(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
09 July 2026 (09.07.2026)

WIPO | PCT

(10) International Publication Number
WO 2026/143319 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *C07K 16/28* (2006.01)
C07K 14/725 (2006.01) *C12N 5/10* (2006.01)

(21) International Application Number:
PCT/CN2024/143635

(22) International Filing Date:
30 December 2024 (30.12.2024)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant: **STARNA THERAPEUTICS** [CN/CN]; 201, Building 2, No. 99, Jingu Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123 (CN).

(72) Inventors: **HU, Rongkuan**; 201, Building 2, No. 99, Jingu Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123 (CN). **YU, Xueliang**; 201, Building 2, No. 99, Jingu Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123 (CN). **REN, Zhaoxiang**; 201, Building 2, No. 99, Jingu Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123 (CN). **SUN, Yimeng**; 201, Building 2, No. 99, Jingu Road, Suzhou Industrial Park, Suzhou, Jiangsu 215123 (CN).

(74) Agent: **CHINA SINDA INTELLECTUAL PROPERTY LIMITED**; 11/F, Tower B, Focus Place, 19 Financial Street, Xicheng District, Beijing 100033 (CN).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: IN VIVO CAR-T THERAPY COMPRISING TARGETING DELIVERY OF RNA BY A NOVEL ANTIBODY-CONJUGATED LNP

(57) Abstract: T-cell targeting antibody-conjugated lipid nanoparticle (LNP) and uses thereof.

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In vivo CAR-T therapy comprising targeting delivery of RNA by a novel antibody-conjugated LNP

Technical Field

The present invention belongs to the pharmaceutical field, and in particular relates to a T-cell targeting antibody-conjugated LNP.

Background Art

Chimeric antigen receptor (CAR) T cell therapy is a type of adoptive cell therapy that uses synthetic receptors to specifically target antigens, and has demonstrated excellent tumor removal effect in the clinical treatment of hematological tumors, especially B-cell lymphomas. Currently, CAR-T therapy has the best efficacy and is the most widely used in the treatment of acute lymphoblastic leukemia (ALL). Among them, CD19-targeting CAR has achieved significant efficacy in the treatment of adult and pediatric ALL. It has been reported in the literature that the complete response rate of CD19 CAR-T in the treatment of relapsed/refractory ALL can be as high as 90%. Therefore, in just a few years, many CAR-T therapies have been approved for marketing worldwide.

CAR-T can be divided into two preparation forms: autologous or allogeneic. The currently approved CAR-T therapies are all autologous CAR-T. CAR-T is a method that collects and isolates T cells from the peripheral blood of a patient, and uses viral or non-viral vectors to transduce a sequence encoding a single-chain fragment variable (scFv) that can recognize specific antigens and an intracellular signaling domain related to activation into isolated T cells, thereby conferring T cells the ability to recognize and kill tumor cells expressing corresponding antigens. For example, currently the most studied method is to specifically kill

CD19-positive tumors through CD19 CAR-T cells. In addition, there are many targets being explored in clinical and preclinical settings, such as BCMA, PSMA, etc.

In addition to exploring tumor treatment, the application of CAR-T in the fields of autoimmune diseases and fibrotic diseases is also actively explored, with CD19, CD20 or FAP CAR-T as the main research targets respectively. Although CAR-T therapy has demonstrated excellent anti-tumor effects in the clinical treatment of hematomas, there are still many shortcomings that limit the further development of CAR-T. 1. At present, CAR-T is predominantly prepared using the autologous method. It involves preparing personalized CAR-T cells after blood collection for subsequent treatment for each patient. This preparation method is highly individualized, complex and time-consuming; resulting in elevated treatment expenses and only a few patients can afford it. 2. CAR-T treatment has serious side effects, such as cytokine release syndrome (CRS), neurotoxicity, etc. 3. Secondary T lymphoma caused by CAR-T therapy has also received great attention, mainly caused by DNA integration into host T cells. 4. Prior to CAR-T therapy, patients must undergo lymphodepletion treatment, which greatly decreases patient tolerance and raises the susceptibility to infections. 5. In addition to the above mentioned, the therapeutic effect of CAR-T in solid tumors is very limited, which may be related to the difficulty of CAR-T cells infiltrating deep into solid tumors and being effectively activated.

Therefore, a novel CAR-T therapy with better versatility, simpler preparation method, higher safety, more convenient administration and no need for lymphodepletion treatment is the main direction of next-generation research and development.

Summary of the Invention

In one aspect, the present invention provides an antibody specifically binding to a cell surface antigen of T cells, wherein the cell surface antigen of T cells is selected from CD3, CD5, CD7, CD8, or CD2.

In certain embodiments, the present antibody specifically binds to CD2, preferably human CD2. In certain embodiments, VH of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 5, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 5. In certain embodiments, the VL of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 7, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 7. In certain embodiments, heavy chain of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 2, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 2. In certain embodiments, light chain of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 4, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 4.

In certain embodiments, the present antibody specifically binds to CD5, preferably human CD5. In certain embodiments, VH of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 20, SEQ ID NO: 21 or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 20, SEQ ID NO: 21. In certain embodiments, the VL of the present antibody comprises an

amino acid sequence set forth in SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 18 or SEQ ID NO: 19, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 18 or SEQ ID NO: 19.

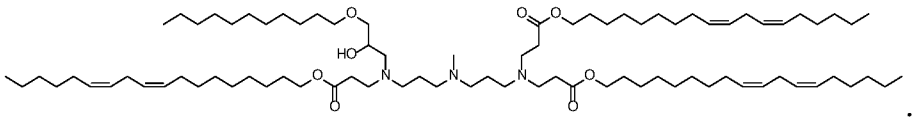
In certain embodiments, the present antibody specifically binds to CD7, preferably human CD7. In certain embodiments, heavy chain of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 24, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 24. In certain embodiments, light chain of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 26, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 26.

In certain embodiments, the present antibody specifically binds to CD8, preferably human CD8. In certain embodiments, VH of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, or SEQ ID NO: 50, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37 or SEQ ID NO: 50. In certain embodiments, the VL of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, or SEQ ID NO: 51 or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34,

SEQ ID NO: 36, or SEQ ID NO: 51.

In certain embodiments, CH of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 6 or SEQ ID NO: 22, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 6 or SEQ ID NO: 22. In certain embodiments, the CL of the present antibody comprises an amino acid sequence set forth in SEQ ID NO: 8, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 8.

In one aspect, the present invention provides a lipid nanoparticle (LNP), wherein the LNP comprises an ionizable lipid of the following structure,



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In one aspect, the present invention provides a lipid nanoparticle (LNP), wherein the LNP comprises an ionizable lipid, a phospholipid, a steroid, and a PEGylated lipid, wherein the molar ratio of ionizable lipid : phospholipid : steroid : PEGylated lipid is a : b : c : d,

wherein a+b+c+d=100,

wherein a is in the range of 40.0-55.0, preferably a is 40.0, 41.0, 42.0, 43.0, 44.0, 45.0, 45.1, 45.2, 45.3, 45.4, 45.5, 45.6, 45.7, 45.8, 45.9, 46.0, 46.1, 46.2, 46.3, 46.4, 46.5, 46.6, 46.7, 46.8, 46.9, 47.0, 48.0, 49.0, 49.1, 49.2, 49.3, 49.4, 49.5, 49.6, 49.7, 49.8, 49.9, 50.0, 50.1, 50.2, 50.3, 50.4, 50.5, 50.6, 50.7, 50.8, 50.9, 51.0, 52.0, 53.0, 54.0, or 55.0;

wherein b is in the range of 5.0-15.0, preferably b is 5.0, 6.0, 7.0, 8.0, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, 10.9, 11.0, 12.0, 13.0, 14.0, or 15.0

wherein c is in the range of 35.0-50.0, preferably c is 35.0, 36.0, 37.0, 37.5, 37.6, 37.7, 37.8, 37.9, 38.0, 38.1, 38.2, 38.3, 38.4, 38.5, 38.6, 38.7, 38.8, 38.9, 39.0, 39.1, 39.2, 39.3, 39.4, 39.5, 40.0, 41.0, 41.1, 41.2, 41.3, 41.4, 41.5, 41.6, 41.7, 41.8, 41.9, 42.0, 42.1, 42.2, 42.3, 42.4, 42.5, 42.6, 42.7, 42.8, 42.9, 43.0, 43.1, 43.2, 43.3, 43.4, 43.5, 43.6, 43.7, 43.8, 43.9, 44.0, 45.0, 46.0, 47.0, 48.0, 49.0, or 50.0; and/or

wherein d is in the range of 0.1 to 3.0, preferably d is 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3.0.

In certain embodiments, the present phospholipid is DSPC, DOPE, DLPC, DSPE, DMPC, DOPC, DPPC, DUPC, or POPC. In certain embodiments, the present steroid is cholesterol or its derivatives, ergosterol, lanosterol, stigmasterol, sitosterol, or any mixtures thereof. In certain embodiments, the present PEGylated lipid is PEG-modified phosphatidylethanolamine, PEG-modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG-modified diacylglycerol, or any mixtures thereof.

In certain embodiments, one or more PEGylated lipid contained in the present LNP is functionalized with a first coupling group, wherein the molar ratio of PEGylated lipid functionalized with a first coupling group relative to the total lipids is in the range of 0.001%-1.000%, preferably 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.010%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, more preferably in the range of 0.02-0.04%, or 0.004%-0.006%.

In certain embodiments, the first coupling group is selected from the

group consisting of maleimides, N-hydroxysuccinimide (NHS) esters, carbodiimides, hydrazide, pentafluorophenyl (PFP) esters, phosphines, hydroxymethyl phosphines, psoralen, imidoesters, pyridyl disulfide, isocyanates, vinyl sulfones, alpha-haloacetyls, aryl azides, acyl azides, alkyl azides, diazirines, benzophenone, epoxides, carbonates, anhydrides, sulfonyl chlorides, cyclooctynes, aldehydes, and sulfhydryl groups. In certain embodiments, the first coupling group is a functional group that is reactive toward sulfhydryl groups, such as maleimide, pyridyl disulfide, or a haloacetyl. In certain embodiments, the first coupling group is a maleimide.

In one aspect, the present invention provides a lipid nanoparticle (LNP), wherein the LNP comprises one or more PEGylated lipid functionalized with a first coupling group, wherein the molar ratio of PEGylated lipid functionalized with a first coupling group relative to the total lipids is in the range of 0.001%-1.000%, preferably 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.010%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, more preferably in the range of 0.02-0.04%, or 0.004%-0.006%.

In one aspect, the present invention provides a nucleic acid encoding a chimeric antigen receptor (CAR), comprising a nucleotide sequence set forth in any one of SEQ ID NO: 52-57, or a nucleotide sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to any one of SEQ ID NO: 52-57.

In certain embodiments, the present nucleic acid is a linear RNA. In certain embodiments, the present nucleic acid further comprises a 5'UTR, preferably a nucleotide sequence set forth in SEQ ID NO: 58, or a

nucleotide sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 58. In certain embodiments, the present nucleic acid further comprises a 3'UTR, preferably a nucleotide sequence set forth in SEQ ID NO: 59, or a nucleotide sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 59. In certain embodiments, the present nucleic acid further comprises a polyA, preferably a nucleotide sequence set forth in SEQ ID NO: 60, or a nucleotide sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 60.

In certain embodiments, the present nucleic acid is circular RNA. In certain embodiments, the present nucleic acid further comprises a nucleotide sequence set forth in SEQ ID NO: 61, or a nucleotide sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 61. In certain embodiments, the present nucleic acid further comprises a IRES, preferably a nucleotide sequence set forth in any one of SEQ ID NO: 62-65, or a nucleotide sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to any one of SEQ ID NO: 62-65.

In certain embodiments, the present nucleic acid is transcribed by a nucleotide sequence set forth in any one of SEQ ID NO: 40-49, or a nucleotide sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to any one of SEQ ID NO: 40-49.

In one aspect, the present invention provides an antibody-conjugated lipid nanoparticle (LNP), wherein the antibody is the present antibody as described above, and/or wherein the LNP is the present LNP as described above.

In certain embodiments, the antibody-conjugated LNP further comprises a molecule encoding a chimeric antigen receptor (CAR), preferably a linear RNA or circular RNA encoding a CAR. In certain embodiments, the CAR specifically binds to a tumor antigen or an antigen selected from a group consisting of CD19, CD20, FAP, BCMA, and PSMA, preferably CD19 or BCMA. In certain embodiments, the chimeric antigen receptor (CAR) is encoded by a nucleic acid as described above.

In one aspect, the present invention provides a method of treating or preventing a disease or disorder in a subject in need thereof, wherein the method comprises administering to the subject in need thereof the present antibody-conjugated LNP.

In certain embodiments, the disease or disorder is selected from antigen-positive tumors, autoimmune diseases or fibrotic diseases.

In one aspect, the present invention provides a pharmaceutical composition comprising the present antibody-conjugated LNP and a pharmaceutically acceptable excipient.

In one aspect, the present invention provides a use of the present antibody-conjugated LNP in the preparation of a medicament for treating or preventing antigen-positive tumors, autoimmune diseases or fibrotic disease.

The purpose of the present invention is to provide a T-cell targeting antibody-conjugated LNP (Ab-LNP). The Ab-LNP encapsulates CAR mRNA to generate CAR-T cells in situ in the body. This method can reduce production costs while maintaining efficacy, improve patient

accessibility and clinical use convenience (no lymphodepletion is required before administration), and is a new type of in vivo and in situ CAR-T therapy suitable for promotion and application.

The present invention uses an antibody-conjugated LNP as a carrier to deliver mRNA specifically to T cell in vivo. The present method generates CAR-T cells in situ in the body and can be used to treat antigen-positive tumors, autoimmune diseases or fibrotic diseases. The present method can significantly overcome the disadvantages of conventional autologous CAR-T therapy. Compared with conventional CAR-T therapy, the present invention can be used as an off-the-shelf therapy, significantly simplifying the complex ex vivo CAR-T preparation process, reducing production costs, and improving patient accessibility. At the same time, using mRNA as CAR expression material can eliminate secondary T lymphoma caused by conventional CAR-T. In vivo and in situ CAR-T cell therapy mediated by Ab-LNP can eliminate the need for patients to undergo lymphodepletion before administration, reduce patient pain, and improve the convenience of clinical application. Compared with similar in situ generation of CAR-T therapies, the present invention uses spleen-targeted LNP-conjugated T cell-targeting antibodies to further improve the ability of T cells to target CAR expression and reduce off-target toxicity in the liver and other organs. The present invention can significantly improve the shortcomings of conventional CAR-T therapy, has high clinical application value, and is suitable for popularization and application.

Brief Description of the Drawings

To clearly indicate the technical solution of the present invention, a brief introduction thereto is provided below in reference to the Figures. Apparently, these Figures are merely some embodiments recorded in this

application. The present invention includes but is not limited to these Figures.

Figures 1a-1c respectively show the particle size, polydispersity coefficient (pdi) and encapsulation efficiency (ee) of LNP after antibody conjugation.

Figure 2 shows the in vivo distribution of mCD5-LNP/Luciferase.

Figure 3a shows the tdTomato/Cre mouse model and Figures 3b-3c show that mCD5-LNP can specifically target mRNA to T cells in vivo.

Figures 4a-4b show the B clearance ability of Ab-LNP/CD-19 CAR.

Figures 5a-5c show the expression of hCD19-CAR circRNA, hCD19-CAR linear mRNA, and hBCMA-CAR mRNA.

Figure 6 shows the expression time of hCD2/hCD5-LNP encapsulating circRNA and linear RNA.

Figure 7 shows the B cell clearance efficiency of hCD2-LNP or hCD5-LNP encapsulating CD19-CAR mRNA in isolated PBMCs.

Figure 8 shows the expression and B cell clearance efficacy of hCD5-LNP encapsulating CD19-CAR mRNA in the peripheral blood of mice with reconstituted human immune systems.

Figure 9 shows the T cell transfection efficiency of LNPs containing different test lipids.

Figures 10 and 11 show the optimization of Ab-LNP conjugates.

Figure 12 shows the expression of CAR in CD3 T cells and B cell clearance using optimized preparation.

Figure 13 shows the improved T cell targeting as well as B cell clearance of spleen-targeting LNP as compared with liver-targeting LNP.

Figure 14 shows the B cell clearance efficiency of conjugated LNPs encapsulating CD19-CAR mRNA in isolated PBMCs.

Figures 15a-15b shows the affinity of anti-hCD5 antibodies or anti-hCD8 antibodies to antigen.

Figure 16 shows the B cell clearance efficiency of anti-hCD8

antibody-conjugated LNP encapsulating CD19-CAR mRNA in isolated PBMCs.

Figures 17a-17b show the B cell clearance efficiency of anti-hCD8 antibody-conjugated LNP encapsulating CD19-CAR mRNA in mice models.

Figures 18a-18d shows the tolerance of present antibody-conjugated LNPs in cynomolgus monkeys.

Figure 19 shows the in vitro anti-tumor efficacy of CD8-STR0511-CD19.

Figure 20 shows the in vivo anti-tumor efficacy of CD8-STR0511-CD19.

Detailed Description

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

"A" or "an" herein refers to one or more, and thus an antigen may include one or more antigen.

"Comprising" or "comprises" is utilized in relation to compositions, methods, and their respective components that are part of a particular embodiment, while allowing for the addition of unspecified elements.

"Approximately" or "about" as applied to one or more values of interest, refers to a value that is similar in magnitude and/or within a similar range to a stated reference value. In certain embodiments, the term "approximately" or "about" may refer to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%,

8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context.

"Antibody" herein refers to any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen. "Antibody" herein encompasses an intact antibody, an antigen fragment thereof, or any modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies exhibit the desired biological activity. For example, formats of antibody herein includes Fv fragment, scFv fragment, Fab fragment, scFab fragment, F(ab)2 fragment and scFv-Fc fusion protein. There are five types of mammalian Ig heavy chain isotypes denoted by the Greek letters alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ). The type of heavy chain defines the class of antibody, i.e., IgA, IgD, IgE, IgG, and IgM, respectively. The γ and α classes are further divided into subclasses on the basis of differences in the constant domain sequence and function, e.g., IgG1, hIgG2, mIgG2A, mIgG2B, IgG3, IgG4, IgA1 and IgA2. In mammals, there are two types of immunoglobulin light chains, λ and κ.

