells were passaged at least 5 times under the selective pressure of 600 μg/mL G418 geneticin to achieve selection of a stably transfected CD19scFv-NK92 cell line.

2.3. Isolation and Characterization of Extracellular Vesicles

V-CD19-Exo were secreted by the NK-92 cell line stably expressing the CD19scFv-LAMP2B fusion protein. This cell line was routinely cultured and passaged under standard conditions (37°C, 5% CO) using complete α-MEM medium supplemented with 12.5% fetal bovine serum, 12.5% horse serum, 1% penicillin (100 U/mL) and streptomycin (100 μg/mL) (P/S), and 200 U/mL recombinant human interleukin-2 (IL-2).

To collect engineered extracellular vesicles, cells at approximately 80% confluence were switched to serum-free medium and cultured for an additional 48 hours. The conditioned medium was collected and subjected to differential centrifugation at 4°C: first at 300×g for 10 minutes to remove live cells, then at 2000×g for 15 minutes to remove dead cells and large debris, and finally at 14,000×g for 30 minutes to remove organelles and large vesicles. The supernatant was filtered through a 0.22 μm PES membrane and initially concentrated using 50 kDa molecular weight cut-off centrifugal filter units. The concentrated solution was mixed with Exosome Precipitation Solution at a 4:1 volume ratio, incubated overnight at 4°C, and then centrifuged at 12,000×g for 30 minutes at 4°C. The pellet was resuspended in 200 μL Solution A, centrifuged again at 12,000×g for 5 minutes at 4°C, and the supernatant (containing vesicles) was collected, aliquoted, and stored at −80°C.

Particle size distribution and concentration of the obtained extracellular vesicles were determined using a Marvin NTA NS300. Typical cup-shaped morphology was observed via transmission electron microscopy. Expression of exosomal marker proteins CD9, CD63, and CD81 was detected by Western Blot to confirm vesicle purity.

2.4. In Vivo Treatment

Sixteen-week-old female MRL/lpr mice and sixteen-week-old female C57BL/6 mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Animals were housed in the specific pathogen-free facility of the Animal Experiment Center at Qingdao University of Science and Technology. Experiments were conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of Qingdao University of Science and Technology. Sixteen-week-old MRL/lpr mice, serving as the SLE model, were randomly divided into a normal saline (NC) group (n=5), an NK92-Exo group (n=5), and a V-CD19-Exo group (n=5). Sixteen-week-old female C57BL/6 mice served as normal controls (n=5). Mice were treated with normal saline, NK92-Exo (10 particles), or V-CD19-Exo (10 particles), respectively. Mice were treated twice weekly for 3 weeks. During the treatment period, urine and serum were collected twice weekly for subsequent detection of relevant indicators. Urine was collected using the “bladder massage” method, and serum was separated from blood collected via orbital sinus. For survival studies, the same interventions and groupings (saline group, NK92-Exo group, V-CD19-Exo group) were applied, and mouse survival was observed for as long as possible. All interventions in this study, including normal saline, NK92-Exo, and V-CD19-Exo, were administered via intraperitoneal injection. Intervention details are shown in Fig. 3A. By week 19, treatment ended, mice were euthanized, and serum, kidneys, and spleens were collected from all mice. At the end of the experiment, euthanasia was performed using sodium pentobarbital according to the AVMA Guidelines for the Euthanasia of Animals (2020 Edition). Animal carcasses were disposed of following the regulations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.5. Flow Cytometry Analysis

Mouse spleen leukocytes were isolated and prepared using a mouse spleen lymphocyte isolation kit (Tianjin Haoyang Technology Co., Ltd.) strictly following the manufacturer’s instructions to obtain a highly viable single-cell suspension. After counting cells using a hemocytometer, cells

were resuspended in PBS containing 1% (w/v) bovine serum albumin (BSA, Gibco) and incubated at room temperature for 15 minutes to block non-specific binding sites. 1×10 cells were incubated with FITC-conjugated anti-mouse CD19 antibody and APC-conjugated anti-mouse CD20 antibody (purchased from BioLegend) for 30 minutes at 4°C in the dark. Cells were then washed twice with PBS containing 1% BSA and finally resuspended in 300 μL PBS buffer for analysis. Flow cytometry data acquisition was performed using a CytoFLEX flow cytometer (Beckman Coulter, USA). Subsequent data analysis was conducted using FlowJo software (Tree Star, USA).

2.6. Western Blot Analysis

Samples were mixed with loading buffer at a 5:1 ratio and heated at 95°C for 5 minutes in a metal bath. The treated samples were loaded onto 10% Bis-Tris SDS-PAGE gels and run sequentially in electrophoresis buffer at 80 V for 15 minutes, then 120 V for 1 hour.

Subsequently, the gel was removed, and a transfer sandwich was assembled in the order: black clamp – sponge – filter paper – protein gel – PVDF membrane – filter paper – sponge – red clamp. The transfer cassette was placed into a transfer tank immersed in ice water, and transfer was performed at a constant current of 250 mA for 2 hours. After electrophoresis, the PVDF membrane was incubated with 5% non-fat milk on a shaker (60–70 rpm) at 4°C for 2 hours to block non-specific sites. After blocking, membranes were incubated with primary antibodies against CD9, CD63, and CD81 overnight at 4°C with shaking (60–70 rpm). Membranes were then washed with 1×TBST for 10 minutes, repeated 3 times. HRP-conjugated secondary antibodies were added and incubated at room temperature for 2 hours. After incubation, membranes were washed 3 times with 1×TBST, each for 10 minutes. Immunoreactive bands were visualized using enhanced chemiluminescence (ECL, Thermo Fisher Scientific, Cat#WP20005) and imaged using a Tanon imaging system.

2.7. Cytokine Detection

After blood collection from the mouse orbital venous plexus, whole blood samples were allowed to clot by standing at room temperature for 2 hours. Samples were then centrifuged at 3000 rpm for 30 minutes at 4°C, and the supernatant (serum) was carefully collected. Cytokine levels were measured using ELISA kits (FANKEW) strictly following the manufacturer's instructions. Levels of anti-double-stranded DNA antibody (anti-dsDNA), anti-nuclear antibody (ANA), immunoglobulin E (IgE), interleukin-17A (IL-17A), and interferon-γ (IFN-γ) in mouse serum were detected. All samples were run in duplicate, and concentrations were calculated based on standard curves.

2.8. Histological Examination

Mouse kidney tissues were fixed in formalin solution for 2 days, then dehydrated and embedded. Sections with a thickness of 4 μm were prepared. Hematoxylin and eosin (HE) staining was performed to observe pathological changes. Images were captured using a light microscope. A semi-quantitative scoring system was used to assess basic pathological changes including congestion, blood stasis, hemorrhage, edema, degeneration, necrosis, proliferation, fibrosis, tissue organization, granulation tissue formation, and inflammatory changes.

Glomerular injury was scored according to an established glomerular scoring system by evaluating 50 glomeruli and taking the average. Specific scoring categories were as follows: normal (0 points), cell proliferation or infiltration (1 point), membranoproliferation, hyaline deposition, or lobulation (2 points), global hyalinosis or crescent formation (3 points) [47].

2.9. Statistical Analysis

Statistical analysis of the data in this study was performed using GraphPad Prism 10 software. All values are expressed as mean ± standard deviation (SD). Data were assessed for normal distribution and similar variance between groups. One-way ANOVA was used for statistical analysis of multiple comparisons. For sample data not following a normal distribution, the Kruskal–Wallis test with post-hoc multiple comparisons was used. Pearson correlation coefficient

was used to evaluate linear correlations between data, and Spearman correlation coefficient was used for non-linear correlation analysis to ensure accuracy and reliability of results. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, with p < 0.05 considered statistically significant.

3. Results

3.1. Preparation of Engineered NK-92 Extracellular Vesicles Expressing Anti-CD19 Chimeric Antigen Receptor Protein

To endow NK-92 cell-derived extracellular vesicles with B-cell targeting capability, we employed an engineering strategy to modify the vesicle surface. The gene encoding the CD19 single-chain antibody fragment (scFv) was linked to the N-terminus of the vesicle surface marker protein LAMP-2B, fused with the GFP gene sequence, and cloned into the pLVX-IRES vector (Fig. 1A). Recombinant lentiviral particles were produced by transfecting HEK293T cells.

Subsequently, NK92 cells were transduced with the prepared lentivirus. Following antibiotic selection, an engineered cell line stably expressing the CD19scFv-LAMP-2B fusion protein was established. Observation under a fluorescence microscope with a blue light source revealed abundant green fluorescence within cell clusters, approximately 60%, confirming successful expression of CD19scFv-LAMP-2B-GFP (Fig. 1B). Subsequently, extracellular vesicles (V-CD19-Exo) were isolated from the supernatant of the CD19scFv-NK-92 cell line using an exosome extraction kit (precipitation method). To comprehensively verify the successful isolation of intact V-CD19-Exo, we characterized the obtained vesicles using Western Blot, NTA, and TEM. Western Blot results showed high expression of the exosomal marker proteins CD63, CD9, and CD81 in the obtained vesicle preparation. The endoplasmic reticulum resident protein

Calnexin was present only in the positive control parent cell lysate and was completely absent in the extracellular vesicle sample. This result confirmed the high purity of our extracellular vesicle preparation, essentially excluding contamination by cell debris or organellar components, and indicated that CD19scFv-LAMP-2B-GFP was successfully incorporated into the vesicles.

Nanoparticle tracking analysis showed that the vesicle concentration was adjusted to 1.0×10^{1} particles/mL, with a highly concentrated particle size distribution; over 90% of particles had diameters between 50 and 150 nm (Fig. 1D), consistent with the classical size range of exosomes. Finally, transmission electron microscopy revealed that V-CD19-Exo exhibited a cup-shaped membranous vesicle morphology (Fig. 1E), characteristic of typical exosomes. In summary, the combined results from Western Blot, NTA analysis, and TEM observation collectively demonstrated the successful acquisition of engineered V-CD19-Exo vesicles.

These results collectively indicate that we successfully constructed an NK-92 cell line stably expressing CD19scFv and prepared engineered extracellular vesicles (V-CD19-Exo) with defined targeting epitopes, laying the material foundation for subsequent functional and efficacy studies.