An antibody "specifically binds" to a target antigen if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. "Specific binding" does not necessarily require exclusive binding. Generally, but not necessarily, reference to binding means specific binding. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of this disclosure.

"Fab fragments" herein refer to antigen-binding fragments generated

from native immunoglobulin molecules by papain digestion which cleaves the antibody molecule in the hinge region, on the amino-terminal side of the interchains disulfide bonds, thus releasing two identical antigen-binding arms. Pepsin also cleaves the antibody molecule in the hinge region, but on the carboxy-terminal side of the interchains disulfide bonds, releasing fragments consisting of two identical Fab fragments and remaining linked through disulfide bonds; reduction of disulfide bonds in the F(ab')2 fragments generates Fab' fragments.

"Single-chain fragment variable" or "scFv" herein refers to antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains, which enables the scFv to form the desired structure for antigen binding. The scFv fragment retains the specificity of the original antibody, despite removal of the constant regions and the introduction of the linker.

"Antigen" herein refers to any molecule, typically a peptide or protein, containing at least one epitope capable of triggering an immune response or being targeted by an immune response.

"Circular RNA" or "circRNA" herein refers to a class of closed RNA without 5' or 3' ends. The unique covalently-closed structures of circRNA prevent RNA degradation by exonucleases, thereby empowering them with high biostability relative to standard-of-care linear mRNA.

"Subject" or "patient" herein refers to an organism to be treated by the methods of the present invention. Such organisms are preferably mammals (e.g., murines, simians, equines, bovines, porcines, canines,

felines, and the like), and more preferably mice or humans.

"Cationic lipid" herein refers to a lipid that is cationic or becomes cationic (protonated) as the pH is lowered below the pK of the ionizable group of the lipid, but is progressively more neutral at higher pH values. At pH values below the pK, the lipid is then able to associate with negatively charged nucleic acids.

"CD" or "cluster of differentiation" herein refers to a system of nomenclature used to identify and categorize antigens present on the cell surface of leukocytes. It is recognized by those skilled in the field that leukocytes contain cell surface antigens called CD antigens, which are unique to different types of leukocytes and play a role in defining distinct subpopulations of these cells.

Lipid nanoparticle

Liposome nanoparticle (LNP) is a non-viral nanoparticle delivery carrier. The two Covid-19 mRNA vaccines currently on the market use LNP as a carrier, and it has been verified in a large number of people that LNP can be used as the preferred carrier for mRNA delivery. In the present invention, a variety of LNPs suitable for delivering CAR mRNA are screened out through in vivo and in vitro experiments, including LNPs targeted to organs such as the liver and spleen.

In order to further improve the efficiency and specificity of T cell-targeted delivery of CAR mRNA, the present invention conjugates antibodies that recognize cell surface antigen of T cells with LNPs obtained from preliminary screening (including spleen-targeting and liver-targeting).

The present LNP may comprise ionizable lipid. As used herein, the term "cationic lipid" or "ionizable lipid" includes lipids that are positively charged at acidic pH to condense RNAs into LNPs, but are neutral at physiological pH to minimize toxicity. Ionizable lipids may have one or more protonatable or deprotonatable group. The ionizable lipid can be included in the lipid nanoparticle in different amounts. For example, based on the amount of total lipids, the lipid nanoparticle can include about 10 mol% to about 100 mol%, such as about 10 mol% to about 70 mol%, about 20 mol% to about 60 mol%, about 30 mol% to about 50 mol% of ionizable lipid.

In certain embodiments, the LNP comprises at least one helper lipid. As used herein, the term "helper lipid" refers to a lipid that contributes to the stability and delivery efficiency of LNP, in addition to the ionizable lipid. Examples of helper lipids include, but are not limited to, phospholipids, steroids, PEGylated lipids, etc.

Examples of phospholipid include, but not limited to, 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC) , 1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) , 1, 2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC) , 1, 2-Distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE) , 1, 2-dimyristoyl-sn-glycero-phosphocholine (DMPC) , 1, 2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) , 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) , 1, 2-diundecanoyl-sn-glycero-phosphocholine (DUPC) , 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) , 1, 2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18: 0 Diether PC) , 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC) , 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC) ,

1, 2-dilinolenoyl-sn-glycero-3-phosphocholine, 1, 2-diarachidonoyl-sn-glycero-3-phosphocholine, 1, 2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1, 2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE) , 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine, 1, 2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1, 2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1, 2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1, 2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1, 2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG) , sphingomyelin, and any mixtures thereof. In some embodiments, the phospholipid is in a molar fraction of about 0 to 40%, such as about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 15%, 20%, 25%, 30%, 35% or 40% of the total lipids present in the lipid nanoparticle.

Examples of steroids include, but are not limited to, cholesterol and its derivatives, ergosterol, lanosterol, stigmasterol, sitosterol, and any mixtures thereof. In some embodiments, the steroid is in a molar fraction of about 0 mol% to about 70 mol%, such as about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or 70% of the total lipids present in the lipid nanoparticle.

In the present invention, PEGylated lipid refers to any PEG-modified lipid. Examples of PEGylated lipids include, but are not limited to, PEG-modified phosphatidylethanolamine, PEG-modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG-modified diacylglycerol, and any mixtures thereof. In some embodiments, the PEGylated lipid is PEG-modified distearoylphosphatidylethanolamine or PEG-modified dimyristoyl-sn-glycerol. In some embodiments, the PEG

modification has a molecular weight of about 100 to about 15,000. In some embodiments, the molecular weight of the PEG modification is about 100, 200, 400, 500, 600, 800, 1,000, 1,250, 1,500, 1,750, 2,000, 2,250, 2,500, 2,750, 3,000, 3,500, 4,000, 4,500, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 12,500 to about 15,000. In some embodiments, the steroid is in a molar fraction of about 0 mol% to about 10 mol%, such as about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% of the total lipids present in the lipid nanoparticle. In certain embodiments, one or more PEGylated lipid contained in the present LNP is functionalized with a first coupling group as disclosed in the conjugation section.

Cell surface antigen of T cells

T cells, along with B cells and NK cells, belong to the group of white blood cells known as lymphocytes. They play a central role in cell-mediated immunity and are distinguished by their T cell receptor (TCR), a special receptor on their cell surface. T cells originate in the bone marrow, mature in the thymus and travel in the blood to other lymphoid tissues, such as the tonsils, spleen and lymph nodes. The function and identity of a given T cell can be broadly determined by its cell surface proteins, beginning in early development.

In certain embodiments, the cell surface antigen of T cells is CD3, CD5, CD7, CD8 or CD2, in particular mouse or human CD3, CD5, CD7, CD8 or CD2.

CD3 is a member of the immunoglobulin superfamily and functions as a co-receptor on all T cells. Present at every stage of T cell development—even before unassigned T cells enter the thymus—CD3 is used to broadly identify T cells.

Another well-known marker for T cells is CD5, which functions as a negative regulator of TCR and BCR signaling. CD2, also part of the immunoglobulin supergene family, is a transmembrane molecule crucial for T-cell activation.

CD2 is also an effective pan-T cell marker and one of the earliest antigens observed in T lymphocytes, appearing after CD7 but before CD1.

CD7 is a 40 kDa transmembrane glycoprotein of the Ig superfamily, which is expressed on most T cells.

CD8 is a cell surface glycoprotein found on cytotoxic T lymphocytes, which play a crucial role in cellular immunity, particularly in responding to cancer and chronic infections. CD8 acts as a co-receptor for cytotoxic T cells, facilitating the interaction between the T cell receptor (TCR) and peptide major histocompatibility complex (pMHC).

Formats of antibodies which specifically bind to the cell surface antigen of T cells include, but are not limited to, full length IgG, Fab or scFv. In vivo and in vitro experiments have proven that antibody-conjugated LNP (Ab-LNP) can efficiently deliver CAR-expressing mRNA to T cells and exert antigen-specific cell killing, including tumor cells, B cells, and fibroblasts. If the LNP used is spleen-targeted LNP, the antibody-coupled LNP can be targeted to spleen T cells, further improving the in vivo CAR targeting and transfection efficiency.

Any antibody specifically binds to a cell surface antigen of T cells known in the art can be used in the present invention, for example, anti-CD7 antibodies disclosed in US20220324968A1, or any commercially

available antibodies such as those listed in the following Tables 1-5.

Table 1. Exemplary anti-CD5 antibodies available commercially

CD5 Recombinant Rabbit Monoclonal Antibody (RM314)	Invitrogen	MA5-27903
CD5 Monoclonal Antibody (UCHT2), PE-Cyanine7, eBioscience™	Invitrogen	25-0059-42
CD5 Monoclonal Antibody (UCHT2), PE, eBioscience™	Invitrogen	12-0059-42
CD5 Monoclonal Antibody (UCHT2), FITC, eBioscience™	Invitrogen	11-0059-42
CD5 Monoclonal Antibody (UCHT2), PerCP-eFluor™ 710, eBioscience™	Invitrogen	46-0059-42
CD5 Monoclonal Antibody (UCHT2), eBioscience™	Invitrogen	14-0059-82
CD5 Monoclonal Antibody (UCHT2), APC-eFluor™ 780, eBioscience™	Invitrogen	47-0059-42
CD5 Monoclonal Antibody (UCHT2), Biotin, eBioscience™	Invitrogen	13-0059-82
CD5 Monoclonal Antibody (UCHT2), Alexa Fluor™ 700, eBioscience™	Invitrogen	56-0059-42
CD5 Monoclonal Antibody (UCHT2), APC, eBioscience™	Invitrogen	17-0059-42
CD5 Monoclonal Antibody (SP19)	Invitrogen	MA5-14560
CD5 Monoclonal Antibody (L17F12), FITC, eBioscience™	Invitrogen	11-0058-42
CD5 Polyclonal Antibody	Invitrogen	PA5-83657
CD5 Polyclonal Antibody	Invitrogen	PA5-84470
CD5 Recombinant Rabbit Monoclonal Antibody (RM354)	Invitrogen	MA5-33060
CD5 Recombinant Rabbit Monoclonal Antibody (JE25-41)	Invitrogen	MA5-37807
CD5 Recombinant Rabbit Monoclonal Antibody (PD00-01)	Invitrogen	MA5-44673
CD5 Recombinant Rabbit Monoclonal Antibody (PD00-01)	Invitrogen	MA5-50681
CD5 Recombinant Rabbit Monoclonal Antibody (505)	Invitrogen	MA5-36002
CD5 Recombinant Rabbit Monoclonal Antibody (039)	Invitrogen	MA5-29117
CD5 Recombinant Rabbit Monoclonal Antibody (039), APC	Invitrogen	MA5-46714
CD5 Recombinant Rabbit Monoclonal Antibody (039), PerCP	Invitrogen	MA5-46715
CD5 Recombinant Rabbit Monoclonal Antibody (039), FITC	Invitrogen	MA5-46716
CD5 Recombinant Rabbit Monoclonal Antibody (039), PE	Invitrogen	MA5-46717
CD5 Monoclonal Antibody (MEM-32)	Invitrogen	MA1-19076
CD5 Monoclonal Antibody (6A11)	Invitrogen	MA5-17045
CD5 Monoclonal Antibody (CRIS1)	Invitrogen	MA1-19438
CD5 Monoclonal Antibody (CRIS1), FITC	Invitrogen	MA1-19621
CD5 Monoclonal Antibody (L17F12), PE	Invitrogen	MA5-38737
CD5 Monoclonal Antibody (L17F12), DyLight™ 594	Invitrogen	MA5-38739
CD5 Monoclonal Antibody (L17F12), PE-Cyanine5, eBioscience™	Invitrogen	15-0058-42
CD5 Monoclonal Antibody (L17F12), APC, eBioscience™	Invitrogen	17-0058-42
CD5 Monoclonal Antibody (L17F12), PerCP-Cyanine5.5, eBioscience™	Invitrogen	45-0058-42
CD5 Monoclonal Antibody (UCHT2), Super Bright™ 780, eBioscience™	Invitrogen	78-0059-42
CD5 Monoclonal Antibody (L17F12), PE-eFluor™ 610, eBioscience™	Invitrogen	61-0058-42
CD5 Monoclonal Antibody (L17F12), Super Bright™ 436, eBioscience™	Invitrogen	62-0058-42

CD5 Monoclonal Antibody (UCHT2), Super Bright™ 436, eBioscience™	Invitrogen	62-0059-42
CD5 Monoclonal Antibody (L17F12), Super Bright™ 600, eBioscience™	Invitrogen	63-0058-42
CD5 Monoclonal Antibody (UCHT2), Super Bright™ 600, eBioscience™	Invitrogen	63-0059-42
CD5 Monoclonal Antibody (UCHT2), Super Bright™ 645, eBioscience™	Invitrogen	64-0059-42
CD5 Monoclonal Antibody (UCHT2), Super Bright™ 702, eBioscience™	Invitrogen	67-0059-42
CD5 Monoclonal Antibody (CRIS1), APC	Invitrogen	MA1-19474
CD5 Monoclonal Antibody (L17F12), Biotin	Invitrogen	MA5-38736
CD5 Monoclonal Antibody (L17F12), PE-Cyanine7	Invitrogen	MA5-38738
CD5 Monoclonal Antibody (L17F12), PerCP	Invitrogen	MA5-38740
CD5 Polyclonal Antibody	Invitrogen	PA5-78990
CD5 Polyclonal Antibody	Invitrogen	PA5-80437
CD5 Polyclonal Antibody	Invitrogen	PA5-119015
CD5 Polyclonal Antibody	Invitrogen	PA5-78989
CD5 Monoclonal Antibody (CD5-5D7), PE-Cyanine5.5	Invitrogen	MHCD0518

Table 2 Exemplary anti-CD3 antibodies available commercially

CD3 Monoclonal Antibody (OKT3), eBioscience™	Invitrogen	14-0037-82
CD3 Monoclonal Antibody (OKT3), Functional Grade, eBioscience™	Invitrogen	16-0037-81
CD3 Monoclonal Antibody (OKT3), Alexa Fluor™ 488, eBioscience™	Invitrogen	53-0037-42
CD3 Monoclonal Antibody (UCHT1), eBioscience™	Invitrogen	14-0038-82
CD3e Monoclonal Antibody (SP7)	Invitrogen	MA5-14524
CD3 Monoclonal Antibody (HIT3a), eBioscience™	Invitrogen	14-0039-82
CD3 Monoclonal Antibody (4D10A6)	Invitrogen	MA5-17043
CD3e Monoclonal Antibody (SP7)	Invitrogen	MA1-90582
CD3 Monoclonal Antibody (7D6)	Invitrogen	MHCD0300
CD3 Monoclonal Antibody (OKT3)	Invitrogen	MA1-10175
CD3 Monoclonal Antibody (RIV9)	Invitrogen	MA1-7630
CD3e Monoclonal Antibody (OKT3)	Invitrogen	MA1-10176
CD3 Monoclonal Antibody (OKT3), Biotin, eBioscience™	Invitrogen	13-0037-82
CD3 Monoclonal Antibody (UCHT1), PE, eBioscience™	Invitrogen	12-0038-42
CD3 Monoclonal Antibody (SK7), APC-eFluor™ 780, eBioscience™	Invitrogen	47-0036-42
CD3 Monoclonal Antibody (UCHT1), APC-eFluor™ 780, eBioscience™	Invitrogen	47-0038-42
CD3 Monoclonal Antibody (UCHT1), Super Bright™ 436, eBioscience™	Invitrogen	62-0038-42
CD3 Monoclonal Antibody (SK7), Super Bright™ 436, eBioscience™	Invitrogen	62-0036-42
CD3e Monoclonal Antibody (UCHT1), FITC	Invitrogen	MA1-10178
CD3 Monoclonal Antibody (S4.1), Pacific Blue™	Invitrogen	MHCD0328
CD3e Monoclonal Antibody (UCHT1), Pacific Orange™	Invitrogen	CD0330
CD3 Monoclonal Antibody (S4.1), Qdot™ 655	Invitrogen	Q10012
CD3e Monoclonal Antibody (UCHT1), Alexa Fluor™ 700	Invitrogen	CD0329
CD3 Monoclonal Antibody (7D6), FITC	Invitrogen	MHCD0301

CD3 Monoclonal Antibody (7D6), PE-Texas Red	Invitrogen	MHCD0317
CD3 Monoclonal Antibody (HIT3a), PE, eBioscience™	Invitrogen	12-0039-42
CD3 Monoclonal Antibody (UCHT1), Super Bright™ 600, eBioscience™	Invitrogen	63-0038-42
CD3 Monoclonal Antibody (HIT3a), Functional Grade, eBioscience™	Invitrogen	16-0039-81
CD3 Monoclonal Antibody (OKT3), PE-eFluor™ 610, eBioscience™	Invitrogen	61-0037-42
CD3 Monoclonal Antibody (HIT3a), FITC, eBioscience™	Invitrogen	11-0039-42
CD3 Monoclonal Antibody (OKT3), eFluor™ 506, eBioscience™	Invitrogen	69-0037-42
CD3 Monoclonal Antibody (UCHT1), PE-Cyanine7, eBioscience™	Invitrogen	25-0038-42
CD3e Monoclonal Antibody (UCHT1), FITC	Invitrogen	CD0301
CD3 Monoclonal Antibody (7D6), APC-Alexa Fluor™ 750	Invitrogen	MHCD0327
CD3e Monoclonal Antibody (UCHT1), Alexa Fluor™ 405	Invitrogen	CD0326
CD3e Monoclonal Antibody (UCHT1), Pacific Blue™	Invitrogen	CD0328
CD3 Monoclonal Antibody (7D6), PE-Cyanine7	Invitrogen	MHCD0312
CD3e Monoclonal Antibody (UCHT1), PE	Invitrogen	CD0304
CD3 Monoclonal Antibody (MEM-57), PE	Invitrogen	MA1-19624
CD3 Monoclonal Antibody (MEM-57), APC	Invitrogen	MA1-19454
CD3e Monoclonal Antibody (CD3-12)	Invitrogen	MA5-16622
CD3e Monoclonal Antibody (UCHT1), APC	Invitrogen	MA1-10177
CD3 Monoclonal Antibody (SK7), Super Bright™ 436, eBioscience™	Invitrogen	62-0036-42
CD3 Monoclonal Antibody (S4.1 (7D6)), PerCP	Invitrogen	MHCD0331
CD3 Monoclonal Antibody (UCHT1), Functional Grade, eBioscience™	Invitrogen	16-0038-81
CD3 Monoclonal Antibody (UCHT1), Biotin, eBioscience™	Invitrogen	13-0038-82
CD3 Monoclonal Antibody (OKT3), APC-eFluor™ 780, eBioscience™	Invitrogen	47-0037-42
CD3 Monoclonal Antibody (OKT3), APC, eBioscience™	Invitrogen	17-0037-42
CD3 Monoclonal Antibody (OKT3), PerCP-eFluor™ 710, eBioscience™	Invitrogen	46-0037-42
CD3 Monoclonal Antibody (OKT3), PE, eBioscience™	Invitrogen	12-0037-42

Table 3 Exemplary anti-CD8 antibodies available commercially

CD8a Monoclonal Antibody (RPA-T8), PE, eBioscience™	Invitrogen	12-0088-42
CD8a Monoclonal Antibody (OKT8 (OKT-8)), Alexa Fluor™ 488, eBioscience™	Invitrogen	53-0086-42
CD8a Monoclonal Antibody (RPA-T8), APC, eBioscience™	Invitrogen	17-0088-42
CD8 Monoclonal Antibody (SP16)	Invitrogen	MA5-14548
CD8a Monoclonal Antibody (OKT8 (OKT-8)), eBioscience™	Invitrogen	14-0086-80
CD8a Monoclonal Antibody (OKT8 (OKT-8)), FITC, eBioscience™	Invitrogen	11-0086-42
CD8a Monoclonal Antibody (OKT8 (OKT-8)), Biotin, eBioscience™	Invitrogen	13-0086-82
CD8a Monoclonal Antibody (RPA-T8), PE-Cyanine7, eBioscience™	Invitrogen	25-0088-42
CD8a Monoclonal Antibody (RPA-T8), PerCP-Cyanine5.5, eBioscience™	Invitrogen	45-0088-42
CD8a Monoclonal Antibody (RPA-T8), FITC, eBioscience™	Invitrogen	11-0088-42
CD8a Monoclonal Antibody (RPA-T8), Super Bright™ 780, eBioscience™	Invitrogen	78-0088-42

CD8 Monoclonal Antibody (3B5), PE-Texas Red	Invitrogen	MHCD0817
CD8 Monoclonal Antibody (3B5), Alexa Fluor™ 405	Invitrogen	MHCD0826
CD8a Monoclonal Antibody (AMC908), eFluor™ 660, eBioscience™	Invitrogen	50-0008-82
CD8 Monoclonal Antibody (3B5), FITC	Invitrogen	MHCD0801
CD8a Monoclonal Antibody (AMC908), eBioscience™	Invitrogen	14-0008-82
CD8 Monoclonal Antibody (3B5), PE	Invitrogen	MHCD0804
CD8 Monoclonal Antibody (3B5), Pacific Orange™	Invitrogen	MHCD0830
CD8 Monoclonal Antibody (4B11)	Invitrogen	MA1-80231
CD8a Monoclonal Antibody (C8/144B), eBioscience™	Invitrogen	14-0085-82
CD8a Monoclonal Antibody (OKT8 (OKT-8)), Super Bright™ 780, eBioscience™	Invitrogen	78-0086-42
CD8a Monoclonal Antibody (OKT8 (OKT-8)), Super Bright™ 436, eBioscience™	Invitrogen	62-0086-42
CD8a Monoclonal Antibody (OKT8 (OKT-8)), Super Bright™ 645, eBioscience™	Invitrogen	64-0086-42
CD8a Monoclonal Antibody (HIT8a), FITC, eBioscience™	Invitrogen	11-0089-42
CD8a Monoclonal Antibody (HIT8a), PE, eBioscience™	Invitrogen	12-0089-42
CD8a Monoclonal Antibody (HIT8a), eBioscience™	Invitrogen	14-0089-82
CD8a Monoclonal Antibody (HIT8a), Biotin, eBioscience™	Invitrogen	13-0089-82
CD8a Monoclonal Antibody (AMC908), Biotin, eBioscience™	Invitrogen	13-0008-80
CD8a Monoclonal Antibody (HIT8a), Super Bright™ 436, eBioscience™	Invitrogen	62-0089-42
CD8 Monoclonal Antibody (SP16)	Invitrogen	MA1-39566
CD8a Monoclonal Antibody (SK1), PE-Cyanine7, eBioscience™	Invitrogen	25-0087-42
CD8a Monoclonal Antibody (SK1), eFluor™ 450, eBioscience™	Invitrogen	48-0087-42
CD8a Monoclonal Antibody (SK1), APC, eBioscience™	Invitrogen	17-0087-42
CD8a Monoclonal Antibody (SK1), FITC, eBioscience™	Invitrogen	11-0087-42
CD8a Monoclonal Antibody (RPA-T8), Alexa Fluor™ 561, eBioscience™	Invitrogen	505-0088-42
CD8a Monoclonal Antibody (SK1), Super Bright™ 600, eBioscience™	Invitrogen	63-0087-42
CD8a Monoclonal Antibody (RPA-T8), Alexa Fluor™ 700, eBioscience™	Invitrogen	56-0088-42
CD8a Monoclonal Antibody (RPA-T8), Super Bright™ 600, eBioscience™	Invitrogen	63-0088-42
CD8a Monoclonal Antibody (OKT8 (OKT-8)), Alexa Fluor™ 700, eBioscience™	Invitrogen	56-0086-42
CD8a Monoclonal Antibody (OKT8 (OKT-8)), Functional Grade, eBioscience™	Invitrogen	16-0086-85
CD8a Monoclonal Antibody (RPA-T8), Super Bright™ 645, eBioscience™	Invitrogen	64-0088-42
CD8a Monoclonal Antibody (AMC908), Alexa Fluor™ 488, eBioscience™	Invitrogen	53-0008-82
CD8a Monoclonal Antibody (OKT8 (OKT-8)), Super Bright™ 702, eBioscience™	Invitrogen	67-0086-42
CD8 Monoclonal Antibody (RFT8), APC-Cyanine7	Invitrogen	A15448
CD8a Monoclonal Antibody (HIT8a), Super Bright™ 780, eBioscience™	Invitrogen	78-0089-42
CD8a Monoclonal Antibody (SK1), PerCP-eFluor™ 710, eBioscience™	Invitrogen	46-0087-42
CD8a Monoclonal Antibody (SK1), Super Bright™ 702, eBioscience™	Invitrogen	67-0087-42
CD8a Monoclonal Antibody (RPA-T8), eFluor™ 506, eBioscience™	Invitrogen	69-0088-42
CD8a Monoclonal Antibody (RPA-T8), PE-eFluor™ 610, eBioscience™	Invitrogen	61-0088-42

CD8 alpha Polyclonal Antibody	Invitrogen	PA5-11453

Table 4 Exemplary anti-CD2 antibodies available commercially

CD2 Monoclonal Antibody (RPA-2.10), APC, eBioscience™	Invitrogen	17-0029-42
CD2 Monoclonal Antibody (RPA-2.10), eFluor™ 450, eBioscience™	Invitrogen	48-0029-42
CD2 Monoclonal Antibody (RPA-2.10), eBioscience™	Invitrogen	14-0029-82
CD2 Monoclonal Antibody (TS2/18)	Invitrogen	MA0200
CD2 Monoclonal Antibody (AB75)	Invitrogen	MA5-11373
CD2 Monoclonal Antibody (RPA-2.10), Biotin, eBioscience™	Invitrogen	13-0029-82
CD2 Monoclonal Antibody (RPA-2.10), PE, eBioscience™	Invitrogen	12-0029-42
CD2 Monoclonal Antibody (RPA-2.10), FITC, eBioscience™	Invitrogen	11-0029-42
CD2 Monoclonal Antibody (S5.5), Qdot™ 605	Invitrogen	Q10172
CD2 Monoclonal Antibody (RPA-2.10), PE-Cyanine5, eBioscience™	Invitrogen	15-0029-42
CD2 Monoclonal Antibody (RPA-2.10), Functional Grade, eBioscience™	Invitrogen	16-0029-81
CD2 Polyclonal Antibody	Invitrogen	PA5-82119
CD2 Monoclonal Antibody (RPA-2.10), PerCP-eFluor™ 710, eBioscience™	Invitrogen	46-0029-42
CD2 Chimeric Recombinant Rabbit Monoclonal Antibody (YTH 655)	Invitrogen	MA5-47805
CD2 Recombinant Rabbit Monoclonal Antibody (118)	Invitrogen	MA5-36000
CD2 Monoclonal Antibody (RPA-2.10), PE-Cyanine7, eBioscience™	Invitrogen	25-0029-42
CD2 Monoclonal Antibody (TS1/8)	Invitrogen	MA5-44080
CD2 Monoclonal Antibody (LT2), PE	Invitrogen	MA1-10134
CD2 Monoclonal Antibody (MEM-65)	Invitrogen	MA1-19070
CD2 Monoclonal Antibody (LT2), FITC	Invitrogen	MA1-19622
CD2 Monoclonal Antibody (MEM-65), Biotin	Invitrogen	MA1-19480
CD2 Monoclonal Antibody (RPA-2.10), APC-eFluor™ 780, eBioscience™	Invitrogen	47-0029-42
CD2 Monoclonal Antibody (S5.5), PerCP	Invitrogen	CD0231
CD2 Monoclonal Antibody (MEM-65), PE	Invitrogen	MA1-19623
CD2 Monoclonal Antibody (LT2)	Invitrogen	MA1-10131
CD2 Monoclonal Antibody (S5.5), PE	Invitrogen	CD0204
CD2 Monoclonal Antibody (RPA-2.10), Alexa Fluor™ 700, eBioscience™	Invitrogen	56-0029-42
CD2 Monoclonal Antibody (LT2), APC	Invitrogen	MA1-10132
CD2 Monoclonal Antibody (S5.5), APC	Invitrogen	CD0205
CD2 Polyclonal Antibody	Invitrogen	PA5-114972
CD2 Monoclonal Antibody (RPA-2.10), Super Bright™ 436, eBioscience™	Invitrogen	62-0029-42
CD2 Monoclonal Antibody (S5.5), FITC	Invitrogen	CD0201
CD2 Polyclonal Antibody	Invitrogen	PA5-77893
CD2 Recombinant Rabbit Monoclonal Antibody (118), PE	Invitrogen	MA5-46705
CD2 Monoclonal Antibody (RPA-2.10), Super Bright™ 600, eBioscience™	Invitrogen	63-0029-42
CD2 Recombinant Rabbit Monoclonal Antibody (118), PerCP	Invitrogen	MA5-46703

CD2 Monoclonal Antibody (RPA-2.10), Super Bright™ 645, eBioscience™	Invitrogen	64-0029-42
CD2 Recombinant Rabbit Monoclonal Antibody (118), FITC	Invitrogen	MA5-46704
CD2 Monoclonal Antibody (RPA-2.10), Super Bright™ 702, eBioscience™	Invitrogen	67-0029-42
CD2 Recombinant Rabbit Monoclonal Antibody (118), APC	Invitrogen	MA5-46702
CD2 Monoclonal Antibody (RPA-2.10), Super Bright™ 780, eBioscience™	Invitrogen	78-0029-42
FITC anti-human CD2	BioLegend	984902
Purified anti-human CD2 Antibody	BioLegend	300202
Biotin anti-human CD2 Antibody	BioLegend	300203
Purified anti-human CD2 Antibody	BioLegend	309202
PE/Cyanine7 anti-human CD2 Antibody	BioLegend	309214
PerCP/Cyanine5.5 anti-human CD2	BioLegend	984912
Purified anti-human CD2 (Maxpar® Ready) Antibody	BioLegend	309219
Alexa Fluor® 700 anti-human CD2 Antibody	BioLegend	300238
PE anti-human CD2 Antibody	BioLegend	309207

Table 5 Exemplary anti-CD7 antibodies available commercially

CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), PE, eBioscience™	Invitrogen	12-0079-42
CD7 Monoclonal Antibody (CD7-6B7), APC	Invitrogen	MHCD0705
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), APC, eBioscience™	Invitrogen	17-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), eBioscience™	Invitrogen	14-0079-82
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), Biotin, eBioscience™	Invitrogen	13-0079-82
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), APC-eFluor™ 780, eBioscience™	Invitrogen	47-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), Alexa Fluor™ 700, eBioscience™	Invitrogen	56-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), eFluor™ 450, eBioscience™	Invitrogen	48-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), FITC, eBioscience™	Invitrogen	11-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), PE-Cyanine7, eBioscience™	Invitrogen	25-0079-42
CD7 Monoclonal Antibody (4H9), FITC, eBioscience™	Invitrogen	11-0078-42
CD7 Monoclonal Antibody (4H9), PE, eBioscience™	Invitrogen	12-0078-42
CD7 Monoclonal Antibody (4H9), PerCP-eFluor™ 710, eBioscience™	Invitrogen	46-0078-42
CD7 Monoclonal Antibody (CD7-6B7), FITC	Invitrogen	MHCD0701
CD7 Monoclonal Antibody (4H9), APC, eBioscience™	Invitrogen	17-0078-42
CD7 Polyclonal Antibody	Invitrogen	PA5-83447
CD7 Recombinant Rabbit Monoclonal Antibody (ARC1634)	Invitrogen	MA5-35808
CD7 Recombinant Rabbit Monoclonal Antibody (307)	Invitrogen	MA5-36003
CD7 Recombinant Rabbit Monoclonal Antibody (15)	Invitrogen	MA5-43071
CD7 Chimeric Recombinant Rabbit Monoclonal Antibody (3A1E)	Invitrogen	MA5-47858
CD7 Recombinant Rabbit Monoclonal Antibody (JE57-72)	Invitrogen	MA5-44897
CD7 Monoclonal Antibody (LT7)	Invitrogen	MA1-34142

CD7 Monoclonal Antibody (21)	Invitrogen	MA5-41062
CD7 Monoclonal Antibody (MEM-186)	Invitrogen	MA1-19080
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), PE-Cyanine5.5, eBioscience™	Invitrogen	35-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), Brilliant Ultra Violet™ 737, eBioscience™	Invitrogen	367-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), Brilliant Violet™ 650, eBioscience™	Invitrogen	416-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), Alexa Fluor™ 532, eBioscience™	Invitrogen	58-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), Super Bright™ 436, eBioscience™	Invitrogen	62-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), Super Bright™ 600, eBioscience™	Invitrogen	63-0079-42
CD7 Monoclonal Antibody (eBio124-1D1 (124-1D1)), Super Bright™ 702, eBioscience™	Invitrogen	67-0079-42
CD7 Monoclonal Antibody (MEM-186), FITC	Invitrogen	MA1-19555
CD7 Monoclonal Antibody (MEM-186), PE	Invitrogen	MA1-19787
CD7 Monoclonal Antibody (MEM-186), APC	Invitrogen	MA1-19788
CD7 Polyclonal Antibody	Invitrogen	PA5-78998
CD7 Polyclonal Antibody	Invitrogen	PA5-117100
CD7 Polyclonal Antibody	Invitrogen	PA5-114990
CD7 Monoclonal Antibody (CD7-6B7), PE	Invitrogen	MHCD0704
CD7 Monoclonal Antibody (CD7-6B7), TRI-COLOR™	Invitrogen	MHCD0706
CD7 Polyclonal Antibody	Invitrogen	PA5-142814
CD7 Polyclonal Antibody	Invitrogen	PA5-32332
CD7 Monoclonal Antibody (MG34)	Invitrogen	MA1-12023
CD7 Polyclonal Antibody	Invitrogen	PA5-87015
Recombinant Human CD7 (carrier-free)	BioLegend	588304
Purified anti-human CD7 Antibody	BioLegend	395602
Purified anti-human CD7 Antibody	BioLegend	343102
Biotinylated Recombinant Human CD7 (carrier-free)	BioLegend	796304
APC anti-human CD7 Antibody	BioLegend	385809
FITC anti-human CD7 Antibody	BioLegend	395611
Purified anti-human CD7 Antibody	BioLegend	385802

Sequence information of exemplary antibodies is provided in Table 6.

Table 6. Sequence information of exemplary antibodies.