3.2. V-CD19-Exo Specifically Kill Tumor Cells and Target $CD19^{+}$ B Cells In Vivo

To verify whether the engineered extracellular vesicles retained the effector functions of their parent NK-92 cells after modification and to assess their potential impact on non-target cells, we first evaluated the in vitro cytotoxicity of V-CD19-Exo. They were co-cultured with tumor cells (A549) and normal mesenchymal stem cells (MSCs) for 24 hours. Assessment was performed via real-time microscopy, trypan blue exclusion counting, and cell morphological analysis. Results showed that V-CD19-Exo exhibited strong killing activity against A549 cells. After 24 hours of co-culture, microscopy revealed numerous A549 cells rounding up, detaching, and floating in the medium, with a marked reduction in adherent cell numbers (Fig. 2A), achieving a cell death rate of approximately 80%. In contrast, under identical treatment conditions, the growth status of normal MSCs was not significantly affected. Microscopy showed MSCs retained their typical spindle-shaped adherent morphology, with cell density comparable to the control group. This

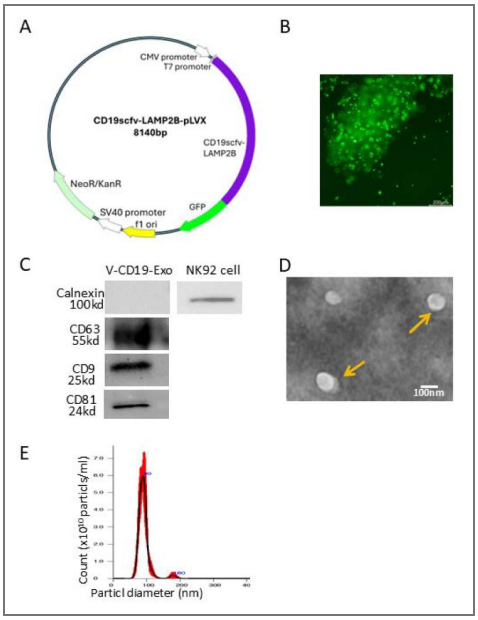


Figure 1. Construction and characterization of CD19-targeting NK-92-derived extracellular vesicles.

(A) Construction of the CD19-targeting CD19scFv-LAMP2B-pLVX plasmid (CD19 plasmid). The CD19-targeting receptor gene was generated by fusing an anti-CD19 single-chain variable fragment (scFv) to the N-terminus of the exosomal membrane protein LAMP2B and linking it to the N-terminus of GFP. The fusion gene sequence was cloned into the multiple cloning site of the plasmid. (B) Fluorescence microscopy image of NK-92 cells 72 hours after transfection with the lentivirally packaged CD19 plasmid (V-CD19). Scale bar = 200 μm. (C) Western blot analysis of exosomal markers in V-CD19-Exo. The exosomes positively expressed CD63 (55 kDa), CD9 (25 kDa), and CD81 (24 kDa), while the endoplasmic reticulum protein Calnexin (100 kDa) was negative. (D) Transmission electron microscopy (TEM) image of extracellular vesicles (V-CD19-Exo) isolated from the supernatant of NK-92 cells after V-CD19 plasmid transfection, negatively stained. Arrows indicate cup-shaped vesicles. Scale bar = 100 nm. (E) Nanoparticle tracking analysis (NTA) of the particle size distribution and concentration of V-CD19-Exo. The exosomes exhibited a unimodal size distribution with a peak diameter of approximately 100 nm and a corresponding concentration of approximately 6.0×10^{10} particles/mL.

indicates that the engineered V-CD19-Exo successfully preserved the cytotoxicity of NK-92 cells and exhibited selective killing action, and the engineering process did not affect their cytotoxicity. Subsequently, we further validated the in vivo targeting efficacy of these vesicles in the MRL/lpr mouse model. MRL/lpr mice were randomly grouped and subjected to systemic administration for three weeks. V-CD19-Exo was administered via intraperitoneal injection at 1.0 × 10 particles/mL based on body weight. After treatment, mice were euthanized, splenic lymphocytes from each group were isolated, and changes in B cell subsets were analyzed by flow cytometry. Single-cell suspensions were stained with anti-CD19 and anti-CD20 (B-cell specific marker) antibodies to detect the proportion of CD19 CD20 double-positive mature B cells (Fig. 2B, C). Results showed that the percentage of CD19 CD20 double-positive mature B cells in the spleens of mice from the V-CD19-Exo treatment group was 1.51%, significantly lower than that in the PBS control group (10.53%, $p < 0.0001$) and the NK92-Exo control group (8.03%, $p < 0.0001$) (Fig. 2C), both showing highly significant decreases. This result directly demonstrates that V-CD19-Exo vesicles, via the CD19scFv targeting structure on their surface, can effectively home to and act on abnormally activated CD19 B cells in vivo. This finding aligns with recent research directions utilizing engineered extracellular vesicles to target B cells. For instance, similar studies have confirmed that vesicles displaying anti-CD19 single-chain antibodies can specifically recognize and eliminate CD19 B cells in vitro and in vivo [48].

In summary, V-CD19-Exo successfully integrated the inherent cytotoxicity of NK-92 vesicles with the targeting ability mediated by CD19scFv, achieving specific action against CD19 B cells and resulting in a significant reduction of mature B cell populations in the spleen. This provides a basis for its potential systemic therapeutic effect in SLE models and offers key functional evidence for its application in treating B cell-related diseases.

3.3. V-CD19-Exo Effectively Alleviates Lupus Nephritis and Renal Inflammation in MRL/lpr Mice

3.3.1. Evaluation of the Renal Protective Effect of V-CD19-Exo in SLE Model Mice

To assess the renal protective effect of V-CD19-Exo in systemic lupus erythematosus (SLE) model mice (Fig. 3A), we systematically analyzed renal function and pathological changes in MRL/lpr mice. Dynamic monitoring showed that urinary protein levels in untreated MRL/lpr mice continuously increased with disease progression, reaching ~2000 mg/L by the end of the experiment, indicating severe proteinuria, a hallmark of lupus nephritis. In contrast, following V-CD19-Exo treatment, the upward trend of urinary protein was significantly delayed. Throughout the observation period, urinary protein levels in the treatment group remained consistently low, averaging below 1000 mg/L by the end of the experiment, showing a statistically significant difference compared to the NC group (Fig. 3C). Concurrently, the urinary protein/creatinine ratio (UPCR), a more stable indicator of renal function, was also significantly reduced in the V-CD19-Exo treatment group (Fig. 3D). Vesicles obtained from NK-92 cells transfected with the control plasmid did not show this significant therapeutic effect. These results, consistent with the trend in urinary protein content, confirm that V-CD19-Exo treatment effectively reduces protein leakage in MRL/lpr mice, ameliorates the glomerular filtration barrier function, and indicates protection of renal function.

Hematoxylin-eosin (HE) staining of kidney tissue was performed to evaluate the effect of V-CD19-Exo on renal pathological changes in MRL/lpr mice. Histological analysis revealed that kidneys from mice in the NC group exhibited typical active lupus nephritis lesions, including diffuse glomerular lymphocyte infiltration, mesangial matrix proliferation, accompanied by tubular epithelial cell swelling and interstitial edema (Fig. 3E). In contrast, renal histopathological damage was significantly attenuated in the V-CD19-Exo treatment group.

Inflammatory cell infiltrates in the glomerular area were markedly reduced, tubular structures were largely intact, and interstitial edema was improved (Fig. 3E). Subsequent evaluation of glomerular lesions using a semi-quantitative histological scoring system showed that the total

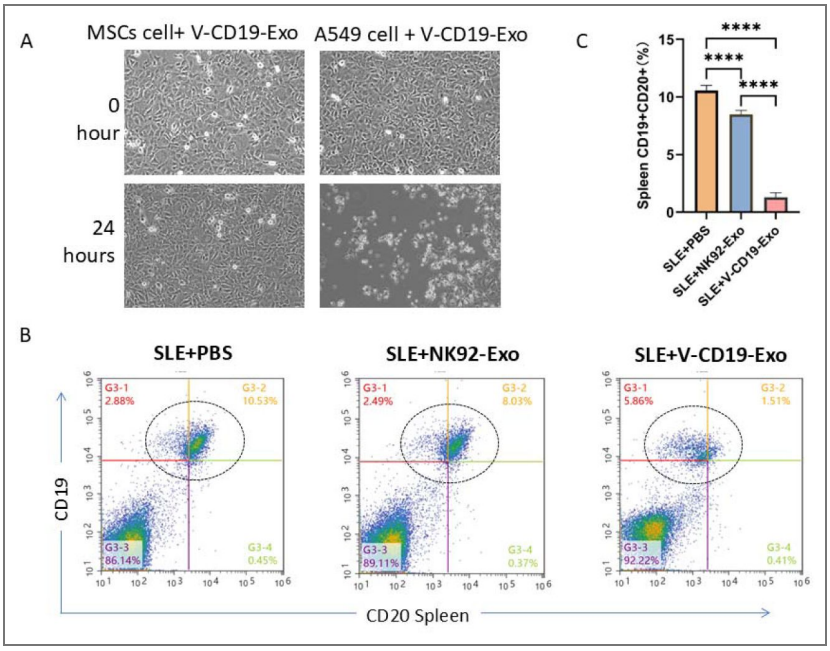
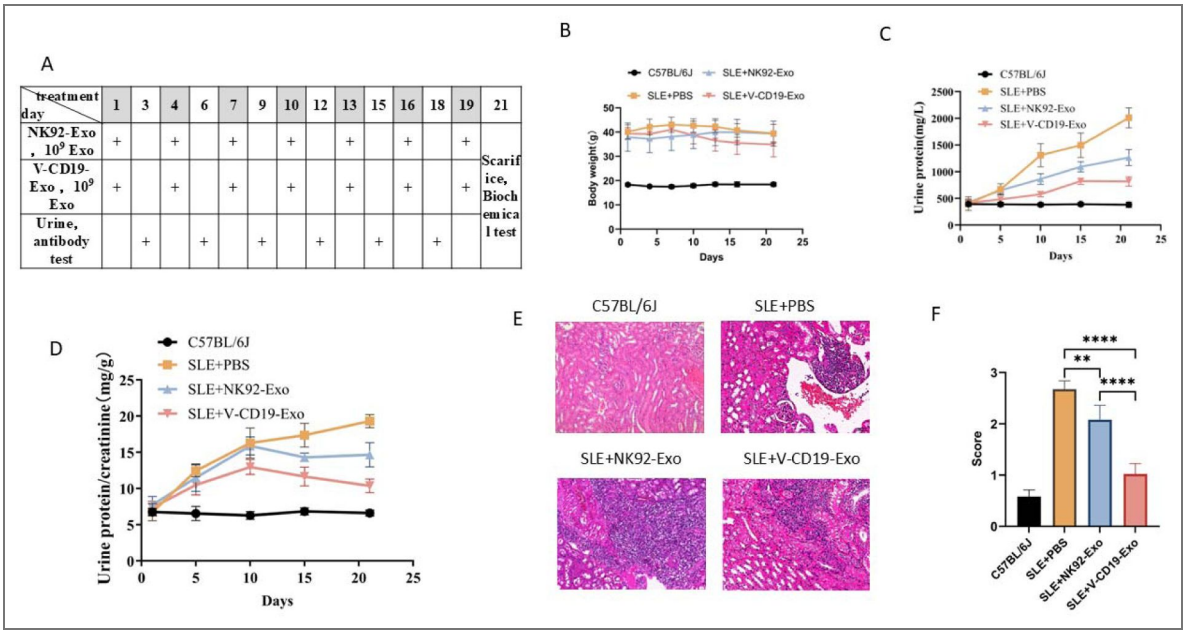


Figure 2. V-CD19-Exo retains NK-92 cytotoxicity against tumor cells and selectively targets CD19$^+$ B cells in vivo.