SEQ ID NO		
SEQ ID NO:	coding sequence of the heavy chain of anti-human CD2	**GCGGCCGC**CACCATGGGATGGAGCTGCATCATCTTGTTCTTGG TGGCCACCGCCACCGGCGTTCATTCT**GAACAGCAGCTGCTGG AGAGCGGAGGAGGATTGGTGCAGCCTGGAGGAAGCCTGAG**

1	antibody (signal peptide-**VH-***CH*(Human IgG1 Mutation))	**GCTGAGTTGTGCCGCTAGCGGAATCGATTTGAGCAGCTACT ACATGAGCTGGGTGAGGCAGGCCCCCGGAAAAGGATTGGA GTGGGTGGGACTGATTTCCGGCAGCGGAACAACCTACTAC GCCAACTGGGCAAAGGGGAGGTTTACCATCTCCAAGGACA ACTCCAAGAATACAGTGTACCTCCAGATGAACAGCCTGCGG GCAGAGGACACAGCCGTTTACTATTGCGCCCGGGTGACCG ACGCCGATGATTGGTCTCTCGACATCTGGGGCCAGGGCAC TCTGGTTACCGTGTCTTCC***GCTAGCACCAAGGGCCCATCGGTC TTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCG GCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG GTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTC CCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC AACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAG CACCTGAAGCCGCAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAA AACCCAAGGACACCCTCATGATCTCCCGGACCCCCGAGGTCACAT GCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCA ACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGC CGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTC CTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAG TGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACC CTGCCCCCATCCCGGGACGAGCTGACCAAGAACCAGGTCAGCCTG ACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAG TGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCA CCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCC TCTCCCTGTCTCCGGGTAAA***TGATTCTAGA**
SEQ ID NO: 2	Amino acid sequence of the heavy chain of anti-human CD2 antibody (signal peptide-**VH-***CH*(Human IgG1 Mutation))	MGWSCIILFLVATATGVHS**EQQLLESGGGLVQPGGSLRLSCAA SGIDLSSYYMSWVRQAPGKGLEWVGLISGSGTTYYANWAKG RFTISKDNSKNTVYLQMNSLRAEDTAVYYCARVTDADDWSLD IWGQGTLVTVSS***ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK***
SEQ ID NO: 3	coding sequence of the light chain of anti-human CD2 antibody (signal peptide-**VL-***CL*(Human Kappa))	GCGGCCGCCACCATGGGATGGAGCTGCATCATCTTGTTCTTGG TGGCCACCGCCACCGGCGTTCATTCT**GATTACCAGATGACCC AGAGCCCCAGCAGCCTGTCTGCAAGTGTGGGAGACAGGGT GACCATTACCTGCCAGGCTTCCCAGAGCATGACCTCCAGCT ACCTGTCCTGGTACCAGCAGAAGCCCGGCAAGGCTCCAAA ACTCCTGATCTACAAGGCCTCCAGGCTGGCCTCCGGAGTTC CTTCTCGATTCTCCGGCTCCGGCTCCGGAACAGATTTCACC CTGACTATCTCCTCCGTGCAGCCCGAGGACTTCGCAACTTA CTACTGCCAGCAGGCCTACACCATCGGCGAGGTTGACAAC CTGTTTGGTGGAGGCACCAAGGTGGAGATTAAG***CGTACGGT GGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTG AAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCC CAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATC GGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACA GCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACT ACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC**

		*TGAGTTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT***TGA**T**TCTAGA**
SEQ ID NO: 4	Amino acid sequence of the light chain of anti-human CD2 antibody (signal peptide-**VL**-*CL*(Human Kappa))	MGWSCIILFLVATATGVHS**DYQMTQSPSSLSASVGDRVTITCQA SQSMTSSYLSWYQQKPGKAPKLLIYKASRLASGVPSRFSGSGS GTDFTLTISSVQPEDFATYYCQQAYTIGEVDNLFGGGTKVEIK** *RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC*
SEQ ID NO: 5	VH of anti-human CD2 antibody	EQQLLESGGGLVQPGGSLRLSCAASGIDLSSYYMSWVRQAPGKG LEWVGLISGSGTTYYANWAKGRFTISKDNSKNTVYLQMNSLRAE DTAVYYCARVTDADDWSLDIWGQGTLVTVSS
SEQ ID NO: 6	CH (Human IgG1 Mutation)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK
SEQ ID NO: 7	VL of anti-human CD2 antibody	DYQMTQSPSSLSASVGDRVTITCQASQSMTSSYLSWYQQKPGKA PKLLIYKASRLASGVPSRFSGSGSGTDFTLTISSVQPEDFATYYCQ QAYTIGEVDNLFGGGTKVEIK
SEQ ID NO: 8	CL (Human Kappa)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
SEQ ID NO: 9	VH of anti-hCD5 (5D7)	QVTLKESGPGILKPSQTLSLTCSFSGFSLSTSGMGVGWIRQPSGKG LEWLAHIWWDDDVYYNPSLKNQLTISKDASRDQVFLKITNLDTA DTATYYCVRRRATGTGFDYWGQGTTLTVSS
SEQ ID NO: 10	VL of anti-hCD5 (5D7)	NIVMTQSHKFMSTSVGDRVSIACKASQDVGTAVAWYQQKPGQSP KLLIYWTSTRHTGVPDRFTGSGSGTDFTLTITNVQSEDLADYFCH QYNSYNTFGSGTRLELK
SEQ ID NO: 11	VH of anti-hCD5 (5D7-H3L1-ab3)	QVTLKESGPVLVKPTETLTLTCTFSGFSLSTSGMGVGWIRQAPGK GLEWVAHIWWDDDVYYNPSLKSRLTITKDASKDQVSLKLSSVTA ADTAVYYCVRRRATGTGFDYWGQGTLVTVSS
SEQ ID NO: 12	VL of anti-hCD5 (5D7-H3L1-ab3)	NIVMTQSPSSLSASVGDRVTITCQASQDVGTAVAWYQQKPDQSP KLLIYWTSTRHTGVPDRFTGSGSGTDFTLTISSLQPEDIATYFCHQ YNSYNTFGSGTKLEIK
SEQ ID NO: 13	VH of anti-hCD5 (5D7-H3L1-hu1, 5D7-H3L1-hu2, and 5D7-H3L1-hu3)	QVTLKESGPTLVKPTQTLTLTCTFSGFSLSTSGMGVGWIRQPPGK ALEWLAHIWWDDDVYYNPSLKSRLTITKDASKNQVVLTMTNMD PVDTATYYCVRRRATGTGFDYWGQGTLVTVSS
SEQ ID NO: 14	VL of anti-hCD5 (5D7-H3L1-hu1)	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQAP RLLIYWTSTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYFCHQY NSYNTFGQGTKVEIK
SEQ ID NO:	VL of anti-hCD5 (5D7-H3L1-hu2)	EIVMTQSPATLSVSPGERATLSCRASQDVGTAVAWYQQKPGQAP RLLIYWTSTRHTGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCHQ YSSYNTFGQGTKVEIK

15		
SEQ ID NO: 16	VL of anti-hCD5 (5D7-H3L1-hu3)	DIQMTQSPSSLSASVGDRVTITCKASQDVGTAVAWYQQKPGKAP KLLIYWTSTRHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCHQ YNSYNTFGQGTKVEIK
SEQ ID NO: 17	VH of anti-hCD5 (ab4-MAT 304)	EVQLQESGPGLVKPSQTLSLTCSVTGYSITSGYYWHWIRQFPGNK LEWMGYISYSGFTNYKTSLINRISITHDTSENQFFLNLNSVTTEDT ATYYCAGDRTGSWFAYWGQGTLVTVSA
SEQ ID NO: 18	VL of anti-hCD5 (ab4-MAT 304)	DIQVTQSPSSLSASLGERISLTCRTSQDISNYLNWFQQKPDGTFKR LIYATSSLDSGVPKRFSGSGSGSDYSLTISSLESEDFADYYCLQYA SYPFTFGSGTKLEIK
SEQ ID NO: 19	VL of anti-hCD5 (ab4-MAT 304-hu1 and ab4-MAT 304-hu2)	DIQMTQSPSSLSASVGDRVTITCRTSQDISNYLNWFQQKPGKAPK RLIYATSSLDSGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCLQY ASYPFTFGGGTKVEIK
SEQ ID NO: 20	VH of anti-hCD5 (ab4-MAT 304-hu1)	QVQLQESGPGLVKPSETLSLTCTVSGYSITSGYYWHWIRQPPGKG LEWMGYISYSGFTNYKTSLINRITISHDTSKNQFSLKLSSVTAADT AVYYCAGDRTGSWFAYWGQGTLVTVSS
SEQ ID NO: 21	VH of anti-hCD5 (ab4-MAT 304-hu2)	QVQLQESGPGLVKPSETLSLTCTVSGYSITSGYYWHWIRQPPGKG LEWMGYISYSGFTNYNPSLKSRVTISHDTSKNQFSLKLSSVTAAD TAVYYCAGDRTGSWFAYWGQGTLVTVSS
SEQ ID NO: 22	CH (Human IgG4 Mutation-FALAP G)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPS NTKVDKRVESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST YRVVSVLTVLHQDWLNGKEYKCKVSNKGLGSSIEKTISKAKGQP REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL HNHYTQKSLSLSLGK
SEQ ID NO: 23	coding sequence of the heavy chain of anti-human CD7 antibody (signal peptide-**VH-***CH(Human IgG4 S228P)*)	GCGGCCGCAAACTACAAGACAGACTTGCAAAAGAAGGCATGC ACAGCTCAGCACTGCTCTGTTGCCTGGTCCTCCTGACTGGGGT GAGGGCC**GAAGTGCAGCTGGTGGAGAGCGGAGGAGGATTG GTGAAGCCAGGAGGGAGTCTGAGGCTGAGTTGCGCTGCTA GCGGATTTAGTTTTCCGTGGCCTGGATGAGCTGGGTGAG GCAGGCTCCTGGAAAGGGACTGGAGTGGGTGGGTAGGATC AAGAGGAAGACAGACGGCGGAACCACCGACTATGCCGCAC CTGTTAAGGGCCGCTTCATTATCTCCCGGGACGACAGCATT AAGGCCCTGTACTTGCAGATGAATAGCTTGAAGACCGAGG ACACAGCCGTGTACTACTGCACCACCGACCCCTTGCTGAGG TACTCTGACTGGCTGAGCCACTCCCTGATGGGCGATGTGTG GGGACAGGGAACCACCGTTACCGTGAGCTCA***GCTAGCACCA AGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCT CCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCC CCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTAC TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACG AAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAG GTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCCACCAT GCCCAGCACCTGAGTTCCTGGGGGGACCATCAGTCTTCCTGTTCC CCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGG TCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTC CAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAG**

		*ACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTC AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGA AAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGT ACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCA GCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCG TGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCA CGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCA GGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCT CATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAA GAGCCTCTCCCTGTCTCTGGGTAAA*ATGATTCTAGA
SEQ ID NO: 24	Amino acid sequence of the heavy chain of anti-human CD7 antibody (signal peptide-**VH-***CH(Human IgG4 S228P)*)	MHSSALLCCLVLLTGVRA**EVQLVESGGGLVKPGGSLRLSCAAS GFSFSVAWMSWVRQAPGKGLEWVGRIKRKTDGGTTDYAAPV KGRFIISRDDSIKALYLQMNSLKTEDTAVYYCTTDPLLRYSDW LSHSLMGDVWGQGTTVTVSS***ASTKGPSVFPLAPCSRSTSESTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE ALHNHYTQKSLSLSLGK*
SEQ ID NO: 25	coding sequence of the light chain of anti-human CD7 antibody (signal peptide-**VL-***CL*(Human Kappa))	GCGGCCGCAAACTACAAGACAGACTTGCAAAAGAAGGCATGC ACAGCTCAGCACTGCTCTGTTGCCTGGTCCTCCTGACTGGGGT GAGGGCC**GATATCCAGATGACCCAGTCCCCCTCCTCCCTGT CTGCTTCTGTGGGAGACCGCGTGGCTATTACCTGTAGGACC TCCCAGAACATTAACAACTACCTGAACTGGTACCAGCAGAA GCCCGGCAAGGCCCCTAAACCACTCATCTACGCCGCCTCTT CCTTGCAGTCCGGCGTTCCTTCCCGCTTTTCCGGAAGCGGA AGCGGAACAGACTTCACCCTCACTATCTCCACCCTGCACCC CGAGGACTTCGCAACATACTACTGCCAGCAGACCTACTCCA TCCCCCTGACCTTTGGCGGCGGTACAAAGGTGGAGATCAA A***CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGAT GAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATA ACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACG CCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACA GCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCA AAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCC ATCAGGGCCTGAGTTCGCCCGTCACAAAGAGCTTCAACAGGGGAG AGTGT***TGATTCTAGA
SEQ ID NO: 26	Amino acid sequence of the light chain of anti-human CD7 antibody (signal peptide-**VL-***CL*(Human Kappa))	*MHSSALLCCLVLLTGVRA***DIQMTQSPSSLSASVGDRVAITCRTSQ NINNYLNWYQQKPGKAPKPLIYAASSLQSGVPSRFSGSGSGTD FTLTISTLHPEDFATYYCQQTYSIPLTFGGGTKVEIK***RTVAAPSV FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC*
SEQ ID NO: 27	VH of anti-hCD8 (STA-RS-TT-002)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSDFGMNWVRQAPGK GLEWVALIYYDGSNKFYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAKPHYDGYYHFFDSWGQGTLVTVSS
SEQ ID NO: 28	VL of anti-hCD8 (STA-RS-TT-002)	DIQMTQSPSSLSASVGDRVTITCKGSQDINNYLAWYQQKPGKAPK LLIYNTDILHTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCYQYN NGYTFGQGTKVEIK
SEQ ID	VH of anti-hCD8 (STA-RS-TT-003)	QVQLLESGPELLKPGASVKMSCKASGYTFTDYNMHWVKQSHGK SLEWIGYIYPYTGGTGYNQKFKNKATLTVDSSSSTAYMELRSLTS

NO: 29		EDSAVYYCARNFRYTYWYFDVWGQGTTVTVSS
SEQ ID NO: 30	VL of anti-hCD8 (STA-RS-TT-003)	DIVMTQSPASLAVSLGQRATISCRASESVDSYDNSLMHWYQQKP GQPPKVLIYLASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATY YCQQNNEDPYTFGGGTKLEIK
SEQ ID NO: 31	VH of anti-hCD8 (STA-RS-TT-004)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMHWVRQAPG QGLEWIGYIYPYTGGTGYAQKFQGRVTLTVDTSTSTAYMELSSLR SEDTAVYYCARNFRYTYWYFDVWGQGTLVTVSS
SEQ ID NO: 32	VL of anti-hCD8 (STA-RS-TT-004)	DIQMTQSPSSLSASVGDRVTITCRASESVDSYDNSLMHWYQQKP GKAPKLLIYLASNLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYY CQQNNEDPYTFGQGTKLEIK
SEQ ID NO: 33	VH of anti-hCD8 (STA-RS-TT-005)	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYIHFVRQRPEQGL EWIGRIDPANDNTLYASKFQGKATITADTSSNTAYMHLCSLTSGD TAVYYCGRGYGYYVFDHWGQGTTLTVSS
SEQ ID NO: 34	VL of anti-hCD8 (STA-RS-TT-005)	DVQINQSPSFLAASPGETITINCRTSRSISQYLAWYQEKPGKTNKL LIYSGSTLQSGIPSRFSGSGSGTDFTLTISGLEPEDFAMYYCQQHNE NPLTFGAGTKLELK
SEQ ID NO: 35	VH of anti-hCD8 (STA-RS-TT-006)	EVQLVQSGAEVKKPGATVKISCKVSGFNIKDTYIHWVQQAPGKG LEWIGRIDPANDNTLYAEKFQGRATITADTSTDTAYMELSSLRSE DTAVYYCGRGYGYYVFDHWGQGTLVTVSS
SEQ ID NO: 36	VL of anti-hCD8 (STA-RS-TT-006 and STA-RS-TT-007)	DIQMTQSPSSLSASVGDRVTITCRTSRSISQYLAWYQQKPGKAPK LLIYSGSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHN ENPLTFGQGTKVEIK
SEQ ID NO: 37	VH of anti-hCD8 (STA-RS-TT-007)	EVQLVQSGAEVKKPGATVKISCKVSGFNIKDTYIHWVQQAPGKG LEWMGRIDPANDNTLYAEKFQGRVTITADTSTDTAYMELSSLRSE DTAVYYCARGYGYYVFDHWGQGTLVTVSS
SEQ ID NO: 50	VH of anti-hCD8 (STA-RS-TT-001)	EVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWVRQAPGQGLEWIG RIDPANDNTLYASKFQGRATITADTSTSTAYLELSSLRSEDTAVYYCGRG YGYYVFDHWGQGTLVTVSS
SEQ ID NO: 51	VL of anti-hCD8 (STA-RS-TT-001)	DVQITQSPSSLSASVGDRVTITCRTSRSISQYLAWYQEKPGKTNKLLIYSG STLQSGIPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNEFPVTFGQGT KVEIK

Conjugation

In certain embodiments, the LNP is conjugated to an antibody, in particular an antibody that specifically binds to a cell surface antigen of T cells. Exemplary methods of conjugation include, but are not limited to, covalent bonds, electrostatic interactions, and hydrophobic interactions.

In certain embodiments, the conjugation comprises a covalent bond between a first coupling group from the LNP and a second coupling group from the antibody. The first coupling group and second coupling group can be any functional groups known to those of skill in the art that together form a covalent bond. In certain embodiments, the first coupling group or second coupling group are selected from the group consisting of maleimides, N-hydroxysuccinimide (NHS) esters, carbodiimides, hydrazide, pentafluorophenyl (PFP) esters, phosphines, hydroxymethyl phosphines, psoralen, imidoesters, pyridyl disulfide, isocyanates, vinyl sulfones, alpha-haloacetyls, aryl azides, acyl azides, alkyl azides, diazirines, benzophenone, epoxides, carbonates, anhydrides, sulfonyl chlorides, cyclooctynes, aldehydes, and sulfhydryl groups, for example, as described in Greg Hermanson, Bioconjugate Techniques 3rd Edition 2013; Curr. Org. Chem. 2010, 14(2): 138-147. In certain embodiments, the first coupling group or second coupling group is selected from the group consisting of free amines (-NH2), free sulfhydryl groups (-SH), free hydroxide groups (-OH), carboxylates, hydrazides, and alkoxyamines. In certain embodiments, the first coupling group is a functional group that is reactive toward sulfhydryl groups, such as maleimide, pyridyl disulfide, or a haloacetyl. In certain embodiments, the first coupling group is a maleimide.

In certain embodiments, the first coupling group is bound to the polyethylene glycol portion of the pegylated lipid. In certain embodiments, the pegylated lipid functionalized with the first coupling group can include, but are not limited to, DSPE-PEG(2000)-succinyl (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[succinyl(polyethylene glycol)-2000](ammonium salt)); DSPE-PEG(2000)-cyanur (l,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[cyanur(poly

ethylene glycol)-2000](ammonium salt)), DSPE-PEG(2000)-maleimide (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)-2000](ammonium salt)), DSPE-PEG(2000)-PDP (l,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[PDP(polyethylene glycol)-2000] (ammonium salt)), DSPE-PEG(2000)-amine (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt)); DSPE-PEG(2000)-biotin (I,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000] (ammonium salt)); DSPE-PEG(5000)-amine (l,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-5000] (ammonium salt)); DSPE-PEG(2000)-carboxylic acid (I,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (sodium salt)); DSPE-PEG(2000)-square (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[square(polyethylene glycol)-2000] (sodium salt)); DSPE-PEG(2000)-carboxy NHS ([carboxy (poly ethylene glycol)-2000, NHS ester] (sodium salt); DSPE-PEG(5000)-maleimide(l,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide (polyethylene glycol)-5000] (ammonium salt); DSPE-PEG(5000)-DBCO(phosphoethanolamine-n-[dibenzocyclooctyl(polyethylene glycol)-5000] (ammonium salt));DSPE-PEG(5000)-azide (l,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[azido(polyethylene glycol)-5000] (ammonium salt); DSPE-PEG(2000)-azide (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[azido(polyethylene glycol)-2000] (ammonium salt)); DSPE-PEG(2000)-DBCG (l,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[dibenzocyclooctyl(polyethylene glycol)-2000] (ammonium salt)); DPPE-PEG(2000)-azide (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[azido(polyethylene glycol)-2000 (ammonium salt)), DOPE-PEG(2000)-azide (l,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[azido(polyethylene glycol)-2000] (ammonium salt)), DOPE-PEG(2000)-amine

(1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt));DOPE-PEG(2000)-carboxylic acid (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (sodium salt)); and DGPE-PEG(2000)-Halo-Tag(1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[polyethylene glycol-2000]-N-[chlorohexyltriethylene glycol] (ammonium salt)). Preferably, the pegylated lipid functionalized with the first coupling group is DSPE-PEG(2000)-maleimide, or DSPE-PEG-MAL.

In certain embodiments, the second coupling group is a sulfhydryl group. The sulfhydryl group can be installed on the antibody using any method known to those of skill in the art, for example, as described by Greg Hermanson, Bioconjugate Techniques 3rd Edition 2013 and Bioconjugate Chem. 2021, 32, 3, 595-606. In certain embodiments, the sulfhydryl group is present on a free cysteine residue. In one embodiment, the sulfhydryl group is revealed via reduction of a disulfide on the antibody, such as through reaction with 2-mercaptoethylamine. In certain embodiments, the sulfhydryl group is installed via a chemical reaction, such as the reaction between a free amine and 2-iminothilane or N-succinimidyl S-acetylthioacetate (SATA).

Chimeric antigen receptor

"Chimeric antigen receptor" or "CAR" herein refers to an artificial T cell receptor that is programmed to target specific antigens. Chimeric antigen receptors usually consist of an extracellular domain that binds to a specific antigen, a transmembrane domain and intracellular signaling domains that provide signals for T-cell activation.

In certain embodiments, the CAR specifically binds to a tumor antigen including a tumor-associated antigen or a tumor-specific antigen.

In certain embodiments, the tumor antigens of the present invention are derived from cancers including, but not limited to, primary or metastatic melanoma, mesothelioma, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, nonHodgkin's lymphoma, Hodgkins lymphoma, leukemias, uterine cancer, cervical cancer, bladder cancer, kidney cancer and adenocarcinomas such as breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, and the like.

In certain embodiments, the CAR specifically binds to an antigen characteristic of an autoimmune diseases/disorders and/or inflammatory diseases/disorders, such as an antigen associated with an autoimmune or inflammatory disorder, or an antigen which is expressed by a cell associated with an autoimmune diseases/disorders and/or inflammatory diseases/disorders. In certain embodiments, the autoimmune disease include, but is not limited to, myositis, Lupus, myasthenia gravis, systemic lupus erythematosus, multiple sclerosis, systemic sclerosis, vasculitis, Stiff-Person syndrome, rheumatoid arthritis, inflammatory bowel disease (including ulcerative colitis and Crohn's disease), Type 1 diabetes, psoriasis, Graves' disease, Hashimoto's thyroiditis, chronic inflammatory demyelinating polyneuropathy, Guillain-Barre syndrome, glomerulonephritis.

In certain embodiments, the CAR specifically binds to an antigen selected from a group consisting of CD19, CD20, Fibroblast Activation Protein (FAP), B-cell maturation antigen (BCMA), and Prostate specific membrane antigen (PSMA).

In certain embodiments, the CAR specifically binds to CD19. In certain embodiments, the CAR specifically binds to mouse or human CD19. In certain embodiments, the extracellular domain of CAR is a

CD19-targeted single-chain fragment variable (scFv). In certain embodiments, the intracellular domain of CAR is 4-1BB (CD137) and/or CD3ζ. "CD19-CAR" herein refers to a recombinant T cell receptor that specifically binds to CD19. A molecule encoding CD19-CAR can be a linear mRNA or circular RNA.

Examples

To further understand the present invention, preferable solutions of the present invention are described in details below in reference to examples. However, these examples are merely used to illustrate the characteristics and merits of the present invention, but not to limit the protection scope of the present invention.

Example 1 Preparation of mRNA

The kit used for in vitro transcription is Transcript Aid T7 High Yield Transcription Kit (Cat. No. #K0441) from Thermo scientific. The reaction system is shown in Table 7.

Table 7. IVT reaction system

DPEC water	Up to 20μl
A/C/GTP, N1-Me-pUTP or pseudo-UTP, clean cap	2 μl
5x Reaction Buffer	4μl
Enzyme Mix	2μl
Template	1μg
Total	20μl

After mixing, the reaction was carried out at 37°C for 30 minutes. 2 μl DNase I was added to the transcript product at the end of the reaction and then the mixture was digested at 37°C for 20 min. An equal volume of LiCl Precipitation Solution 22 μl was then added. The mixture was then thoroughly mixed and cooled at -20°C for 1 hour to terminate and

precipitate the RNA. Then the sample was centrifuged at the highest speed for 15 minutes at 4°C to precipitate the RNA and remove the supernatant. The pellet was washed with 1 mL of 70% ethanol (DEPC water), centrifuged again, and the supernatant was removed. The pellet was dried, and an appropriate solution was added as needed to dissolve RNA, and the mRNA was stored at -70°C.

Example 2 Preparation of circular RNA

The T7 High Yield Transcription Kit (Cat. No. #K0441) was used to prepare the circular RNA. According to the following reaction system, enzyme-free water, buffer, NTP and enzyme were added to the EP tube in sequence, and the mixture was reacted at 37°C for 1-4 hours. The transcript product at the end of the reaction was digested with DnaseI at 37°C. The reaction system can be scaled up in equal proportions.

Table 8. Reaction system to produce circular RNA

Component	Volume (μl)
10 x transcription buffer	2
ATP	2
UTP	2
CTP	2
GTP	2
T7RNAP	0.8
PPase	0.04
RRI	0.5
Template (1mg/ml)	1
Injection water	To 20

After the above reaction proceeded for about 1 hour, transcription buffer and GTP were added, and the mixture was mixed and reacted at 55°C for 5-15 minutes. Purified RNA was obtained by LiCl precipitation. The RNA was digested according to the RNaseR instructions to obtain

purified circular RNA.

Example 3 Preparation of Ab-LNP

Steps for Ab-LNP Preparation:

Step 1: LNP Preparation. mRNA (such as Luc, Cre, and CAR) was dissolved in citrate buffer (10 mM, pH =4.0) and the concentration of mRNA was adjusted to 0.2 mg/mL, so that to obtain the aqueous layer. Testing Lipids, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) or 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol, DMG-PEG2000, and DSPE-PEG2000-Mal were dissolved in desired molar ratios in dry ethanol, with the total concentration of lipids adjusted to 10 mg/mL to obtain the organic layer. The aqueous layer and organic layer were admixed in 3:1 ratio (v/v) by microfluidic device (NanoAssemblr® Ignite™) at total flow rate of 12 mL/min. The mixture was diluted 10-fold with PBS buffer (pH 7.4). Ethanol was separated by tangential flow filtration (TFF). The solution was concentrated to 0.5 mg/mL (mRNA concentration) and filtrated by 0.22 μm Millipore filter to afford mRNA-containing lipid particles.

Step 2: SATA and Ab Reaction and Purification. SATA solution was mixed with antibody, and the mixture was incubated at room temperature for 30 minutes. The reaction solution was diluted with Reaction Buffer (6-10 times), mixed well, and ultrafiltered using a 10k ultrafiltration unit at 4000 rpm for 5 minutes. The ultrafiltration was repeated with Reaction Buffer several times (about 50 times exchange). Note: Antibody can be stored at -20/-80°C (use within 24 hours if stored at 2-8°C) for later use.

Step 3: Deprotection to Obtain SH-AB and Purification. 1/10 volume of Deacetylation Solution was added to SATA-AB solution. The mixture was mixed well with a pipette, incubated at room temperature for 2 hours, transferred to a 10kD ultrafiltration unit, and centrifuged at 4000 rpm. 2 mL of 0.1X Reaction Buffer (containing 10 mM EDTA) was added to the

solution. And the mixture was purified by centrifugation (repeat several times, about 1000 times exchange). SH-AB solution should be used quickly for subsequent LNP reaction to avoid self-polymerization.

Step 4: LNP-Antibody Conjugation. A certain amount of SH-Ab and 2 mL of 0.5 mg/mL mal-LNP (1 mg) were added to a 5.0 mL centrifuge tube. The mixture was mixed gently and thoroughly (invert or pipette gently). Then the mixture was incubated at room temperature for 1 hour, gently inverted to mix 2-3 times, checked for any particulates. The conjugated product was purified using a 300kD ultrafiltration unit (do not exceed 3900g). The product was then diluted with PBS 3-5 times, and centrifuged (3-5 times) to achieve over 100 times exchange.

After LNP preparation (SL990:DSPC:Chol:DMG-PEG:DSPE-PEG-Mal = 50:10:38.5:1.0:0.5; unless otherwise specified, this lipid ratio is used in the present Examples), three different antibodies (Anti-hCD5(5D7), 5D7-H3L1-ab3, Anti-hCD5 ab-4) were conjugated to the LNP. As can be seen from Figure 1a, the particle size of the LNPs increased significantly after antibody conjugation, indicating that the increase in particle size is due to the conjugation of antibodies to the LNP surface. Furthermore, the polydispersity coefficients of the LNPs after antibody conjugation were all lower than 0.1, and the encapsulation rates were all higher than 90% (Figures 1b and 1c). There are no significant changes in either the polydispersity coefficient or encapsulation rate compared to the naked LNPs prior to conjugation, suggesting that the uniformity and encapsulation rate of the LNPs will not be affected after antibody conjugation.

Example 4 In vivo distribution of Ab-LNP

To test the in vivo distribution of antibody-conjugated LNPs, LNPs encapsulating Luciferase mRNA were prepared as in Example 3, and

conjugated with mCD5 antibody (Purified anti-mouse CD5 Antibody, Biolegend, 100602). The template sequence for generating Luciferase mRNA is set forth in SEQ ID NO: 38. After intravenous administration of LNP/Luciferase or mCD5-LNP/Luciferase in C57BL/6 mice, various organs of the mice were collected after 24 hours, and the distribution of LNPs or mCD5-LNPs in the mice were detected by IVIS imaging. The results (Figure 2) show that in the LNP group, Luciferase signals were mainly concentrated in the liver, with a small amount expressed in the spleen. In the mCD5-LNP group, Luciferase signals were more enriched in the spleen, and the expression in the liver was significantly reduced. In addition to liver and spleen, a small amount of signal enrichment was also detected in lymph nodes.

To further explore the in vivo targeting of conjugated LNPs, Ai9-Cre-tdTomato mice (Figure 3a) were utilized. PBS, LNP/Cre or mCD5-LNP/Cre (with the template sequence for generating Cre recombinase mRNA set forth in SEQ ID NO: 39) were injected through the tail vein. Peripheral blood mononuclear cells, spleens and lymph nodes of the mice were collected 72 hours later. After preparing the collected tissue into a single cell suspension, the cells were incubated with the target antibodies. Flow cytometry was then used to detect the proportion of tdTomato-positive cells in different cell subpopulations (Figure 3b). The antibodies used for flow cytometry in the present study are shown in Table 9. CD3+ cells represent T cells. CD45+ cells represent total white blood cells (leukocytes). CD19+ cells represent B cells, which belong to a subgroup of CD3-CD11b-. Since the CD19+ ratio is too low to be analyzed, CD3-CD11b- cells are used to represent B cells. More than 90% of the toTomato-positive cell population is T cell, and the positive proportion of B cells is less than 1%, indicating that mCD5-LNP can specifically deliver mRNA to T cells (Figure 3c).

Table 9 Antibodies used for flow cytometry

	Name	brand	Cat.
mCD3 mAb	InVivoMAb anti-mouse CD3ε	BioXcell	BE0001-1
	InVivoMAb anti-mouse CD3ε F(ab')2 fragment	bioXcell	BE0001-1FAB
mouse FACS Antibody	CD45 Monoclonal Antibody (I3/2.3), APC-Cyanine7	Invitrogen	A15395
	CD3 Monoclonal Antibody (17A2), FITC, eBioscience™	Invitrogen	11-0032-82
	PE Rat Anti-CD11b	BD Pharmingen	557397
	Brilliant Violet 421 anti-mouse CD5	BioLegend	100629
	BV650 Rat Anti-Mouse CD45R/B220	BD Horizo	563893
human FACS Antibody	APC-Cy™7 Mouse Anti-Human CD45	BD Pharmingen	557833
	CD3 Monoclonal Antibody (OKT3), FITC, eBioscience™	Invitrogen	11-0037-42
	CD4 Monoclonal Antibody (RPA-T4), PerCP-Cyanine5.5, eBioscience™	Invitrogen	45-0049-42
	CD8a Monoclonal Antibody (RPA-T8), eFluor™ 506, eBioscience™	Invitrogen	69-0088-42
	PerCP/Cyanine5.5 anti-human CD19	BioLegend	302229
CD19 CAR	CD19 Protein, Human, Recombinant (ECD, His Tag)	Sinobio	11880-H08H
	Alexa Fluor® 488 anti-His Tag Antibody	BioLegend	652509
	SureLight® APC Anti-DDDDK tag (Binds to FLAG® tag sequence)	Abcam	ab72569

Example 5 Preliminary Efficacy of CAR-T generated by Ab-LNP in vivo

In order to further verify the efficacy, the B cell clearance ability in peripheral blood was detected after intravenous injection of mCD5-LNP/mCD19-CAR mRNA in wild-type C57BL/6 mice. The template sequence for generating mCD19 CAR is set forth in SEQ ID NO: 40, and coding region of mCD19 CAR mRNA is set forth in SEQ ID NO: 52. The results (Figure 4a) indicate that significant clearance of peripheral blood B cells 24 hours after a single dose of mCD5-LNP/mCD19-CAR mRNA. This clearance can be maintained for up to one week after repeated doses.

In addition, the B cell clearance ability in peripheral blood was detected after intravenous injection of mCD3 mAb-LNP/mCD19-CAR

mRNA and mCD3 Fab-LNP/mCD19-CAR mRNA into wild-type C57BL/6 mice at day 2 (mCD3 mAb: InVivoMAb anti-mouse CD3ε BioXcell, BE0001-1; mCD3 F(ab')2: InVivoMAb anti-mouse CD3ε F(ab')2 fragment, bioXcell, BE0001-1FAB). As shown in Figure 4b, significant clearance of peripheral blood B cells can be observed with both mCD3 mAb-LNP/mCD19-CAR mRNA and mCD3 Fab-LNP/mCD19-CAR mRNA.

Example 6 Expression and efficacy of hCD19-CAR delivered by Ab-LNP

First, the expression of circRNA and linear mRNA encoding hCD19-CAR was verified. The transfection reagent Lipo2000 was used to transfect 293T cells with the corresponding RNA, as shown in the figure (Figure 5a). The horizontal axis of Figure 5a is the expression level of hCD19-CAR, and the vertical axis is the number of cells. The corresponding sequence information of hCD19 is shown in Table 10. After 24 hours, the cells were collected and the expression of hCD-19+CAR on the cell surface was detected. The results (Figure 5a) show that both the circRNA-CAR and linear mRNA-CAR tested can effectively express hCD-19 CAR molecules.

Table 10 Sequence information of hCD19 or hBCMA

Sequence Name	Template sequence	Coding region of mRNA
hCD19-CARflag-41BB-CD3z (hCD19-CAR-linear mRNA)	SEQ ID NO: 41	SEQ ID NO: 53
hCD19-CAR -41BB-CD3z	SEQ ID NO: 42	SEQ ID NO: 54
hCD19-CAR-flag-41BB-CD3z-ORNA (hCD19-CAR circRNA 1)	SEQ ID NO: 43	SEQ ID NO: 53
hCD19-CAR-flag-41BB-CD3z-17-2 ORNA (hCD19-CAR circRNA 2)	SEQ ID NO: 44	SEQ ID NO: 53
hCD19-CAR-flag-41BB-CD3z-76-2 ORNA (hCD19-CAR circRNA 3)	SEQ ID NO: 45	SEQ ID NO: 53
hCD19-CAR flag-41BB-CD3z-79-2 ORNA	SEQ ID NO: 46	SEQ ID NO: 53

(hCD19-CAR circRNA 4)		
hBCMA-CAR seq -1 scFv	SEQ ID NO: 47	SEQ ID NO: 55
hBCMA-CAR seq -2 VHH	SEQ ID NO: 48	SEQ ID NO: 56
hCD19/BCMA- dual-CAR	SEQ ID NO: 49	SEQ ID NO: 57

In the CD3+ T cells isolated from hPBMCs, CD3/CD28-activated magnetic beads were used to activate the cells for 48 hours. Following activation, an electroporation instrument was used to transfect linear mRNA or circRNA expressing hCD19-CAR into the T cells. The expression of hCD19-CAR was then detected at 24 hours and 96 hours after transfection. The results (Figure 5b) show that both linear CAR and circRNA could highly express hCD19-CAR on the surface of T cells 24 hours after transfection, while circCAR could maintain this high expression until 96 hours. Unless otherwise specified, hCD19-CAR linear mRNA of the present invention refers to hCD19-CARflag-41BB-CD3z, and hCD19-CAR circRNA refers to hCD19-CAR-flag-41BB-CD3z-17-2 ORNA.

In a separate experiment, 3μg of BCMA CAR (scFv or VHH) encoding mRNA was transfected into HEK293 cells using 3μl LipoMAX. After 24 hours of transfection, the cells were collected, and the expression of BCMA CAR was detected by flow cytometry. The detection reagents are listed in Table 11.As shown in Figure 5c, BCMA scFv-CAR mRNA (middle panel) and BCMA-VHH CAR mRNA (right panel) were efficiently expressed as compared to control (left panel).

Table 11. Reagents for BCMA flow cytometry

		brand	cat.	Lot.
CD19	Recombinant Human CD19 Protein (ECD, His & AVI Tag), Biotinylated	Sinobio	11880-H49H-B	LC17MY1917
	PE Streptavidin	BioLegend	405203	B380982
BCMA	Human BCMA / TNFRSF17 Protein, Fc Tag, premium grade	ACRO	BC7-H5254-100ug	554-222TW1-1LP
	PE anti-human IgG Fc Antibody	Biolegend	410708	B425941
	APC anti-human IgG Fc Antibody	Biolegend	410712	B405408

Furthermore, hCD2 or hCD5-LNP (unless otherwise specified, the anti-hCD5 antibody used in the present Examples refers to ab4-MAT 304-hu2) was used to package linear CAR or circular CAR RNA respectively, and transfection and expression verification were performed in the Jurkat T cell line. The expression of CAR in Jurkat cellswas detected after 24, 96 and 144 hours of incubation. The results (Figure 6) show that at 24 hours after incubation, all groups achieved high transfection efficiency, and hCD2/hCD5-LNP encapsulating circRNA significantly extended the expression time of hCD19-CAR.