(A) Cytotoxic effect of V-CD19-Exo on A549 tumor cells. Tumor cells were seeded in 6-well plates containing DMEM + 10% FBS (1x10^6 A549 cells/2 mL/well), cultured for 24 hours, then V-CD19-Exo was added, mixed, and cultured for another 24 hours. (B) Effect of V-CD19-Exo on B cells in MRL/lpr mice. 16-week-old female MRL/lpr mice were intraperitoneally injected with V-CD19-Exo (10^9 particles/mouse/time). Mice were euthanized 3 weeks later, spleens were collected to prepare single-cell suspensions, and splenic B cell populations were analyzed by flow cytometry using anti-CD20-FITC and anti-CD19-APC antibodies. NC, control group; NK92-EXO, MRL/lpr mice injected with NK92 cell exosomes; CD19scfv-NK92-Exos, MRL/lpr mice injected with V-CD19-Exo exosomes. The dotted box indicates CD20 and CD19 double-positive B cells. (C) Quantitative analysis of CD20 and CD19 double-positive B cells (cells within the circular gate in Figure B) in the spleens from mice treated with NC, control, and NK92-EXO groups, corresponding to Figure B.



treatment / day	1	3	4	6	7	9	10	12	13	15	16	18	19	21
NK92-Exo, 10^9 Exo	+		+		+		+		+		+		+	Scarifice, Biochemical test
V-CD19-Exo, 10^9 Exo	+		+		+		+		+		+		+	
Urine, antibody test		+		+		+		+		+		+		

Figure 3. Effects of V-CD19-Exo on body weight, proteinuria, and renal histopathology in MRL/lpr mice.

Treatment and detection timelines are as indicated. (A) Drugs were injected according to the experimental design timeline, or mouse urine and serum were collected to monitor urinary protein, urinary creatinine, and serum autoantibody levels. Mice were euthanized at the experimental endpoint, and kidneys were collected for pathological analysis. Experimental data analysis is shown as follows: (B) Dynamic changes in mean body weight of mice in each group, (C) Kidney function indicator - urinary protein concentration, (D) Urinary protein to urinary creatinine ratio. Data presented as mean ± SEM. **p < 0.01, ***p < 0.001 using two-way ANOVA analysis. (E) Representative images of renal histopathology at the experimental endpoint (H&E staining, scale bar = 100 μm), showing glomerular necrosis (black arrows) and lymphocyte infiltration (white arrows). (F) Kidney injury score, **p<0.001, n=5; **** p<0.0001, n=5.

renal pathology score in the V-CD19-Exo treatment group was significantly lower than that in the NC group (Fig. 3F, $p < 0.001$, n=5; $p < 0.0001$, n=5). These results indicate that V-CD19-Exo treatment effectively inhibits local lymphocyte infiltration and activation in the kidney, reduces the inflammatory response mediated by immune complex deposition, and thereby significantly ameliorates renal pathological damage in SLE model mice.

The above results demonstrate that V-CD19-Exo treatment, while targeting autoreactive B cells, can alleviate the progression of lupus nephritis at the pathological structural level, exhibiting excellent renal protective effects.

3.3.2. Unveiling the Systemic Immunomodulatory Effects of V-CD19-Exo

To investigate whether V-CD19-Exo exerts systemic immunomodulatory effects, we evaluated its impact on spleen weight, serum cytokine levels (IgE, IL-17A, and IFN-γ), and anti-double-stranded DNA IgG autoantibody levels in MRL/lpr mice. The results showed that the treatment effectively reversed SLE-associated abnormal immune states at multiple levels. First, we observed that V-CD19-Exo treatment significantly ameliorated the pathological phenotype of immune organs in model mice. Compared to the PBS control group, which exhibited significant splenomegaly, V-CD19-Exo treatment restored spleen size and weight to near-normal levels (Fig. 4A, B). Pathological splenomegaly, a direct indicator of systemic immune hyperactivation in MRL/lpr mice, was thus reversed. This result indicates that V-CD19-Exo treatment significantly ameliorated systemic immune inflammation in model mice. Simultaneously, we used ELISA to measure serum levels of IgE, IL-17A, and IFN-γ, finding that V-CD19-Exo treatment significantly downregulated these pro-inflammatory factors. Compared with the NC group and the control NK92-Exo treatment group, V-CD19-Exo treatment significantly reduced serum levels of IgE, IL-17A, and IFN-γ in MRL/lpr mice (Fig. 4C–E). This change reflects an overall improvement in the body's immune inflammatory status.

More critically, serological analysis showed that V-CD19-Exo treatment significantly reduced the titers of two key SLE autoantibodies—anti-double-stranded DNA (dsDNA) antibody and anti-nuclear antibody (ANA)—showing a downward trend (Fig. 5A, B). This effect was significantly superior to the non-targeted NK92-Exo control group, demonstrating that CD19scFv-mediated targeted delivery offers a specific advantage in inhibiting autoantibody production. In summary, V-CD19-Exo achieved multi-level systemic immunomodulation by restoring immune organ homeostasis, downregulating broad-spectrum pro-inflammatory cytokines, and specifically inhibiting pathogenic autoantibody production.

3.3.3. Does Immune Improvement Translate into Long-Term Survival Benefits?

Survival rate is the most direct and critical indicator for evaluating SLE treatment efficacy. We monitored the survival of mice in each group for up to 30 weeks, revealing significant differences in survival among the different treatment groups. Mice in the NC group began to die from week 17, with a survival rate of only 25% by the experimental endpoint. The survival curve of the control NK92-Exo group was similar. In stark contrast, the survival rate of mice in the V-CD19-Exo treatment group was significantly improved, reaching approximately 80% (Fig. 5C), indicating that this treatment significantly prolongs the lifespan of model mice.

In summary, long-term survival observations, together with the previously observed renal function protection and reduced pathological damage, strongly demonstrate that V-CD19-Exo treatment not only effectively regulates autoimmune activity but also fundamentally and significantly prolongs the survival of disease model animals, demonstrating clear therapeutic potential and protective effects.

4. Discussion

Natural killer (NK) cells are a crucial component of the innate immune system, capable of specifically killing target cells without prior antigen presentation. NK cells and their derived extracellular vesicles play important roles in tumor immunotherapy, maintaining immune

Figure 4. **V-CD19-Exo treatment alleviates splenomegaly and systemic inflammation in MRL/lpr mice.**

(A) Representative images of spleens at the treatment endpoint (week 19). Spleens from healthy control C57BL/6J mice, untreated MRL/lpr mice, control NK92-Exo treatment group, and V-CD19-Exo treatment group. Scale bar: 1 cm. (B-E) Quantitative analysis of spleen weight and serum inflammatory cytokines at the treatment endpoint. (B) Spleen weight. (C) Serum total IgE concentration. (D) Serum IL-17A concentration. (E) Serum IFN-γ concentration. Data are presented as mean ± standard error of the mean (n=X per group). Statistical analysis was performed using one-way ANOVA with Tukey's post-hoc test. *p < 0.05, **p < 0.01, ***p < 0.001; ns, not statistically significant.

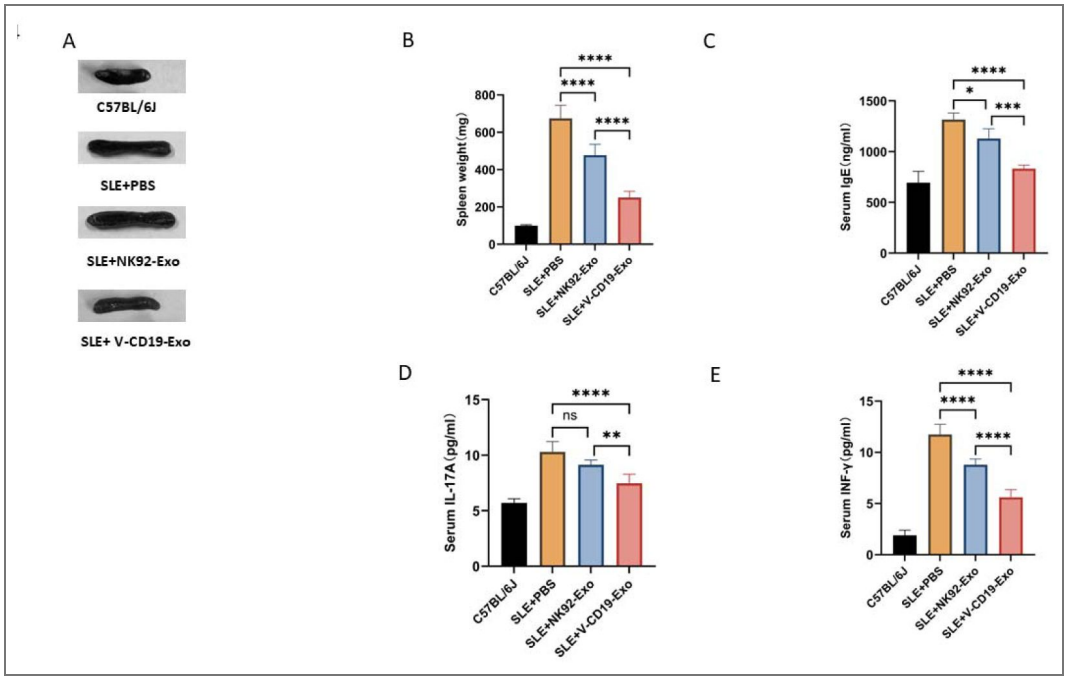
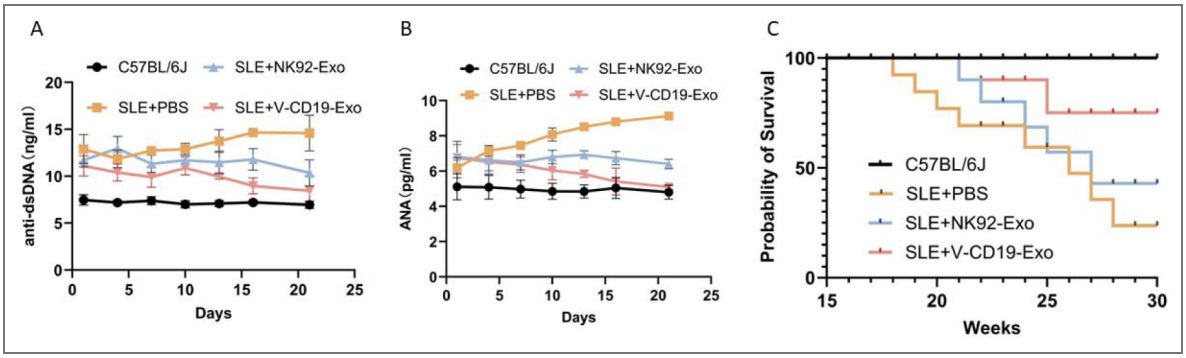


Figure 5. **Autoantibody levels and survival during V-CD19-Exo treatment in MRL/lpr mice.**

(A) Serum anti-double-stranded DNA IgG antibody levels, and (B) Anti-nuclear antibody titers (n=5). (C) Kaplan-Meier survival curve of MRL/lpr mice over a 30-week observation period (n=10).



homeostasis, and regulating aberrant immune responses.

NK-92 cells are an immortalized human NK cell line derived from a lymphoma patient and are widely used in tumor immunotherapy research. In recent years, extracellular vesicles derived from NK-92 cells have gained increasing attention. They are considered to inherit, to some extent, the immunomodulatory and targeted killing functions of the parent cells while avoiding the safety concerns associated with live cell therapy. However, no reports have yet been published on the application of NK-92-derived extracellular vesicles in the treatment of autoimmune diseases.

This study demonstrates for the first time that engineered NK-92 cell-derived extracellular vesicles can significantly reduce the number of CD19 B cells in vivo. In contrast, vesicles produced by NK-92 cells transfected with a control plasmid showed no significant therapeutic effect, suggesting that vesicles targeting CD19 B cells can effectively regulate abnormal B cell activation or proliferation. This finding provides a novel cell-free therapeutic strategy for B cell-mediated autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, significantly expanding the application scope of NK cell therapy.

This study successfully constructed engineered extracellular vesicles (V-CD19-Exo) derived from NK-92 cells, conferring targeting ability via CD19scFv expression, and confirmed their significant therapeutic efficacy in the MRL/lpr lupus mouse model. This strategy effectively alleviated renal pathology, systemic immune dysregulation, and autoantibody production, ultimately prolonging survival. These results indicate that targeted therapeutic strategies based on engineered extracellular vesicles offer a new interventional approach for SLE.

Compared to cell therapy, extracellular vesicles offer distinct advantages. NK cell culture is time-consuming; in vitro established NK cell lines after plasmid transfection may exhibit reduced cytotoxicity, limited passage numbers, and risks like cytokine storms and immunogenicity. NK-92 cells, as an immortalized cell line, carry potential tumorigenicity risks and require irradiation before use, which affects their in vivo survival time and therapeutic persistence. Both cell types lack crucial homing receptors, limiting their ability to target specific cells or tissues. In contrast, targeted modified NK-92 vesicles lack proliferative capacity, possess low immunogenicity, offer higher safety, and are amenable to engineering modifications and large-scale production. Therefore, this approach completely eliminates dependence on live cell infusion, avoiding safety risks associated with cell therapy. Simultaneously, based on existing cell culture and vesicle isolation technologies, this platform holds potential for scalable production, cost control, and high batch-to-batch consistency, providing a novel and translationally feasible solution for targeted therapy of autoimmune diseases with promising clinical prospects.

This study established a modular engineered extracellular vesicle delivery platform. By anchoring the targeting molecule CD19 single-chain antibody fragment (CD19 scFv) onto the vesicle membrane surface, the vesicles were endowed with active recognition, specific binding, and homing capabilities towards CD19 B cells, achieving precise targeted delivery. This design enables vesicles to efficiently act on pathogenic B cells, providing a new technological pathway for targeted intervention in B cell-related diseases. Through directed engineering of EVs, their yield, targeting ability, and therapeutic specificity can be systematically enhanced, thereby overcoming the limitations of natural EVs [38, 39]. The CD19 scFv “targeting module” can be flexibly replaced with other targeting molecules (e.g., scFvs against different tumor antigens or immune cell markers) depending on the disease type. Concurrently, the internal cavity of the vesicle can be loaded with various therapeutic payloads, including small molecule drugs, nucleic acids, or functional proteins. This forms a multifunctional delivery system with a “replaceable target head and variable payload,” granting the platform broad disease adaptability and good industrialization potential [19, 49, 50].

The core innovation of this study lies in adapting the CAR targeting design concept, replacing immune cell therapy with extracellular vesicles. By constructing a stable cell line, continuously and stably produced targeted extracellular vesicles are applied for treatment, offering a novel solution for precise intervention in autoimmune diseases and B cell-related disorders.

Despite the significant potential shown by engineered NK-92 vesicles in targeted therapy, their clinical translation still faces multiple challenges. Large-scale, high-purity production of extracellular vesicles remains a critical bottleneck for industrialization. Current yields from cell culture systems are limited, and preparations are prone to contamination with other extracellular vesicles or impurities, potentially affecting product uniformity and therapeutic efficacy.

This study clearly demonstrates the effectiveness of targeting B cells therapeutically. However, the precise cellular mechanism of action warrants further investigation. Firstly, whether the vesicle-mediated reduction in B cells results from direct cell elimination (killing) or downregulation of CD19 gene activity remains unclear. This study observed a sharp decline in target cell proportion in the spleen (from 10.53% to 1.51%) accompanied by improvements in systemic immune parameters, suggesting a potential direct cytotoxic effect, given that the plasmid itself lacks the function to recognize the CD19 gene. The observed outcome is likely mediated by cytotoxic proteins (e.g., perforin, granzymes) carried by NK-92 cell-derived vesicles.

Notably, although within the same MRL/lpr model, we observed that identical treatment exhibited more significant efficacy in male mice compared to female mice (data not shown). This could stem from inherent sex differences in the model—females display earlier onset and more robust autoimmune phenotypes, making the same intervention relatively more effective in males with lower inflammatory baselines. Alternatively, it suggests that differences in the immune microenvironment of target organs like the kidney might influence EV efficacy, necessitating deeper mechanistic exploration considering sex as a biological variable in future studies.

Data availability

If necessary, you may contact the corresponding author to request the raw data.

Additional information

CRediT Author Statement

Yu Sun: Conceptualization, Methodology, Investigation, Writing – original draft. **Zeyu Tang:** Investigation, Methodology. **Mengting Guo:** Investigation, Data curation. **Zimeng Zhai:** Investigation. **Zixian Wu:** Investigation. **Xia Wang:** Investigation. **Fang Li:** Investigation. **Weiling An:** Resources. **Xiaowei Dou:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

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References

1. **Siegel CH,** Sammaritano LR (2024) Systemic Lupus Erythematosus. *Jama* **331**:1480-1491 https://doi.org/10.1001/jama.2024.2315 | PubMed

2. **Tian J,** Zhang D, Yao X, Huang Y, Lu Q (2023) Global epidemiology of systemic lupus erythematosus: a comprehensive systematic analysis and modelling study. *Ann Rheum Dis* https://doi.org/10.1136/ard-2022-223035 | PubMed
3. **Pan L,** Lu M.-P, Wang J.-H, Xu M, Yang S.-R (2019) Immunological pathogenesis and treatment of systemic lupus erythematosus. *World Journal of Pediatrics* **16**:19-30 https://doi.org/10.1007/s12519-019-00229-3 | PubMed
4. **Parikh SV,** Almaani S, Brodsky S, Rovin BH (2020) Update on Lupus Nephritis: Core Curriculum 2020. *American Journal of Kidney Diseases* **76**:265-281 https://doi.org/10.1053/j.ajkd.2019.10.017 | PubMed
5. **Obrișcă B,** Sorohan B, Tuță L, Ismail G (2021) Advances in Lupus Nephritis Pathogenesis: From Bench to Bedside. *International Journal of Molecular Sciences* **22** https://doi.org/10.3390/ijms22073766 | PubMed
6. **Yap DYH,** Chan TM (2019) B Cell Abnormalities in Systemic Lupus Erythematosus and Lupus Nephritis—Role in Pathogenesis and Effect of Immunosuppressive Treatments. *International Journal of Molecular Sciences* **20** https://doi.org/10.3390/ijms20246231 | PubMed
7. **Ma K,** Du W, Wang X, et al. (2019) Multiple Functions of B Cells in the Pathogenesis of Systemic Lupus Erythematosus. *International Journal of Molecular Sciences* **20** https://doi.org/10.3390/ijms20236021 | PubMed
8. **Li M,** Zhang Y, Jiang N, et al. (2024) Anti-CD19 CAR T Cells in Refractory Immune Thrombocytopenia of SLE. *N Engl J Med* **391**:376-378 https://doi.org/10.1056/nejmc2403743 | PubMed
9. **Möckel T,** Basta F, Weinmann-Menke J, Schwarting A (2021) B cell activating factor (BAFF): Structure, functions, autoimmunity and clinical implications in Systemic Lupus Erythematosus (SLE). *Autoimmunity Reviews* **20** https://doi.org/10.1016/j.autrev.2020.102736 | PubMed
10. **Lee DSW,** Rojas OL, Gommerman JL (2020) B cell depletion therapies in autoimmune disease: advances and mechanistic insights. *Nature Reviews Drug Discovery* **20**:179-199 https://doi.org/10.1038/s41573-020-00092-2 | PubMed
11. **Abeles I,** Palma C, Meednu N, Payne AS, Looney RJ, Anolik JH (2024) B Cell–Directed Therapy in Autoimmunity. *Annual Review of Immunology* **42**:103-126 https://doi.org/10.1146/annurev-immunol-083122-044829 | PubMed
12. **Stockfelt M,** Teng YKO, Vital EM (2025) Opportunities and limitations of B cell depletion approaches in SLE. *Nature Reviews Rheumatology* **21**:111-126 https://doi.org/10.1038/s41584-024-01210-9 | PubMed
13. **Brudno JN,** Maus MV, Hinrichs CS (2024) CAR T Cells and T-Cell Therapies for Cancer. *Jama* **332** https://doi.org/10.1001/jama.2024.19462 | PubMed
14. **de Oliveira Canedo G,** Roddie C, Amrolia PJ (2025) Dual-targeting CAR T cells for B-cell acute lymphoblastic leukemia and B-cell non-Hodgkin lymphoma. *Blood Advances* **9**:704-721 https://doi.org/10.1182/bloodadvances.2024013586 | PubMed
15. **Baker DJ,** June CH (2024) Off-the-shelf CAR-T cells could prove paradigm shifting for autoimmune diseases. *Cell* **187**:4826-4828 https://doi.org/10.1016/j.cell.2024.07.056 | PubMed
16. **Abdalhadi HM,** Chatham WW, Alduraibi FK (2024) CAR-T-Cell Therapy for Systemic Lupus Erythematosus: A Comprehensive Overview. *International Journal of Molecular Sciences* **25** https://doi.org/10.3390/ijms251910511 | PubMed
17. **Wang S.-y,** An W.-h, Wang Z.-s, et al. (2024) Incidentally cured psoriasis in a patient with refractory/relapsed diffuse large B-cell lymphoma receiving CD19 CAR-T cell therapy: a case report. *Frontiers in Immunology* **15** https://doi.org/10.3389/fimmu.2024.1418768 | PubMed
18. **Gao J,** Li M, Sun M, et al. (2026) Efficacy and safety of allogeneic CD19 CAR NK-cell therapy in systemic lupus erythematosus: a case series in China. *Lancet* **406**:2968-2979 https://doi.org/10.1016/s0140-6736(25)01671-x | PubMed