In verify whether the Ab-LNP/hCD19 CAR can function effectively, the proportion of B cells was detected after incubating PBMCs with hCD2-LNP or hCD5-LNP encapsulating linear CAR or circular CAR RNA for 24hrs. The results (Figure 7) indicate that all groups significantly induced B cell clearance, with hCD5-LNP/linear CAR showing the most efficient B cell clearance.

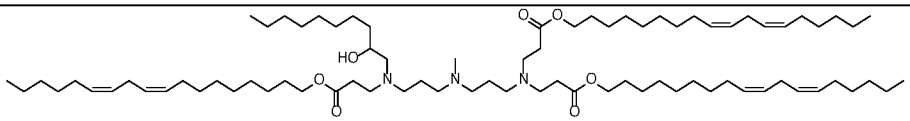
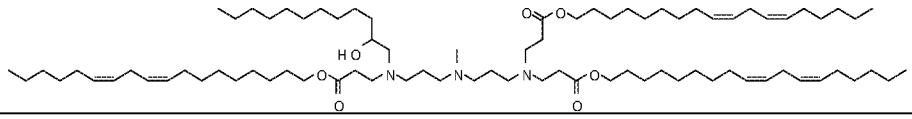
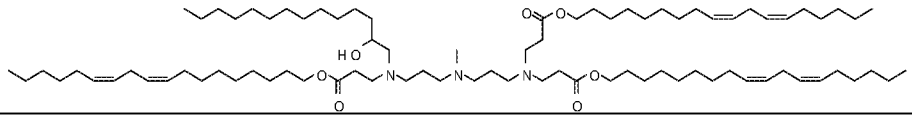
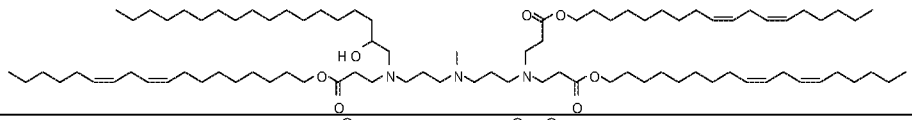
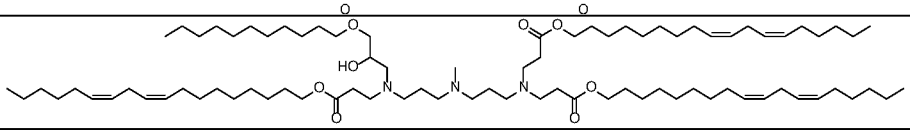
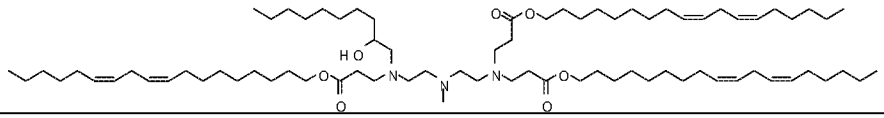
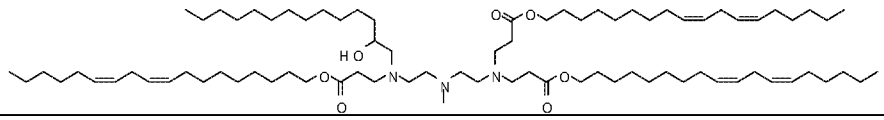
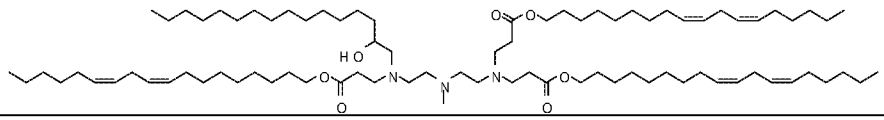
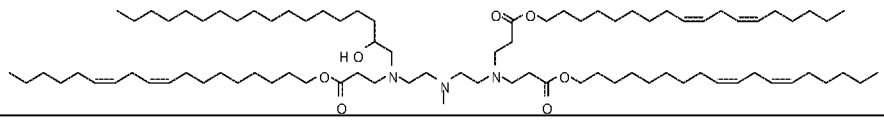
In mice with CD34+ HST immune system reconstitution, peripheral blood PBMCs were collected after intravenous injection of hCD5-LNP/hCD19 CAR mRNA (0.5 mpk) for 48 hours. Unless otherwise specified, hCD19-CAR mRNA in the present Examples refers to hCD19-CARflag-41BB-CD3z. The expression of hCD19 in different cell subpopulations was detected, along with the assessment of B cell clearance efficacy in peripheral blood. The results (Figure 8) show that the expression of hCD-19 CAR can be detected in different proportions in the peripheral blood of mice with reconstituted human immune systems, significantly inducing approximately 90% B cell clearance.

Example 7 Screening of testing lipids

To identify LNPs with superior T cell transfection efficiency, PBMC

cell lines were incubated with LNPs encapsulating mRNA (100 ng for LNPs encapsulating Luciferase mRNA and 1 μg for LNPs encapsulating mOX40L mRNA) (Figure 9). The testing lipids contained in LNP are detailed in Table 12. Unless otherwise specified, testing lipids of the present disclosure refer to ionizable lipids. As can be seen from Figure 9, the expression level of mRNA mediated by LNP containing SL996 was the highest.

Table 12 Structures of testing lipids.

Compd. No.	Structure	Structure Code
SL990		A1EP10O18A
SL991		A1EP12O18A
SL992		A1EP14O18A
SL994		A1EP18O18A
SL996		A1EPO15O18A
SL1288		A20EP10O18A
SL1290		A20EP14O18A
SL1291		A20EP16O18A
SL1292		A20EP18O18A

Example 8 Optimization of Ab-LNP conjugates

To optimize the ratio of Ab: LNP, molar ratios of DSPE-PEG-Mal, as shown in the following table 13, were tested. As shown in the left panel of Figure 10, the expression levels of CD19 CAR are higher when the ratio of DSPE-PEG-Mal is around 0.02%~0.04%.

Table 13 Optimization of Ab-LNP conjugates by varying the molar ratio of DSPE-PEG-Mal.

Entry	SL996: DSPC: Chol: total PEG	Ab:Mal-PEG	mal-PEG %
A	50 : 10 : 38.5 : 1.5	1.8125:1 (wt/wt)	0.5
B	50 : 10 : 38.5 : 1.5	1:1 (mol/mol)	0.5
C	50 : 10 : 38.5 : 1.5	1:1 (mol/mol)	0.1
D	50 : 10 : 38.5 : 1.5	1:1 (mol/mol)	0.04
E	50 : 10 : 38.5 : 1.5	1:1 (mol/mol)	0.02
F	50 : 10 : 38.5 : 1.5	1:1 (mol/mol)	0.01
G	50 : 10 : 38.5 : 1.5	1:1 (mol/mol)	0.005

Next, the following ratios of different lipids in LNP were tested (Table 14). As shown in the right panel of Figure 10, when the formulation is STAR0502X, the expression level of CD19 CAR is significantly higher.

Table 14 Optimization of Ab-LNP conjugates by varying the lipid ratios of LNP.

Entry	SL996: DSPC: Chol: total PEG	Ab:Mal-PEG	mal-PEG %
STAR0299X	50 : 10 : 38.5 : 1.5	1.8125:1 (wt/wt)	0.5
STAR0502X	46 : 10 : 42.4 : 1.6	1:1 (mol/mol)	0.04
STAR0503X	46 : 8 : 43.9 : 2.1	1:1 (mol/mol)	0.04
STAR0504X	45 : 8 : 45.3 : 1.7	1:1 (mol/mol)	0.04
STAR0505X	45 : 6 : 47.4 : 1.6	1:1 (mol/mol)	0.04
STAR0506X	48 : 7 : 43.4 : 1.6	1:1 (mol/mol)	0.04
STAR0507X	46 : 8 : 43.95 : 2.05	1:1 (mol/mol)	0.04

To further optimize the STAR0502X formulation, molar ratios of DSPE-PEG-Mal, as shown in the following table 15, were tested. As shown in Figure 11, when the formulation is STAR0511X, the expression level of CD19 CAR is significantly higher.

Table 15. Optimization of Ab-LNP conjugates by varying the molar ratio of DSPE-PEG-Mal.

Entry	SL996: DSPC: Chol: total PEG	Ab:Mal-PEG	mal-PEG %
STAR0502X	46 : 10 : 42.4 :	1:1	0.04

	1.6	(mol/mol)	
STAR0511X	46 : 10 : 42.4 : 1.6	1:1 (mol/mol)	0.005
STAR0512X	46 : 10 : 42.4 : 1.6	1:1 (mol/mol)	0.0025
STAR0513X	46 : 10 : 42.4 : 1.6	1:1 (mol/mol)	0.00125

The optimized formulation was incubated with PBMCs for 24 hours to detect CAR expression in CD3 T cells (Figure 12, left panel) and to assess B cell clearance (Figure 12, right panel). As can be seen, performance of STAR0511X containing SL996 was better than LNPs containing MC3 and SM-102. Hereafter, unless otherwise specified, the lipid ratios in STAR0511X are used for the tested LNPs.

Example 9 Spleen-targeted LNP enhances in vivo CAR expression and B cell clearance

LNP/hCD19 CAR mRNA was incubated with PBMCs in vitro for 24hrs to detect the CAR expression in different cells in PBMCs. The expression of Splenic T LNP (SL996) was better than that of liver LNP (SL990) (Figure 13, left panel). Furthermore, LNP/hCD19 CAR mRNA was incubated with PBMCs in vitro for 24hrs to assess the B cell clearance efficiency. The results (Figure 13, right panel) show that both LNPs significantly cleared B cells at 24hrs, but Splenic LNP cleared faster.

B cell clearance was further evaluated using anti-human CD5- or CD7- conjugated LNP containing SL996, CD5-conjugated LNP containing DLin-MC3-DMA was used as control. The proportion of B cells was detected after incubating PBMCs with LNPs encapsulating linear CD19

CAR LNP for 24hrs. As can be seen from Figure 14, LNPs containing SL996 demonstrated significantly better clearance efficiency compared with LNP containing DLin-MC3-DMA.

Example 10 Screening of different anti-human CD5 antibodies and anti-human CD8 antibodies

As shown in Figure 15a, different anti-human CD5 antibodies were tested for their affinity. CD5 antigen was coated on the bottom of the 96 well plate and incubated with different anti-hCD5 antibodies to assess the antibody-antigen affinity.

The VH and VL sequences of anti-human CD5 antibodies can be seen in Table 6. The VH of anti-human CD5 antibodies are conjugated to CH (Human IgG4 Mutation-FALAPG) (SEQ ID NO: 22), while the VL of anti-human CD5 antibodies is conjugated to CL (Human Kappa) (SEQ ID NO: 8).

For the optimization of CD8 antibodies, CD8+ T cells were isolated from PBMCs and incubated with different anti-CD8 antibodies for 1 hour. Subsequently, flow cytometry was used to assess the affinity of the antibodies for the CD8+ T cells (Figure 15b).

The VH and VL sequences of anti-human CD8 antibodies can be seen in Table 6. The VH of anti-human CD8 antibodies are conjugated to CH (Human IgG4 Mutation-FALAPG) (SEQ ID NO: 22), while the VL of anti-human CD8 antibodies is conjugated to CL (Human Kappa) (SEQ ID NO: 8).

Example 11 B cell clearance of anti-CD8 antibody conjugated LNP

B cell clearance was assessed using anti-CD8 antibody (STA-RS-TT-001, unless otherwise specified, anti-CD8 antibody in the present invention refers to STA-RS-TT-001) conjugated LNP containing SL996. The proportion of B cells was detected after incubating PBMCs with LNPs encapsulating linear CD19 CAR LNP for 24hrs (Figure 16).

B cell clearance was then assessed in vivo in the HSC mouse model. Human T and B cells were reconstituted from CD34+ HSC in NSG mice. Following reconstitution, treatment began at 12 weeks, with weekly doses administered while peripheral blood was collected at various time points to assess B cell clearance (Figure 17a). Ab-LNP Ctrl refers to CD8(001)-STR0511-tdTomato.

For assessing B cell clearance in tissues and organs, the same mice model with reconstituted human immune systems was used. Following reconstitution, treatment began at 12 weeks, with weekly doses for a total of four doses. 24 hours after the final dose, tissues and organs were harvested to evaluate B cell clearance through histological analysis (Figure 17b).

Example 12 Tolerance in cynomolgus monkeys

After administration, as shown in Figures 18a-18d, cynomolgus monkeys only exhibited transient ALT/AST elevations, while body temperature and weight were hardly affected. This suggests that CD5/CD8 conjugated LNP encapsulating CD 19 CAR mRNA was safe and tolerable.

Example 13 Anti-tumor efficacy of CD8-STR0511-CD19

The anti-tumor efficacy of CD8-STR0511-CD19 was assessed in vitro using CD19+ tumor cell (Raji and Nalm6). CD3 T cells were incubated with CD8-STR0511-CD19 (antibody 001, linear CD19 CAR) for

16 hours, followed by co-culture with CD19-positive tumor cell lines stably transfected with Luciferase for 16 hours, in accordance with the Effector-to-target (E:T) ratios indicated in Figure 19. The specific killing of CD19+ cells was quantified by measuring luciferase activity. Effector cells refer to CD8-STR0511-CD19 incubated T cells, while target cells are CD19+ tumor cells.

In vivo anti-tumor efficacy of CD8-STR0511-CD19 was evaluated in NSG mice inoculated with human PBMCs and NALM6 cells. 24 days before D0 (Day-24), each NSG mouse was inoculated with 5E6 Human PBMCs. On Day-5, each mouse was inoculated with 1E6 NALM6-Fluc cells. Starting from Day 0, the mice were administrated 1.5mpk of CD8-STR0511-CD19 intravenously twice a week, with IVIS imaging twice a week to monitor the tumor growth. As shown in Figure 20, administration of CD8-STR0511-CD19 significantly inhibits the tumor growth in vivo.

Claims

1. An antibody specifically binding to a cell surface antigen of T cells, wherein the cell surface antigen of T cells is selected from CD3, CD5, CD7, CD8, or CD2.

2. The antibody according to claim 1, wherein the antibody specifically binds to CD2, preferably human CD2, wherein the VH of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 5, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 5, and/or the VL of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 7, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 7.

3. The antibody according to claim 2, wherein the heavy chain of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 2, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 2, and/or the light chain of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 4, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 4.

4. The antibody according to claim 1, wherein the antibody specifically binds to CD5, preferably human CD5, and wherein VH of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 20, SEQ ID NO: 21 or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 9, SEQ ID NO: 11, SEQ

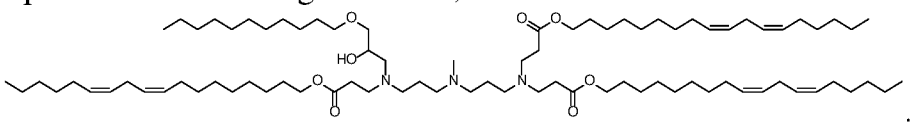
ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 20, SEQ ID NO: 21, and/or VL of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 18 or SEQ ID NO: 19, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 18 or SEQ ID NO: 19.

5. The antibody according to claim 1, wherein the antibody specifically binds to CD7, preferably human CD7, and wherein heavy chain of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 24, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 24, and/or light chain of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 26, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 26.

6. The antibody according to claim 1, wherein the antibody specifically binds to CD8, preferably human CD8, and wherein VH of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, or SEQ ID NO: 50, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, or SEQ ID NO: 50, and/or VL of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, or SEQ ID NO: 51, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, or SEQ ID NO: 51.

7. The antibody according to any one of claims 1-6, wherein CH of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 6 or SEQ ID NO: 22, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 6 or SEQ ID NO: 22, and/or the CL of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 8, or an amino acid sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to SEQ ID NO: 8.

8. A lipid nanoparticle (LNP), wherein the LNP comprises an ionizable lipid of the following structure,



9. A lipid nanoparticle (LNP), wherein the LNP comprises an ionizable lipid, a phospholipid, a steroid, and a PEGylated lipid, wherein the molar ratio of ionizable lipid : phospholipid : steroid : PEGylated lipid is a : b : c : d,

wherein a+b+c+d=100,

wherein a is in the range of 40.0-55.0, preferably a is 40.0, 41.0, 42.0, 43.0, 44.0, 45.0, 45.1, 45.2, 45.3, 45.4, 45.5, 45.6, 45.7, 45.8, 45.9, 46.0, 46.1, 46.2, 46.3, 46.4, 46.5, 46.6, 46.7, 46.8, 46.9, 47.0, 48.0, 49.0, 49.1, 49.2, 49.3, 49.4, 49.5, 49.6, 49.7, 49.8, 49.9, 50.0, 50.1, 50.2, 50.3, 50.4, 50.5, 50.6, 50.7, 50.8, 50.9, 51.0, 52.0, 53.0, 54.0, or 55.0;

wherein b is in the range of 5.0-15.0, preferably b is 5.0, 6.0, 7.0, 8.0, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, 10.9, 11.0, 12.0, 13.0, 14.0, or 15.0

wherein c is in the range of 35.0-50.0, preferably c is 35.0, 36.0, 37.0, 37.5, 37.6, 37.7, 37.8, 37.9, 38.0, 38.1, 38.2, 38.3, 38.4, 38.5, 38.6, 38.7, 38.8,

38.9, 39.0, 39.1, 39.2, 39.3, 39.4, 39.5, 40.0, 41.0, 41.1, 41.2, 41.3, 41.4, 41.5, 41.6, 41.7, 41.8, 41.9, 42.0, 42.1, 42.2, 42.3, 42.4, 42.5, 42.6, 42.7, 42.8, 42.9, 43.0, 43.1, 43.2, 43.3, 43.4, 43.5, 43.6, 43.7, 43.8, 43.9, 44.0, 45.0, 46.0, 47.0, 48.0, 49.0, or 50.0; and/or

wherein d is in the range of 0.1 to 3.0, preferably d is 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3.0.