19. **Wang X,** Wu X, Tan B, et al. (2024) Allogeneic CD19-targeted CAR-T therapy in patients with severe myositis and systemic sclerosis. *Cell* **187**:4890-4904. https://doi.org/10.1016/j.cell.2024.06.027 | PubMed
20. **Scherlinger M,** Kolios AGA, Kyttaris VC, Tsokos GC (2025) Advances in the treatment of systemic lupus erythematosus. *Nature Reviews Drug Discovery* **24**:926-944 https://doi.org/10.1038/s41573-025-01242-0 | PubMed
21. **Doyle LM,** Wang MZ (2019) Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. *Cells* **8** https://doi.org/10.3390/cells8070727 | PubMed
22. **He C,** Zheng S, Luo Y, Wang B (2018) Exosome Theranostics: Biology and Translational Medicine. *Theranostics* **8**:237-255 https://doi.org/10.7150/thno.21945 | PubMed
23. **Li W,** Pang Y, He Q, et al. (2024) Exosome-derived microRNAs: emerging players in vitiligo. *Front Immunol* **15** https://doi.org/10.3389/fimmu.2024.1419660 | PubMed
24. **Su X,** Wang H, Li Q, Chen Z (2025) Extracellular Vesicles: A Review of Their Therapeutic Potentials, Sources, Biodistribution, and Administration Routes. *International Journal of Nanomedicine* **20**:3175-3199 https://doi.org/10.2147/ijn.s502591 | PubMed
25. **Gui Y,** Peng M, Huang Y, et al. (2025) New insights into extracellular vesicles in metastatic cancer: From mechanisms to diagnostics and targeted therapies. *Molecular Therapy* **33**:4731-4747 https://doi.org/10.1016/j.ymthe.2025.07.016 | PubMed
26. **Zhang M,** Johnson-Stephenson TK, Wang W, et al. (2022) Mesenchymal stem cell-derived exosome-educated macrophages alleviate systemic lupus erythematosus by promoting efferocytosis and recruitment of IL-17+ regulatory T cell. *Stem Cell Research & Therapy* **13** https://doi.org/10.1186/s13287-022-03174-7 | PubMed
27. **Abbasi S,** Movassaghpour A, Soleimani M, Asghari Molabashi Z (2025) The Effects of Released Exosomes from NK-92 Cells with IL15 on the Apoptosis of HL-60 Cell Line. *Int J Hematol Oncol Stem Cell Res* **19**:126-137 https://doi.org/10.18502/ijhoscr.v19i2.18550 | PubMed
28. **Zhao Y,** Song W, Yuan Z, et al. (2023) Exosome Derived from Human Umbilical Cord Mesenchymal Cell Exerts Immunomodulatory Effects on B Cells from SLE Patients. *Journal of Immunology Research* **2023**:1-15 https://doi.org/10.1155/2023/3177584 | PubMed
29. **Miyamoto N,** Sakuragi M, Kitade Y (2024) Advanced Nanotechnology-Based Nucleic Acid Medicines. *Pharmaceutics* **16** https://doi.org/10.3390/pharmaceutics16111367 | PubMed
30. **Gharpure S,** Ankamwar B (2021) Use of nanotechnology in combating coronavirus. *3 Biotech* https://doi.org/10.1007/s13205-021-02905-6 | PubMed
31. **Yaghoubi Y,** Movassaghpour A, Zamani M, Talebi M, Mehdizadeh A, Yousefi M (2019) Human umbilical cord mesenchymal stem cells derived-exosomes in diseases treatment. *Life Sci* **233** https://doi.org/10.1016/j.lfs.2019.116733 | PubMed
32. **Zhu L,** Kalimuthu S, Gangadaran P, et al. (2017) Exosomes Derived From Natural Killer Cells Exert Therapeutic Effect in Melanoma. *Theranostics* **7**:2732-2745 https://doi.org/10.7150/thno.18752 | PubMed
33. **Tao B,** Du R, Zhang X, et al. (2023) Engineering CAR-NK cell derived exosome disguised nano-bombs for enhanced HER2 positive breast cancer brain metastasis therapy. *J Control Release* **363**:692-706 https://doi.org/10.1016/j.jconrel.2023.10.007 | PubMed
34. **Si C,** Gao J, Ma X (2024) Natural killer cell-derived exosome-based cancer therapy: from biological roles to clinical significance and implications. *Mol Cancer* **23** https://doi.org/10.1186/s12943-024-02045-4 | PubMed
35. **Xie QH,** Zheng JQ, Ding JY, et al. (2022) Exosome-Mediated Immunosuppression in Tumor Microenvironments. *Cells* **11** https://doi.org/10.3390/cells11121946 | PubMed

36. **Wang S,** Chen J, Cui Y, et al. (2025) Multifunctional extracellular vesicles inhibiting autophagy ameliorate immunotherapy in non-small cell lung cancer. *Acta Pharmaceutica Sinica B* https://doi.org/10.1016/j.apsb.2025.12.013 | PubMed

37. **Sun M,** Zhang H, Ma Y, et al. (2025) In Situ Programming of the Tumor Microenvironment to Alleviate Immunosuppression for Pancreatic Cancer Immunotherapy. *Advanced Science* **12** https://doi.org/10.1002/advs.202504008 | PubMed

38. **Niu L,** Ou Q, Ren Q, et al. (2025) Engineered Foxp1high Exosomes Ameliorates Systemic Lupus Erythematosus. *Advanced Science* **12** https://doi.org/10.1002/advs.202415712 | PubMed

39. **Cai M,** Tian F, Han J, et al. (2025) Genetically engineered extracellular vesicles expressing decoy protein TACI provide a therapeutic effect in systemic lupus erythematosus mouse model. *Journal of Controlled Release* **384** https://doi.org/10.1016/j.jconrel.2025.113886 | PubMed

40. **Fu Y,** Xu Z, Wu C, et al. (2025) Genetically modified CD19-targeting IL-15 secreting NK cells for the treatment of systemic lupus erythematosus. *Annals of the Rheumatic Diseases* **84**:1811-1821 https://doi.org/10.1016/j.ard.2025.07.028 | PubMed

41. **Burger MC,** Forster M.-T, Romanski A, et al. (2023) Intracranial injection of natural killer cells engineered with a HER2-targeted chimeric antigen receptor in patients with recurrent glioblastoma. *Neuro-Oncology* **25**:2058-2071 https://doi.org/10.1093/neuonc/noad087 | PubMed

42. **Rana S,** Thomas L, Kirkham AM, et al. (2025) CAR-NK cells to treat patients with cancer: a systematic scoping review of published studies and registered clinical trials. *Cytotherapy* **27**:1179-1189 https://doi.org/10.1016/j.jcyt.2025.06.006 | PubMed

43. **Zhu L,** Kalimuthu S, Gangadaran P, et al. (2017) Exosomes Derived From Natural Killer Cells Exert Therapeutic Effect in Melanoma. *Theranostics* **7**:2732-2745 https://doi.org/10.7150/thno.18752 | PubMed

44. **Klingemann H** (2023) The NK-92 cell line-30 years later: its impact on natural killer cell research and treatment of cancer. *Cytotherapy* **25**:451-457 https://doi.org/10.1016/j.jcyt.2022.12.003 | PubMed

45. **Si C,** Wang Y, Li Y, et al. (2025) Engineered NK92 cell-derived exosomes inhibit ovarian cancer progression by degrading GPRC5A. *Front Immunol* **16** https://doi.org/10.3389/fimmu.2025.1613178 | PubMed

46. **Burger MC,** Forster MT, Romanski A, et al. (2023) Intracranial injection of natural killer cells engineered with a HER2-targeted chimeric antigen receptor in patients with recurrent glioblastoma. *Neuro Oncol* **25**:2058-2071 https://doi.org/10.1093/neuonc/noad087 | PubMed

47. **Murakami Y,** Fukui R, Tanaka R, et al. (2021) Anti-TLR7 Antibody Protects Against Lupus Nephritis in NZBWF1 Mice by Targeting B Cells and Patrolling Monocytes. *Frontiers in Immunology* **12** https://doi.org/10.3389/fimmu.2021.777197 | PubMed

48. **Zhao M,** Li Q, Chai Y, et al. (2025) An anti-CD19-exosome delivery system navigates the blood–brain barrier for targeting of central nervous system lymphoma. *Journal of Nanobiotechnology* **23** https://doi.org/10.1186/s12951-025-03238-9 | PubMed

49. **Askanase A,** Khalili L, Tang W, et al. (2023) New and future therapies: Changes in the therapeutic armamentarium for SLE. *Best Practice & Research Clinical Rheumatology* **37** https://doi.org/10.1016/j.berh.2023.101865 | PubMed

50. **Wang Q,** Xiao ZX, Zheng X, et al. (2025) In Vivo CD19 CAR T-Cell Therapy for Refractory Systemic Lupus Erythematosus. *N Engl J Med* **393**:1542-1544 https://doi.org/10.1056/nejmc2509522 | PubMed

Peer reviews

Reviewer #1 (Public review):

Summary:

This study constructed engineered NK-92 cell extracellular vesicles displaying CD19 single-chain variable fragment and evaluated their therapeutic efficacy in MRL/lpr mouse models of systemic lupus erythematosus, demonstrating that these vesicles could deplete B cells, alleviate lupus nephritis, and improve mouse survival. However, this strategy lacks significant innovation compared to existing research. The current results are not sufficient to provide strong support for the experimental hypotheses.

Weaknesses:

(1) This study proposes using engineered EVs displaying CD19 scFv to target B cells for SLE treatment. However, similar core therapeutic strategies have been reported in previous studies. For instance, recently, studies have reported engineered EVs for SLE therapy (J Control Release. 2025, 384:113886; Ann Rheum Dis. 2025, 84(11):1811-1821; J Nanobiotechnology. 2026, 24(1):203). Another research team from China also constructed engineered EVs displaying anti-CD19 scFv for SLE treatment, which is highly consistent with the present work in targeting strategy, delivery vehicle, and disease model (Mol Ther. 2026:S1525-0016(26)00080-8). Moreover, the human trial of allogeneic CD19-targeted CAR-NK therapy for SLE has been published (Lancet. 2026, 406(10522):2968-2979). This study has not made original improvements in therapeutic vectors, targeting modules, therapeutic mechanisms, and indications, and thus finds it difficult to meet the requirements of high-level journals for originality and novelty.

(2) Numerous core experiments are missing, including the validation of CD19 scFv fusion protein expression on EVs, systematic characterization of engineered EVs, verification of EVs functions and therapeutic mechanisms, and in vitro and in vivo safety assessments. The available data are insufficient to support complete conclusions.

(3) The stable expression of CD19 scFv on EVs should be further verified by Western blot or flow cytometry. The anchoring of CD19 scFv on the outer membrane surface of EVs must be confirmed. In addition, the loading capacity of CD19 scFv on exosomes should be quantified for the dosage selection in SLE treatment.

(4) In vitro experiments are required to confirm the specific targeting ability of CD19 scFv-EVs to B cells and clarify the precise mechanism of B cell depletion, particularly whether it is mediated by effector molecules carried by exosomes such as perforin and granzyme B.

(5) The key quality control parameters, such as the stability, purity, buoyant density, and particle/protein ratio of engineered exosomes, should be characterized and identified.

(6) For the in vivo treatment experiments, the author needs to explain how the treatment dose of CD19scFv-EVs was determined in order to clarify the dose-effect relationship.

(7) It is necessary to supplement with in vivo imaging and tissue distribution data to prove that the CD19 scFv-EVs can specifically accumulate in B-cell organs such as the spleen or lymph nodes.

(8) The author needs to clarify the mechanism by which CD19 scFv-EVs reduce B cells in vivo and verify the caspase apoptosis pathway.

(9) For the in vivo therapeutic experiments, the clinical first-line drugs and the free CD19scFv should be used to supplement the control group to highlight the advantages of the engineered EVs.

(10) Safety assessment in this manuscript is completely absent. Routine toxicity examinations, including hepatic and renal function tests, routine blood tests, and histopathological analysis of major organs in mice, must be supplemented. In addition, the systemic inflammatory cytokine profile and anti-drug antibody levels should be determined to rule out critical safety

risks such as cytokine release syndrome and immunogenicity. The authors only focused on alterations in B cells; the impacts of the treatment on T cell subsets, NK cells, and monocytes/macrophages should be further investigated.

https://doi.org/10.7554/eLife.111440.1.sa3

Reviewer #2 (Public review):

Summary:

Sun and colleagues report the development of an engineered extracellular vesicle platform derived from NK-92 cells that display an anti-CD19 single-chain variable fragment (scFv) on their surface via fusion with LAMP-2B (V-CD19-Exo). In an MRL/lpr mouse model of SLE, the authors demonstrate that intraperitoneal administration of V-CD19-Exo reduces splenic CD19+CD20+ B cells, attenuates proteinuria and lupus nephritis pathology, downregulates pro-inflammatory cytokines (IL-17A, IFN-γ) and autoantibodies (anti-dsDNA, ANA), and improves survival from approximately 25% to 80%. The authors propose that this "cell-free" targeted extracellular vesicle strategy offers advantages over conventional cell therapies, including lower immunogenicity, scalable production, and no requirement for lymphodepletion.

The study addresses an important question in autoimmune disease therapeutics: how to achieve targeted B cell depletion while avoiding the complexities and safety risks associated with CAR-T/CAR-NK cell therapies. The concept is novel, and the initial in vivo efficacy data are encouraging. However, several significant limitations in experimental design, mechanistic depth, and evidence rigor temper the strength of the conclusions.

Strengths:

(1) Novel conceptual approach.

The adaptation of CAR targeting principles to extracellular vesicles represents a creative and potentially impactful strategy. By displaying CD19 scFv on NK-92-derived vesicles, the authors successfully confer B cell-targeting capability while retaining the cytotoxic effector functions of the parental NK cells. This "cell-free" concept addresses genuine limitations of live cell therapies, including the need for lymphodepletion, risks of cytokine release syndrome, and manufacturing complexity.

(2) Comprehensive in vivo efficacy readouts.

The study evaluates therapeutic effects across multiple clinically relevant endpoints: B cell depletion (flow cytometry), renal function (proteinuria, UPCR), renal histopathology (HE staining with semi-quantitative scoring), systemic inflammation (IgE, IL-17A, IFN-γ), autoantibody production (anti-dsDNA, ANA), and survival. This multi-dimensional characterization strengthens the phenotypic evidence for efficacy.

(3) Appropriate control groups.

The inclusion of non-targeted NK92-Exo as a control allows attribution of the observed effects to CD19-mediated targeting rather than non-specific vesicle-associated activities.

(4) Significant survival benefit.

The improvement in survival from 25% to approximately 80% in V-CD19-Exo-treated mice is substantial and represents arguably the most compelling evidence for therapeutic potential in this model.

Weaknesses:

(1) Mechanism of B-cell reduction remains unclear.

The manuscript reports a dramatic reduction in splenic CD19+CD20+ B cells (from 10.53% to 1.51%) following V-CD19-Exo treatment. However, the authors do not establish whether this results from direct cytotoxicity (e.g., perforin/granzyme-mediated killing, apoptosis induction) or from functional suppression/downregulation of CD19 expression. The authors speculate that the effect is likely mediated by cytotoxic proteins carried by NK-92-derived vesicles, but no data are provided to support this mechanism. Essential experiments would include the detection of apoptosis markers (Annexin V, activated caspase-3/7) in B cells, assessment of perforin/granzyme B content within V-CD19-Exo, or in vitro co-culture assays demonstrating direct B cell killing.

(2) Small sample sizes.

Most experimental endpoints were assessed with n=5 per group, which is marginal for detecting modest effect sizes and may amplify the influence of individual biological variation. While the survival study had n=10 per group, the main mechanistic and endpoint analyses would benefit from larger cohorts (n=8-10) to increase statistical power and robustness.

(3) No dose-response or dosing optimization studies.

All experiments used a single dose (10^9 particles per injection) and a fixed schedule (twice weekly for three weeks). The absence of dose-response data leaves unclear whether the observed effects represent maximal efficacy or could be achieved with lower doses, and whether alternative dosing regimens could improve outcomes or reduce potential off-target effects.

(4) Lack of safety assessment.

The authors emphasize the theoretical safety advantages of extracellular vesicles over cell therapies, but no systematic safety evaluation is presented. Key missing data include: histopathological examination of non-target organs (liver, lung, heart, gastrointestinal tract), assessment of off-target immune activation (T cell responses, cytokine profiles beyond those measured), and evaluation of potential accumulation or toxicity with repeated dosing.

(5) Incomplete characterization of the engineered vesicles beyond targeting.

While the manuscript successfully demonstrates CD19scFv display and vesicle enrichment of exosomal markers, it does not characterize whether V-CD19-Exo retains the full spectrum of NK-92 effector molecules (perforin, granzymes, FasL, TRAIL, cytokines such as IFN-γ) at functional levels. Quantitative or semi-quantitative comparison of cargo between V-CD19-Exo and parental NK-92 cells or non-engineered NK92-Exo would help contextualize the observed in vivo effects.

(6) Sex as a biological variable is not systematically addressed.

The authors note in the Discussion that the same treatment showed more significant efficacy in male mice compared to females (data not shown), yet all main experiments were conducted exclusively in female mice. Given the strong sex bias in SLE epidemiology (approximately 9:1 female-to-male ratio) and potential differences in immune responses between sexes, this observation warrants systematic investigation rather than a footnote. Presenting the sex-differential data or alternatively, conducting adequately powered sex-stratified analyses would substantially strengthen the manuscript.

(7) Translational claims are premature.

The manuscript repeatedly emphasizes advantages over cell therapy (low immunogenicity, scalable production, no requirement for lymphodepletion) as if these are established

properties of V-CD19-Exo. However, no experiments directly compare V-CD19-Exo to CAR-NK or CAR-T cells in terms of efficacy, immunogenicity, or safety. Similarly, claims of "scalable production" and "high batch-to-batch consistency" are not supported by any manufacturing or quality control data. These statements should be toned down or supported with empirical evidence.

https://doi.org/10.7554/eLife.111440.1.sa2

Reviewer #3 (Public review):

Summary:

This manuscript describes the development of engineered NK-92-derived extracellular vesicles (EVs) displaying CD19scFv for targeted treatment of systemic lupus erythematosus (SLE). Using a CD19scFv-LAMP2B fusion strategy, the authors generated EVs intended to selectively target pathogenic B cells in the MRL/lpr lupus mouse model. The study reports reductions in $CD19^{+}CD20^{+}$ B-cell populations, improvements in proteinuria and renal histopathology, decreased inflammatory cytokines and autoantibody levels, reduced splenomegaly, and improved survival outcomes following treatment. The work aims to position engineered EVs as a cell-free alternative to CAR-T/CAR-NK therapies for autoimmune disease treatment. While the concept is interesting and potentially translational, the study currently lacks sufficient methodological rigor, EV purification standards, mechanistic validation, and comprehensive characterization to fully support many of the claims presented.

Strengths:

(1) The study addresses an important unmet clinical need in systemic lupus erythematosus and explores an innovative cell-free therapeutic strategy.

(2) The concept of combining CAR-like targeting approaches with engineered EVs is interesting and potentially translational.

(3) The manuscript includes both in vitro and in vivo experiments, including functional renal assessments, immune profiling, histopathology, and survival studies.

(4) The authors attempt to evaluate multiple disease-associated readouts, including proteinuria, cytokines, autoantibodies, splenomegaly, and survival outcomes, which strengthens the overall biological relevance of the work.

(5) The use of engineered NK92-derived vesicles as a scalable alternative to CAR-NK therapy represents a potentially attractive therapeutic platform.

(6) The in vivo therapeutic observations in the MRL/lpr lupus model are encouraging and warrant further mechanistic investigation.

Weaknesses:

(1) The EV isolation strategy is not sufficiently rigorous for defining the isolated particles as "exosomes" according to current International Society for Extracellular Vesicles/MISEV guidelines. The precipitation-based workflow without density gradient purification or SEC raises major concerns regarding EV purity and identity.

(2) No direct validation was provided demonstrating successful surface localization or functional accessibility of CD19scFv on EV membranes.

(3) The characterization of EVs is incomplete and insufficient. Additional positive/negative EV markers, purity metrics, and orthogonal characterization methods are required.

(4) The absence of density gradient ultracentrifugation is particularly concerning, given the systemic injection of EV preparations into mice, as contaminating soluble factors and non-vesicular particles may contribute to the observed therapeutic effects.

(5) The manuscript lacks adequate mechanistic studies explaining how engineered EVs mediate B-cell depletion or immune modulation.

(6) The in vitro functional assays are weakly designed, particularly the use of A549 cells for evaluating CD19-targeted vesicle function.

(7) Important methodological details are missing, including EV normalization strategies, flow cytometry gating controls, blinding procedures, and randomization approaches.

(8) Several figures, particularly TEM and western blot images, are of low quality and difficult to interpret.

(9) The study does not sufficiently exclude the possibility that observed therapeutic effects result from contaminating soluble immune mediators rather than EV-specific activity.

(10) Broader immune profiling is lacking despite the systemic immune complexity of SLE.

(11) The statistical analysis section includes tests that are not reflected in the Results section, creating concerns regarding data presentation and consistency.

(12) Overall, while the concept is interesting, the manuscript currently falls short of the experimental rigor expected for high-impact translational EV studies.

https://doi.org/10.7554/eLife.111440.1.sa1

Author response:

Public Reviews:

Reviewer #1 (Public review):

Summary:

This study constructed engineered NK-92 cell extracellular vesicles displaying CD19 single-chain variable fragment and evaluated their therapeutic efficacy in MRL/lpr mouse models of systemic lupus erythematosus, demonstrating that these vesicles could deplete B cells, alleviate lupus nephritis, and improve mouse survival. However, this strategy lacks significant innovation compared to existing research. The current results are not sufficient to provide strong support for the experimental hypotheses.