10. The LNP according to claim 9, wherein one or more PEGylated lipid contained in the LNP is functionalized with a first coupling group, wherein the molar ratio of PEGylated lipid functionalized with a first coupling group relative to the total lipids is in the range of 0.001%-1.000%, preferably 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.010%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, more preferably in the range of 0.02-0.04%, or 0.004%-0.006%.

11. A lipid nanoparticle (LNP), wherein the LNP comprises one or more PEGylated lipid functionalized with a first coupling group, wherein the molar ratio of PEGylated lipid functionalized with a first coupling group relative to the total lipids is in the range of 0.001%-1.000%, preferably 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, 0.008%, 0.009%, 0.010%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, more preferably in the range of 0.02-0.04%, or 0.004%-0.006%.

12. A nucleic acid encoding a chimeric antigen receptor (CAR), comprising a nucleotide sequence set forth in any one of SEQ ID NO:

52-57, or a nucleotide sequence having at least 80%, at least 90%, at least 95%, at least 97%, or at least 99% identity to any one of SEQ ID NO: 52-57.

13. An antibody-conjugated lipid nanoparticle (LNP), wherein the antibody is an antibody according to any one of claims 1-7, and/or wherein the LNP is a LNP according to any one of claims 8-11.

14. The antibody-conjugated LNP according to claim 13, wherein the antibody-conjugated LNP further comprises a molecule encoding a chimeric antigen receptor (CAR), preferably a linear RNA or circular RNA encoding a CAR.

15. The antibody-conjugated LNP according to claim 14, wherein the CAR specifically binds to a tumor antigen or an antigen selected from a group consisting of CD19, CD20, FAP, BCMA, and PSMA, preferably CD19 or BCMA.

16. The antibody-conjugated LNP according to claim 15, wherein the chimeric antigen receptor (CAR) is encoded by the nucleic acid according to claim 12.

17. A method of treating or preventing a disease or disorder in a subject in need thereof, wherein the method comprises administering to the subject in need thereof the antibody-conjugated LNP of any one of claims 13-16.

18. The method of claim 17, wherein the disease or disorder is selected from antigen-positive tumors, autoimmune diseases or fibrotic diseases.

19. A pharmaceutical composition comprising the antibody-conjugated

LNP of any one of claims 13-16 and a pharmaceutically acceptable excipient.

20. Use of the antibody-conjugated LNP of any one of claims 13-16 in the preparation of a medicament for treating or preventing antigen-positive tumors, autoimmune diseases or fibrotic diseases.

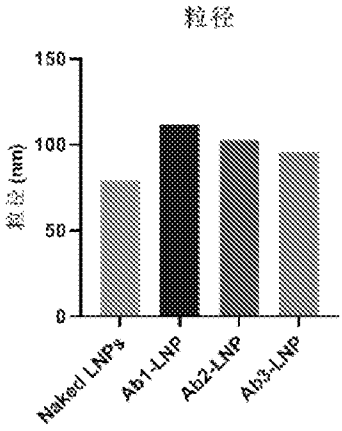


Figure 1a

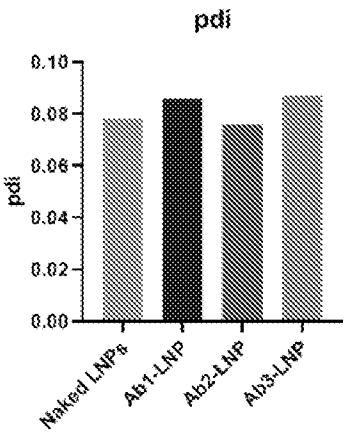


Figure 1b

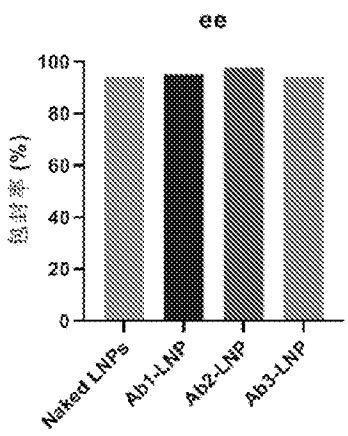


Figure 1c

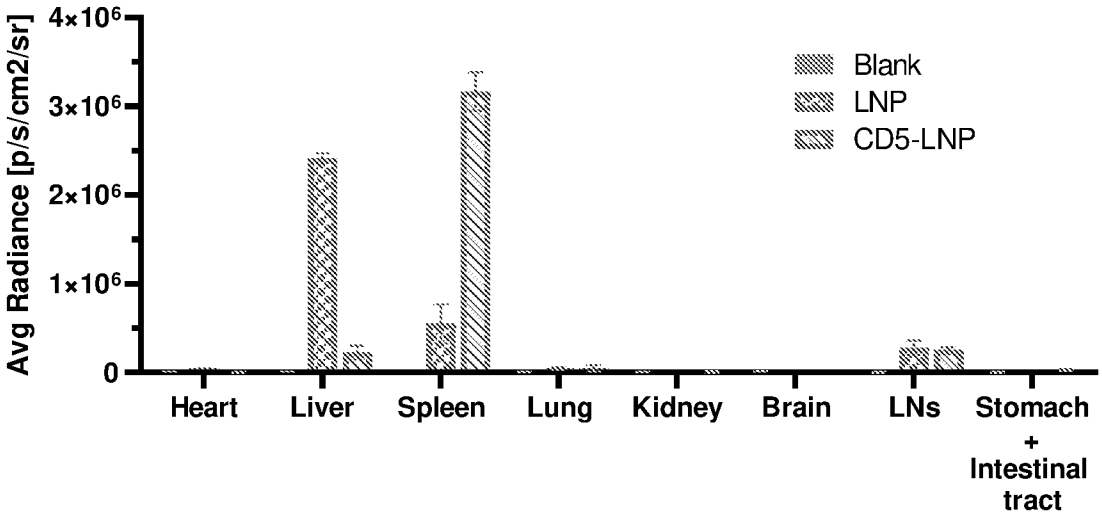


Figure 2

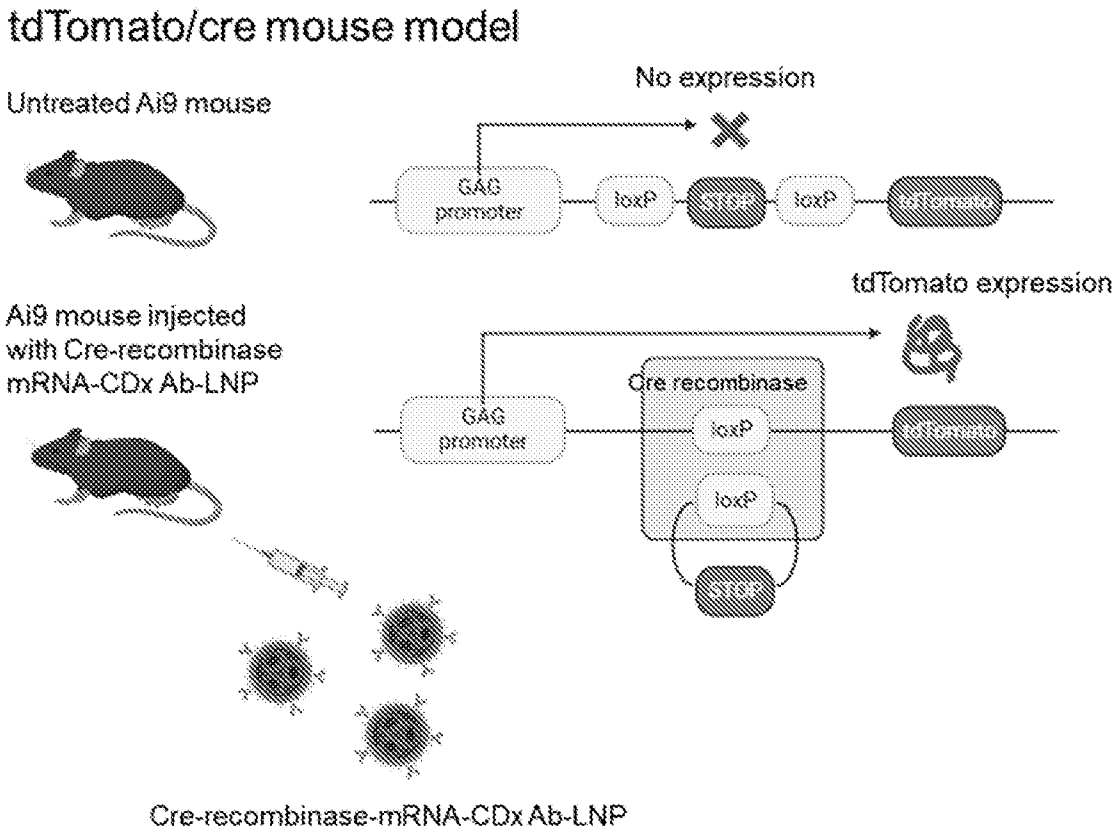


Figure 3a

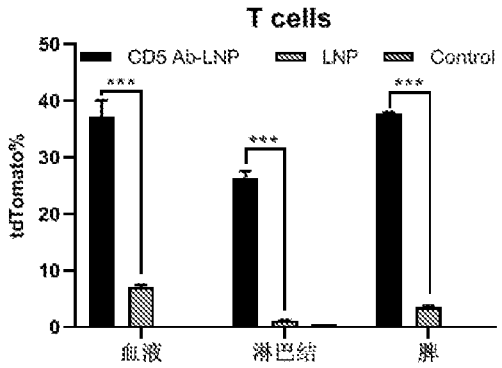


Figure 3b

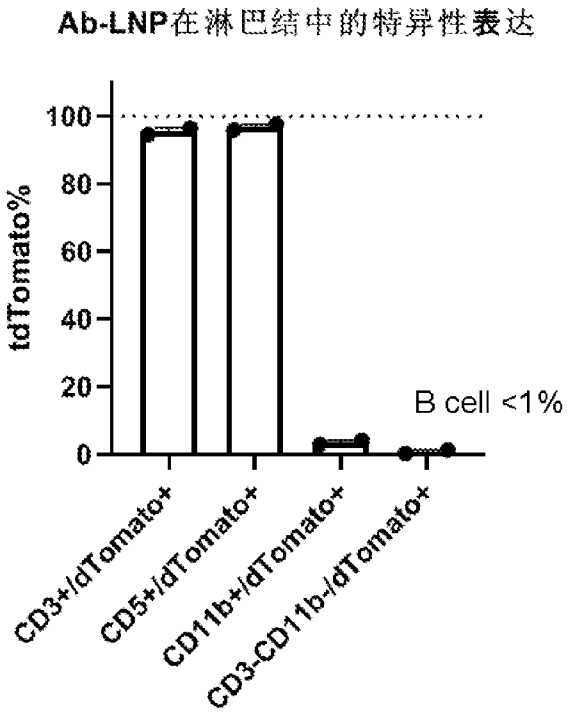


Figure 3c

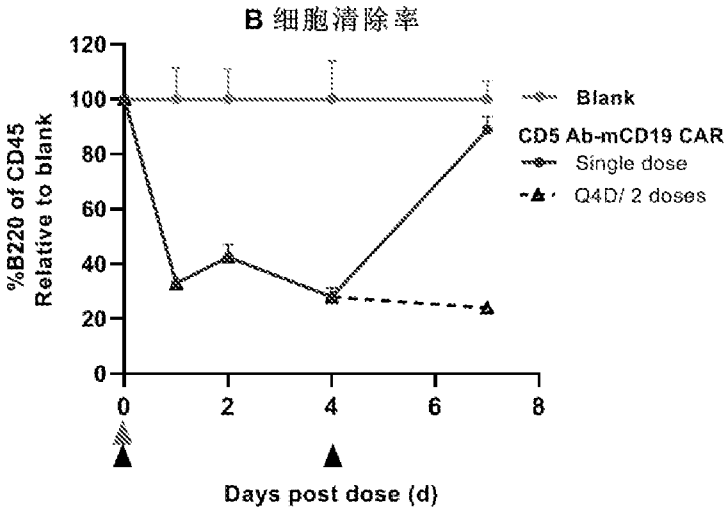


Figure 4a

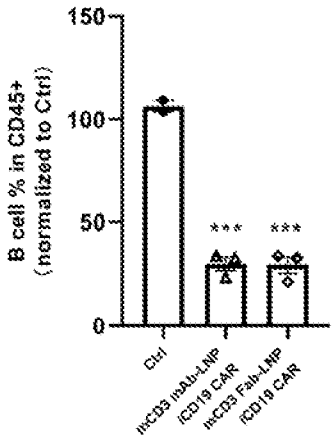


Figure 4b

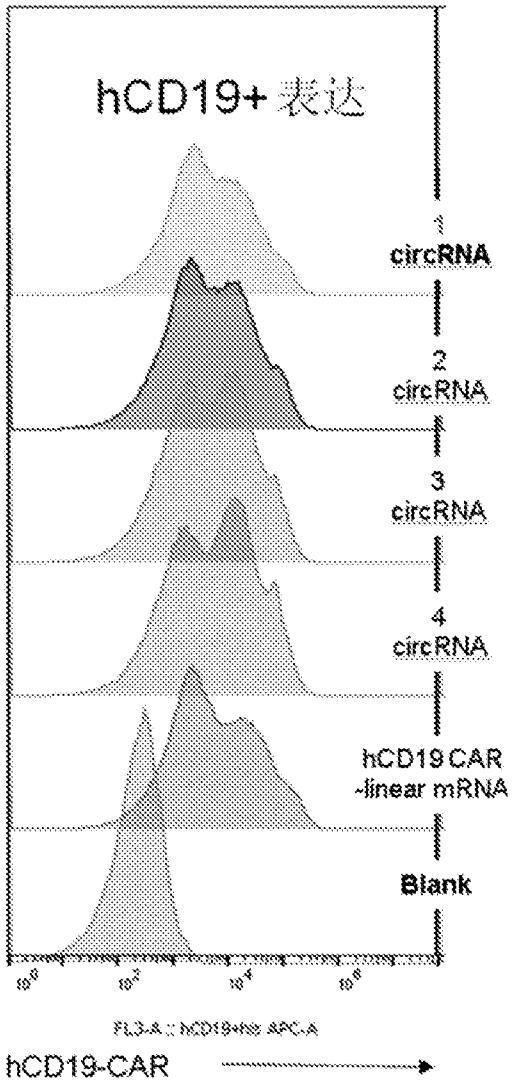

hCD19+表达
1
circRNA
2
circRNA
3
circRNA
4
circRNA
hCD19 CAR
-linear mRNA
Blank
hCD19-CAR