Weaknesses:

(1) This study proposes using engineered EVs displaying CD19 scFv to target B cells for SLE treatment. However, similar core therapeutic strategies have been reported in previous studies. For instance, recently, studies have reported engineered EVs for SLE therapy (J Control Release. 2025, 384:113886; Ann Rheum Dis. 2025, 84(11):1811-1821; J Nanobiotechnology. 2026, 24(1):203). Another research team from China also constructed engineered EVs displaying anti-CD19 scFv for SLE treatment, which is highly consistent with the present work in targeting strategy, delivery vehicle, and disease model (Mol Ther. 2026:S1525-0016(26)00080-8). Moreover, the human trial of allogeneic CD19-targeted CAR-NK therapy for SLE has been published (Lancet. 2026, 406(10522):2968-2979). This study has not made original improvements in therapeutic vectors, targeting modules, therapeutic mechanisms, and indications, and thus finds it difficult to meet the requirements of high-level journals for originality and novelty.

> *J Control Release. 2025, 384:113886; Ann Rheum Dis. 2025, 84(11):1811-1821; J Nanobiotechnology. 2026, 24(1):203). Another research team from China also constructed engineered EVs displaying anti-CD19 scFv for SLE treatment, which is highly consistent with the present work in targeting strategy, delivery vehicle, and disease model (Mol Ther. 2026:S1525-0016(26)00080-8). Moreover, the human trial of allogeneic CD19-targeted CAR-NK therapy for SLE has been published (Lancet. 2026, 406(10522):2968-2979).*

Reviewer 1 mentioned 4 publications

(1) J Control Release. 2025, 384:113886; Genetically engineered extracellular vesicles expressing decoy protein TACI provide a therapeutic effect in systemic lupus erythematosus mouse model

(2) Ann Rheum Dis. 2025, 84(11):1811-1821; J Nanobiotechnology. 2026, 24(1):203） Genetically modified CD19-targeting IL-15 secreting NK cells for the treatment of systemic lupus erythematosus. –but not Evs

(3) Lancet. 2026, 406(10522):2968-2979） Efficacy and safety of allogeneic CD19 CAR NK-cell therapy in systemic lupus erythematosus: a case series in China。

(4) Anti-CD19 engineered exosomes enable B-cell targeted anti-BAFF mRNA delivery to alleviate lupus progression”,

We sincerely thank the reviewers for their valuable and constructive feedback. We fully acknowledge the important contributions made by the publications cited, and we respectfully submit that they do not invalidate our findings. A critical point to emphasize is that our study employed engineered NK-92 cell extracellular vesicles (EVs) not the cells themselves and we would like to respectfully reiterate the fundamental differences between whole cells and non-cellular EVs, particularly in terms of safety and efficiency profiles. Our safety hypothesis is further supported by the clinical use of inactivated NK-92 cells (as demonstrated in this study: [URL]), which we believe provides a strong and relevant precedent. We are also very grateful that the originality and novelty of our approach have been favorably recognized by Reviewers 2 and 3, which we take as an encouraging validation of our work.

> *(2) Numerous core experiments are missing, including the validation of CD19 scFv fusion protein expression on EVs, systematic characterization of engineered EVs, verification of EVs functions and therapeutic mechanisms, and in vitro and in vivo safety assessments. The available data are insufficient to support complete conclusions.*
>
> *(3) The stable expression of CD19 scFv on EVs should be further verified by Western blot or flow cytometry. The anchoring of CD19 scFv on the outer membrane surface of EVs must be confirmed. In addition, the loading capacity of CD19 scFv on exosomes should be quantified for the dosage selection in SLE treatment.*

We sincerely thank the reviewers for raising these important points. We note that points (2) and (3) address essentially the same concern, and we fully agree that further validation of CD19 scFv fusion protein expression on EVs is necessary. We are pleased to confirm that we will present additional data on this in due course. Furthermore, we respectfully acknowledge that several other aspects—including the EVs' functions, therapeutic mechanisms, *in vitro* and *in vivo* safety profiles, and CD19 scFv loading capacity—remain to be thoroughly investigated. We are committed to addressing these important questions in our follow-up studies, and we hope to provide more comprehensive insights in future work.

> *(4) In vitro experiments are required to confirm the specific targeting ability of CD19 scFv-EVs to B cells and clarify the precise mechanism of B cell depletion, particularly*

> *whether it is mediated by effector molecules carried by exosomes such as perforin and granzyme B.*

We are most grateful to the reviewer for raising this important point. We are happy to report that we have successfully obtained data demonstrating the specific targeting of CD19 scFv-EVs to B cells, and we will be pleased to include these findings in our revision. With regard to the mechanism of action, we respectfully acknowledge that perforin and granzyme B are recognized as key mediators of NK cell targeting. Nevertheless, we are not aware of any published evidence to date that supports the presence of this same machinery in NK exosomes. We consider this a valuable question for future exploration, and while it lies beyond the scope of the current work, we are diligently investigating it in related ongoing studies.

> *(5) The key quality control parameters, such as the stability, purity, buoyant density, and particle/protein ratio of engineered exosomes, should be characterized and identified.*

Agreed, We will provide additional characterization data for the engineered EVs in our revision.

> *(6) For the in vivo treatment experiments, the author needs to explain how the treatment dose of CD19scFv-EVs was determined in order to clarify the dose-effect relationship.*

We sincerely thank the reviewer for this valuable suggestion. We fully agree and will be happy to revise the dose calculation accordingly in the updated manuscript.

> *(7) It is necessary to supplement with in vivo imaging and tissue distribution data to prove that the CD19 scFv-EVs can specifically accumulate in B-cell organs such as the spleen or lymph nodes.*

We sincerely thank the reviewer for this valuable suggestion. We fully acknowledge that this is a challenging experiment for several reasons: (1) EV internalization is a rapid process and is therefore difficult to capture; and (2) currently, there is no reliable method available for labeling EVs. Nevertheless, we respectfully assure the reviewer that we will make every effort to attempt this experiment and will report our findings in due course.

> *(8) The author needs to clarify the mechanism by which CD19 scFv-EVs reduce B cells in vivo and verify the caspase apoptosis pathway.*

We sincerely thank the reviewer for these valuable comments. We are pleased to confirm that we have successfully demonstrated the specific targeting ability of CD19 scFv-EVs to B cells, and we will gladly incorporate these results in our revised manuscript.

Regarding the mechanism of action, we fully acknowledge that perforin and granzyme B are well-established mediators of NK cell targeting according to textbook knowledge. However, to the best of our knowledge, there is currently no evidence indicating that NK-derived exosomes are equipped with the same machinery. We respectfully recognize that this is an interesting and important question; while it lies beyond the scope of the present study, we are actively pursuing it in our ongoing parallel work.

We also appreciate the reviewer's comment regarding the apoptosis pathway. We respectfully note that this aspect was not assessed in any of the publications mentioned by Reviewer 1, which suggests that such analysis may be considered optional rather than mandatory. Nevertheless, we fully agree that this is a worthwhile avenue for further investigation, and we are committed to exploring it in our future studies."

> *(9) For the in vivo therapeutic experiments, the clinical first-line drugs and the free CD19scFv should be used to supplement the control group to highlight the advantages of the engineered EVs.*

We sincerely thank the reviewer for this thoughtful and constructive advice. We fully agree that if we were developing this approach for clinical trials, regulatory agencies such as the FDA would require it to demonstrate superiority over current first-line clinical drugs. However, we respectfully wish to clarify that the primary objective of the present study is to provide a proof-of-concept that this strategy is feasible. We fully acknowledge that efficacy and safety will need to be investigated more intensively in future studies before any clinical translation can be considered. We are grateful for this valuable perspective and will be sure to discuss these considerations more explicitly in the revised manuscript.

> *(10) Safety assessment in this manuscript is completely absent. Routine toxicity examinations, including hepatic and renal function tests, routine blood tests, and histopathological analysis of major organs in mice, must be supplemented. In addition, the systemic inflammatory cytokine profile and anti-drug antibody levels should be determined to rule out critical safety risks such as cytokine release syndrome and immunogenicity. The authors only focused on alterations in B cells; the impacts of the treatment on T cell subsets, NK cells, and monocytes/macrophages should be further investigated.*

We sincerely thank the reviewer for this valuable advice. We fully agree and will be happy to provide additional data to address this point in our revised manuscript.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *Sun and colleagues report the development of an engineered extracellular vesicle platform derived from NK-92 cells that display an anti-CD19 single-chain variable fragment (scFv) on their surface via fusion with LAMP-2B (V-CD19-Exo). In an MRL/lpr mouse model of SLE, the authors demonstrate that intraperitoneal administration of V-CD19-Exo reduces splenic CD19+CD20+ B cells, attenuates proteinuria and lupus nephritis pathology, downregulates pro-inflammatory cytokines (IL-17A, IFN-γ) and autoantibodies (anti-dsDNA, ANA), and improves survival from approximately 25% to 80%. The authors propose that this "cell-free" targeted extracellular vesicle strategy offers advantages over conventional cell therapies, including lower immunogenicity, scalable production, and no requirement for lymphodepletion.*
>
> *The study addresses an important question in autoimmune disease therapeutics: how to achieve targeted B cell depletion while avoiding the complexities and safety risks associated with CAR-T/CAR-NK cell therapies. The concept is novel, and the initial in vivo efficacy data are encouraging. However, several significant limitations in experimental design, mechanistic depth, and evidence rigor temper the strength of the conclusions.*
>
> *Strengths:*
>
> *(1) Novel conceptual approach.*
>
> *The adaptation of CAR targeting principles to extracellular vesicles represents a creative and potentially impactful strategy. By displaying CD19 scFv on NK-92-derived vesicles, the authors successfully confer B cell-targeting capability while retaining the cytotoxic effector functions of the parental NK cells. This "cell-free" concept addresses genuine limitations of live cell therapies, including the need for lymphodepletion, risks of cytokine release syndrome, and manufacturing complexity.*

> *(2) Comprehensive in vivo efficacy readouts.*
>
> *The study evaluates therapeutic effects across multiple clinically relevant endpoints: B cell depletion (flow cytometry), renal function (proteinuria, UPCR), renal histopathology (HE staining with semi-quantitative scoring), systemic inflammation (IgE, IL-17A, IFN-γ), autoantibody production (anti-dsDNA, ANA), and survival. This multi-dimensional characterization strengthens the phenotypic evidence for efficacy.*
>
> *(3) Appropriate control groups.*
>
> *The inclusion of non-targeted NK92-Exo as a control allows attribution of the observed effects to CD19-mediated targeting rather than non-specific vesicle-associated activities.*
>
> *(4) Significant survival benefit.*
>
> *The improvement in survival from 25% to approximately 80% in V-CD19-Exo-treated mice is substantial and represents arguably the most compelling evidence for therapeutic potential in this model.*
>
> *Weaknesses:*
>
> *(1) Mechanism of B-cell reduction remains unclear.*
>
> *The manuscript reports a dramatic reduction in splenic CD19+CD20+ B cells (from 10.53% to 1.51%) following V-CD19-Exo treatment. However, the authors do not establish whether this results from direct cytotoxicity (e.g., perforin/granzyme-mediated killing, apoptosis induction) or from functional suppression/downregulation of CD19 expression. The authors speculate that the effect is likely mediated by cytotoxic proteins carried by NK-92-derived vesicles, but no data are provided to support this mechanism. Essential experiments would include the detection of apoptosis markers (Annexin V, activated caspase-3/7) in B cells, assessment of perforin/granzyme B content within V-CD19-Exo, or in vitro co-culture assays demonstrating direct B cell killing.*

We sincerely thank the reviewer for raising this excellent question. We fully agree that it is an important point that truly needs to be addressed. We are pleased to confirm that we have already begun investigating this and hope to obtain meaningful results in due course.

> *(2) Small sample sizes.*
>
> *Most experimental endpoints were assessed with n=5 per group, which is marginal for detecting modest effect sizes and may amplify the influence of individual biological variation. While the survival study had n=10 per group, the main mechanistic and endpoint analyses would benefit from larger cohorts (n=8-10) to increase statistical power and robustness.*

We are most grateful to the reviewer for this thoughtful and constructive comment. We completely agree that the sample size in our current analysis is somewhat limited for robust statistical evaluation. We are pleased to report that we have since collected additional data, which we will incorporate into our revised manuscript to strengthen the statistical power. If further data become available, we will gladly update them in subsequent revisions.

> *(3) No dose-response or dosing optimization studies.*

> *All experiments used a single dose (10^9 particles per injection) and a fixed schedule (twice weekly for three weeks). The absence of dose-response data leaves unclear whether the observed effects represent maximal efficacy or could be achieved with lower doses, and whether alternative dosing regimens could improve outcomes or reduce potential off-target effects.*

We appreciate the reviewer's thoughtful and important question. We completely agree that this needs to be addressed, and we have already started working on it. We will be pleased to update our data in later comments once further results are obtained.

> *(4) Lack of safety assessment.*
>
> *The authors emphasize the theoretical safety advantages of extracellular vesicles over cell therapies, but no systematic safety evaluation is presented. Key missing data include: histopathological examination of non-target organs (liver, lung, heart, gastrointestinal tract), assessment of off-target immune activation (T cell responses, cytokine profiles beyond those measured), and evaluation of potential accumulation or toxicity with repeated dosing.*

We appreciate the reviewer's careful and important observations. We fully agree that a systematic safety assessment is necessary.We are actively conducting these experiments and will update our manuscript with the findings as soon as possible.

> *(5) Incomplete characterization of the engineered vesicles beyond targeting.*
>
> *While the manuscript successfully demonstrates CD19scFv display and vesicle enrichment of exosomal markers, it does not characterize whether V-CD19-Exo retains the full spectrum of NK-92 effector molecules (perforin, granzymes, FasL, TRAIL, cytokines such as IFN-γ) at functional levels. Quantitative or semi-quantitative comparison of cargo between V-CD19-Exo and parental NK-92 cells or non-engineered NK92-Exo would help contextualize the observed in vivo effects.*

We thank the reviewer for this valuable comment. We fully agree that further characterization of the engineered vesicles including NK-92 effector molecules and cargo comparison is needed. We are actively working on this and will update the manuscript as soon as the data become available.

> *(6) Sex as a biological variable is not systematically addressed.*
>
> *The authors note in the Discussion that the same treatment showed more significant efficacy in male mice compared to females (data not shown), yet all main experiments were conducted exclusively in female mice. Given the strong sex bias in SLE epidemiology (approximately 9:1 female-to-male ratio) and potential differences in immune responses between sexes, this observation warrants systematic investigation rather than a footnote. Presenting the sex-differential data or alternatively, conducting adequately powered sex-stratified analyses would substantially strengthen the manuscript.*

We appreciate the reviewer's important comment. We agree that sex is a relevant biological variable, but a systematic analysis is beyond the current scope. We will consider this for future studies and will acknowledge this limitation in the Discussion.

> *(7) Translational claims are premature.*
>
> *The manuscript repeatedly emphasizes advantages over cell therapy (low immunogenicity, scalable production, no requirement for lymphodepletion) as if these are established properties of V-CD19-Exo. However, no experiments directly compare V-CD19-Exo to CAR-NK or CAR-T cells in terms of efficacy, immunogenicity, or safety.*

> *Similarly, claims of "scalable production" and "high batch-to-batch consistency" are not supported by any manufacturing or quality control data. These statements should be toned down or supported with empirical evidence.*

We thank the reviewer for this important observation. We fully agree that our therapeutic claims are premature without direct comparative and manufacturing data. We will revise the manuscript to temper these statements and present them as potential advantages that warrant future investigation.

> ***Reviewer #3 (Public review):***
>
> *Summary:*
>
> *This manuscript describes the development of engineered NK-92-derived extracellular vesicles (EVs) displaying CD19scFv for targeted treatment of systemic lupus erythematosus (SLE). Using a CD19scFv-LAMP2B fusion strategy, the authors generated EVs intended to selectively target pathogenic B cells in the MRL/lpr lupus mouse model. The study reports reductions in $CD19^{+}CD20^{+}$ B-cell populations, improvements in proteinuria and renal histopathology, decreased inflammatory cytokines and autoantibody levels, reduced splenomegaly, and improved survival outcomes following treatment. The work aims to position engineered EVs as a cell-free alternative to CAR-T/CAR-NK therapies for autoimmune disease treatment. While the concept is interesting and potentially translational, the study currently lacks sufficient methodological rigor, EV purification standards, mechanistic validation, and comprehensive characterization to fully support many of the claims presented.*
>
> *Strengths:*
>
> *(1) The study addresses an important unmet clinical need in systemic lupus erythematosus and explores an innovative cell-free therapeutic strategy.*
>
> *(2) The concept of combining CAR-like targeting approaches with engineered EVs is interesting and potentially translational.*
>
> *(3) The manuscript includes both in vitro and in vivo experiments, including functional renal assessments, immune profiling, histopathology, and survival studies.*
>
> *(4) The authors attempt to evaluate multiple disease-associated readouts, including proteinuria, cytokines, autoantibodies, splenomegaly, and survival outcomes, which strengthens the overall biological relevance of the work.*
>
> *(5) The use of engineered NK92-derived vesicles as a scalable alternative to CAR-NK therapy represents a potentially attractive therapeutic platform.*
>
> *(6) The in vivo therapeutic observations in the MRL/lpr lupus model are encouraging and warrant further mechanistic investigation.*
>
> *Weaknesses:*
>
> *(1) The EV isolation strategy is not sufficiently rigorous for defining the isolated particles as "exosomes" according to current International Society for Extracellular Vesicles/MISEV guidelines. The precipitation-based workflow without density gradient purification or SEC raises major concerns regarding EV purity and identity.*

We thank the reviewer for this valuable and timely comment. We fully agree that our precipitation-based isolation does not meet MISEV guidelines for defining particles specifically as 'exosomes.' Since our characterization is based on shape, protein markers, and

size, we will replace 'exosome' with 'extracellular vesicles' throughout the manuscript to more accurately reflect our methodology.

> *(2) No direct validation was provided demonstrating successful surface localization or functional accessibility of CD19scFv on EV membranes.*

We thank the reviewer for this valuable point. We agree, and we are happy to confirm that we have obtained data on surface localization and functional accessibility of CD19 scFv, which we will include in the revision.

> *(3) The characterization of EVs is incomplete and insufficient. Additional positive/negative EV markers, purity metrics, and orthogonal characterization methods are required.*

We thank the reviewer for this important point. We fully agree that more comprehensive EV characterization is needed. We are pleased to confirm that we have obtained data on CD19 scFv surface localization and accessibility, which we will include in the revision. We also acknowledge the need for additional markers and purity metrics, and will address this as a limitation in the Discussion.

> *(4) The absence of density gradient ultracentrifugation is particularly concerning, given the systemic injection of EV preparations into mice, as contaminating soluble factors and non-vesicular particles may contribute to the observed therapeutic effects.*

We sincerely thank the reviewer for raising this important technical concern. We fully agree that density gradient ultracentrifugation is a more rigorous method for EV purification and that contaminating soluble factors or non-vesicular particles cannot be completely ruled out in our current preparation. We also acknowledge that even with gradient ultracentrifugation, absolute purity is not guaranteed. Nevertheless, we respectfully note that the therapeutic effect of CD19 scFv from EVs was evident when compared to appropriate controls, suggesting that the observed efficacy is attributable at least in part to the EVs themselves. We will add a clear statement of this limitation in the Discussion and will consider more stringent purification methods in our future studies.

> *(5) The manuscript lacks adequate mechanistic studies explaining how engineered EVs mediate B-cell depletion or immune modulation.*

We thank the reviewer for this important point. We agree that mechanistic studies would be valuable, but we respectfully note that our current paper focuses on establishing a proof-of-concept. We plan to investigate the mechanisms of B-cell reduction and immune modulation in our future work.

> *(6) The in vitro functional assays are weakly designed, particularly the use of A549 cells for evaluating CD19-targeted vesicle function.*

We thank the reviewer for this comment. We wish to clarify that the A549 experiment was intended to confirm that the engineered EVs retain their native function, not to validate CD19 targeting (which will be addressed in point (2). We will revise the manuscript to make this distinction clearer.

> *(7) Important methodological details are missing, including EV normalization strategies, flow cytometry gating controls, blinding procedures, and randomization approaches.*

We thank the reviewer for this important observation. We agree that several methodological details were missing. We will reorganize and expand the Methods section to include EV normalization, flow cytometry gating controls, blinding, and randomization procedures.

> *(8) Several figures, particularly TEM and western blot images, are of low quality and difficult to interpret.*

We thank the reviewer for this comment. We agree that the TEM and Western blot images are of low quality. We will provide improved, higher-resolution images in the revision

> *(9) The study does not sufficiently exclude the possibility that observed therapeutic effects result from contaminating soluble immune mediators rather than EV-specific activity.*

We appreciate this concern. Based on our data, we believe the effects are EV-specific. We will acknowledge this limitation and plan additional controls in future work.

> *(10) Broader immune profiling is lacking despite the systemic immune complexity of SLE.*

We thank the reviewer for this important point. We agree that broader immune profiling would be valuable, especially for clinical translation. However, our current study is designed as a proof-of-concept to establish feasibility. We will acknowledge this limitation in the Discussion and plan to address immune profiling in our future work.

> *(11) The statistical analysis section includes tests that are not reflected in the Results section, creating concerns regarding data presentation and consistency.*

We thank the reviewer for pointing this out. We agree that the statistical tests in the Methods do not match those in the Results. We will revise both sections to ensure consistency throughout.

> *(12) Overall, while the concept is interesting, the manuscript currently falls short of the experimental rigor expected for high-impact translational EV studies.*

We sincerely thank the reviewer for this thoughtful comment. We fully agree that this is a very early-stage translational study, and we acknowledge that considerable work remains before any clinical application can be envisioned. Nevertheless, we respectfully believe that our findings provide a valuable conceptual framework and an initial proof-of-concept that may inform and guide future translational development."

https://doi.org/10.7554/eLife.111440.1.sa0