Figure 5a

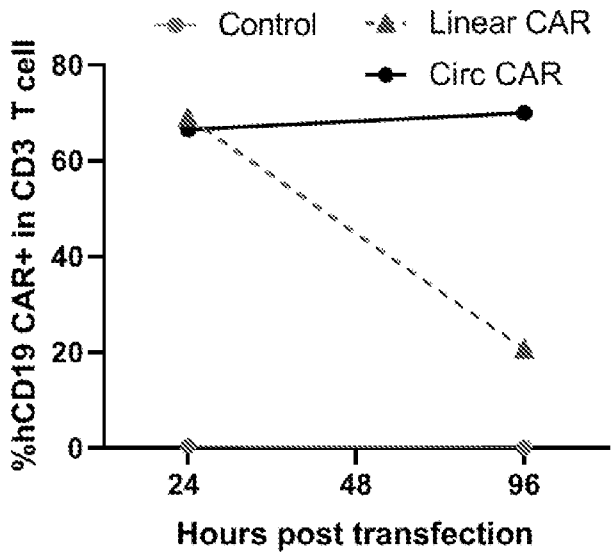


Figure 5b

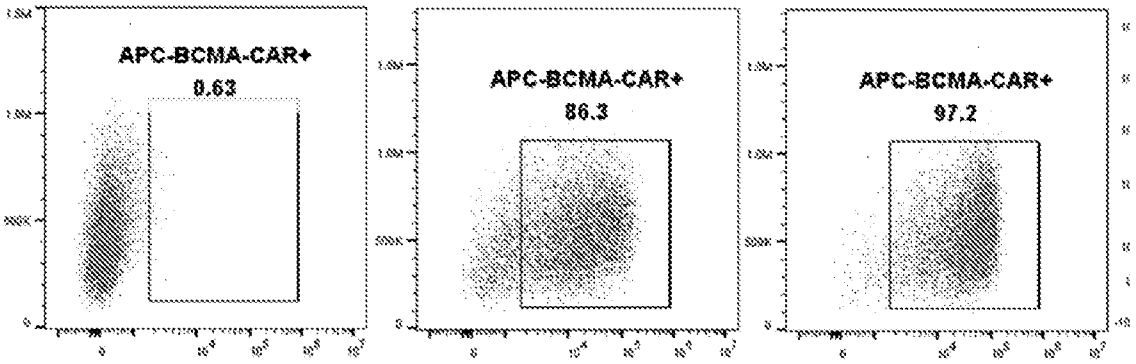


Figure 5c

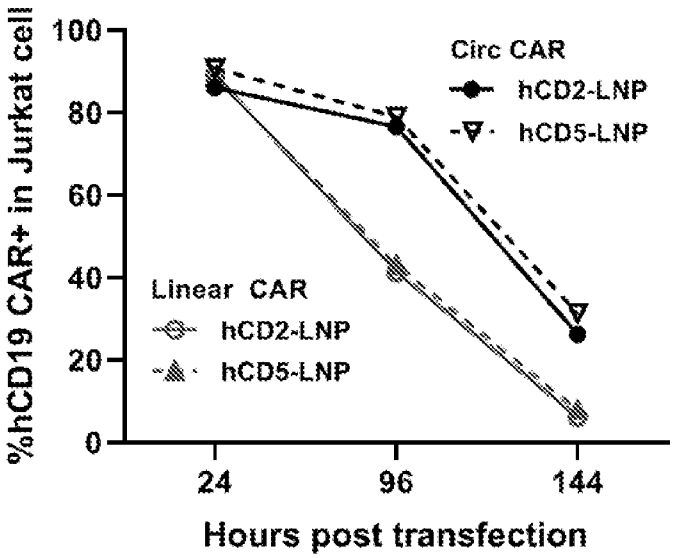


Figure 6

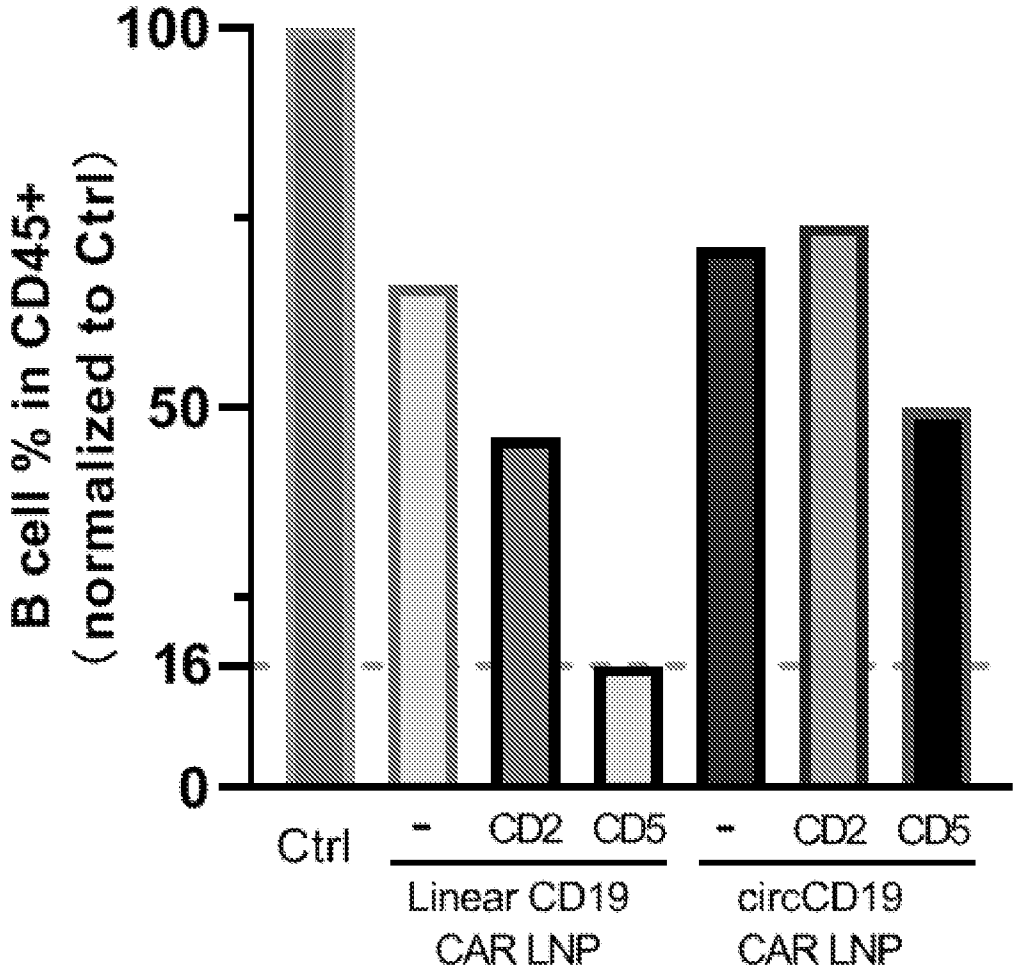

100
50
16
0
B cell % in CD45+
(normalized to Ctrl)
Ctrl
-
CD2
CD5
-
CD2
CD5
Linear CD19
CAR LNP
circCD19
CAR LNP

Figure 7

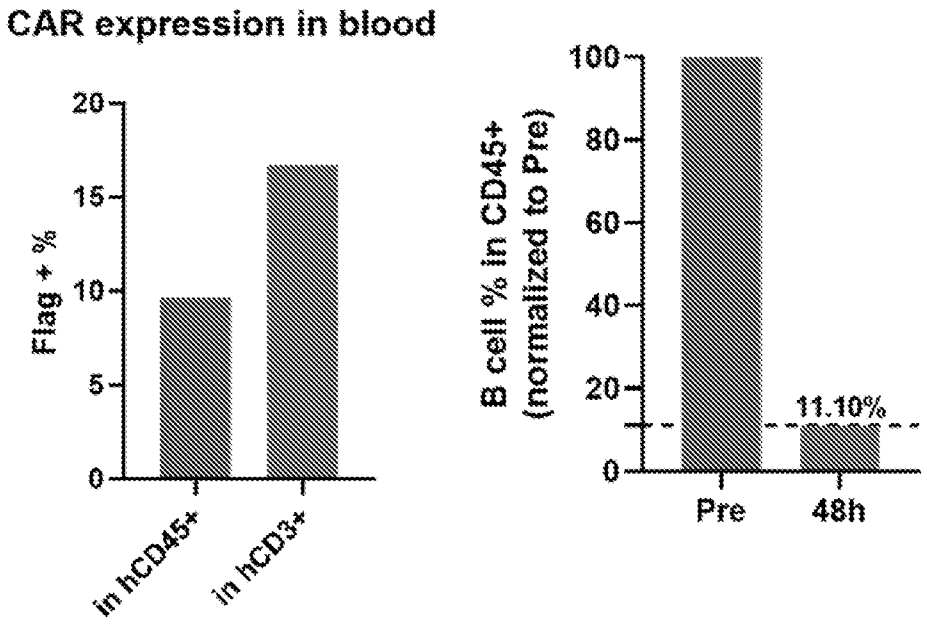


Figure 8

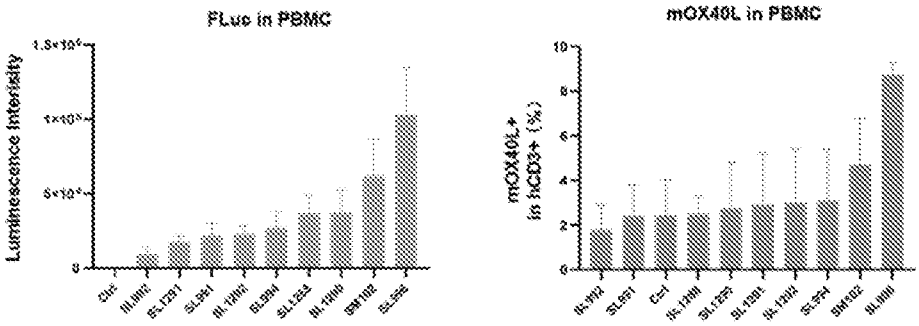


Figure 9

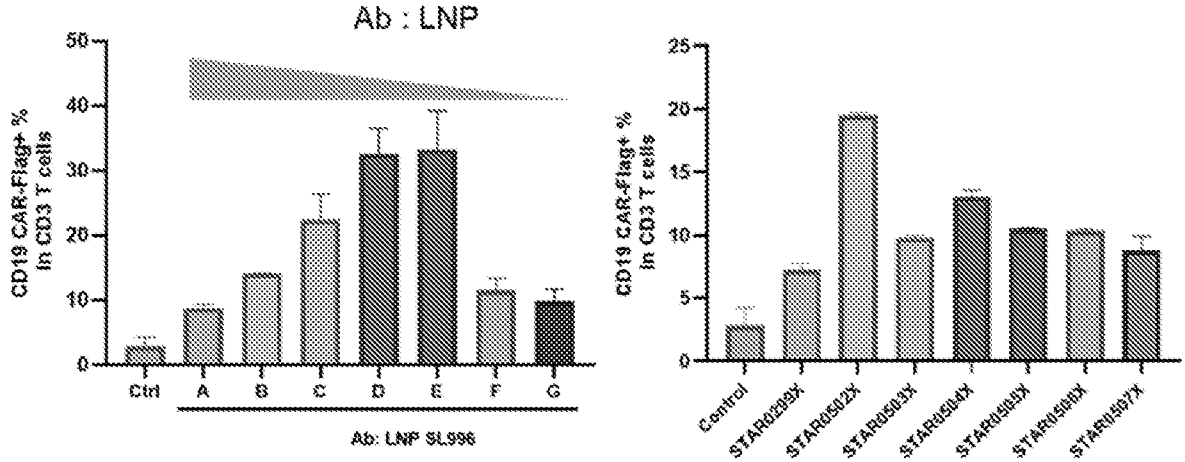


Figure 10

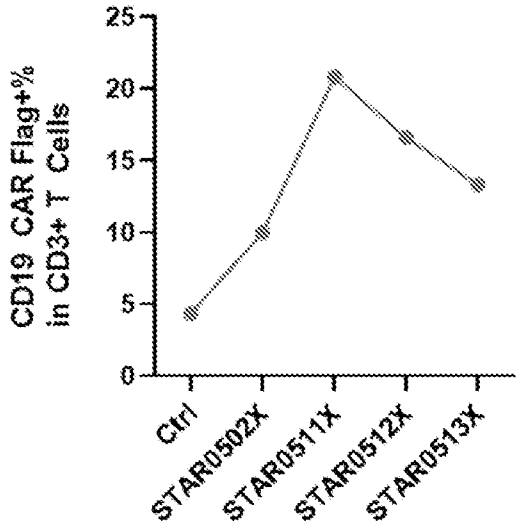


Figure 11

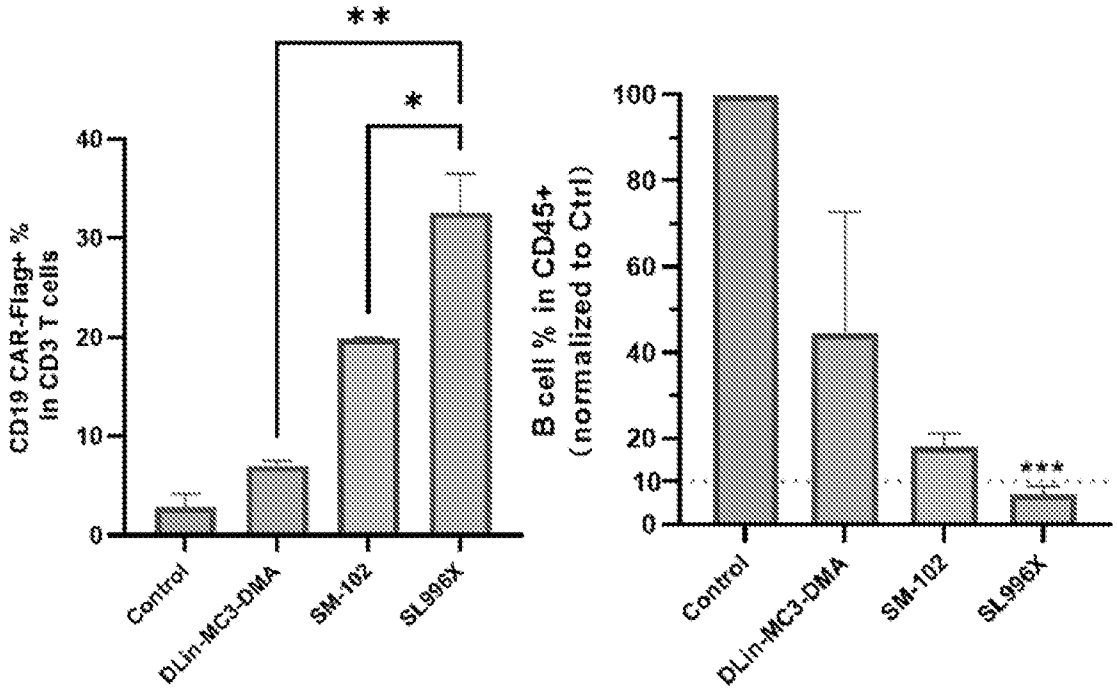


Figure 12

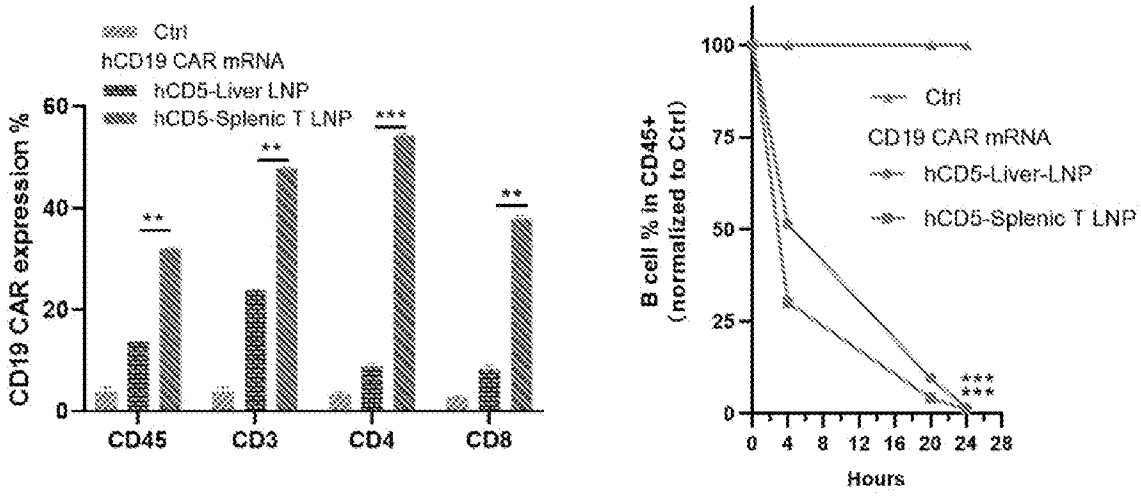


Figure 13

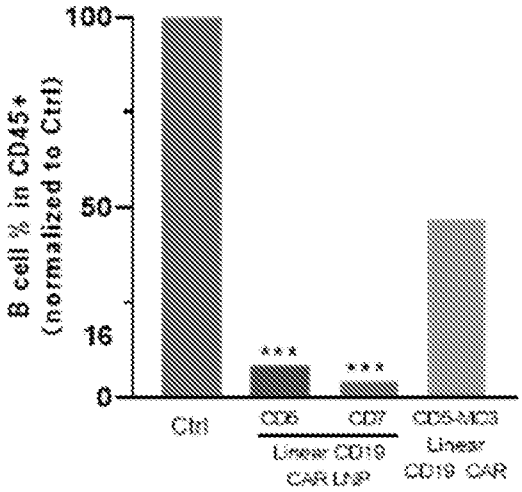


Figure 14

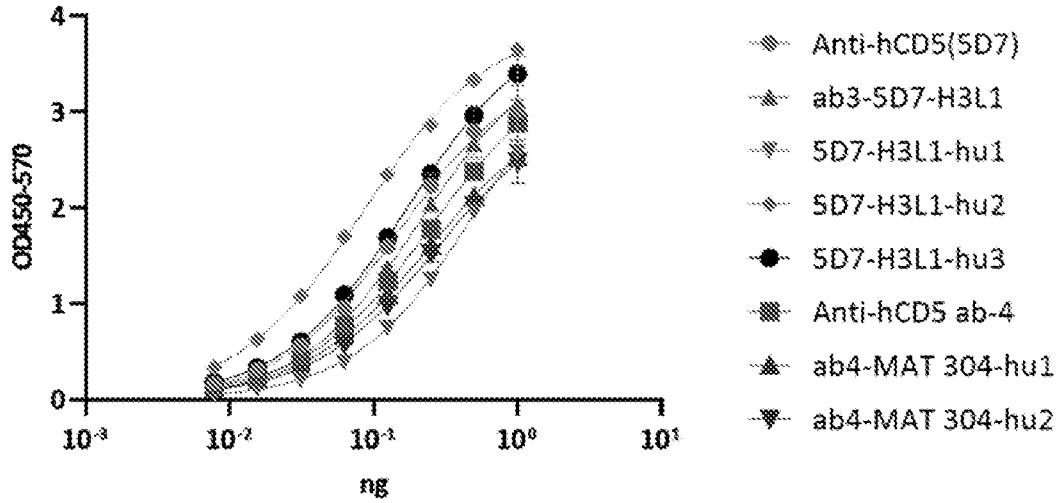
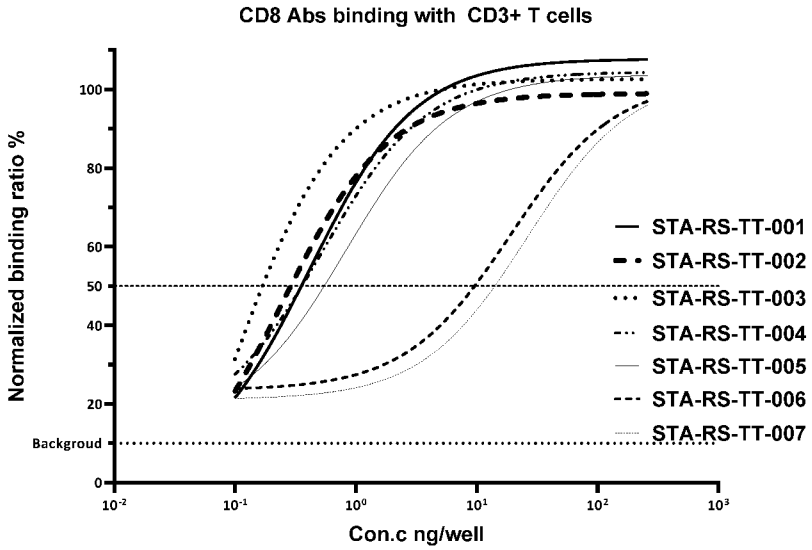


Figure 15a



抗体	EC50 / ng
STA-RS-TT-001	0.42
STA-RS-TT-002	0.25
STA-RS-TT-003	0.09
STA-RS-TT-004	0.53
STA-RS-TT-005	0.83
STA-RS-TT-006	18.99
STA-RS-TT-007	26.11

Figure 15b

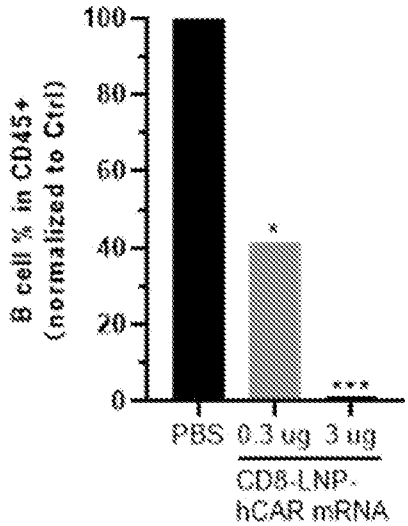


Figure 16

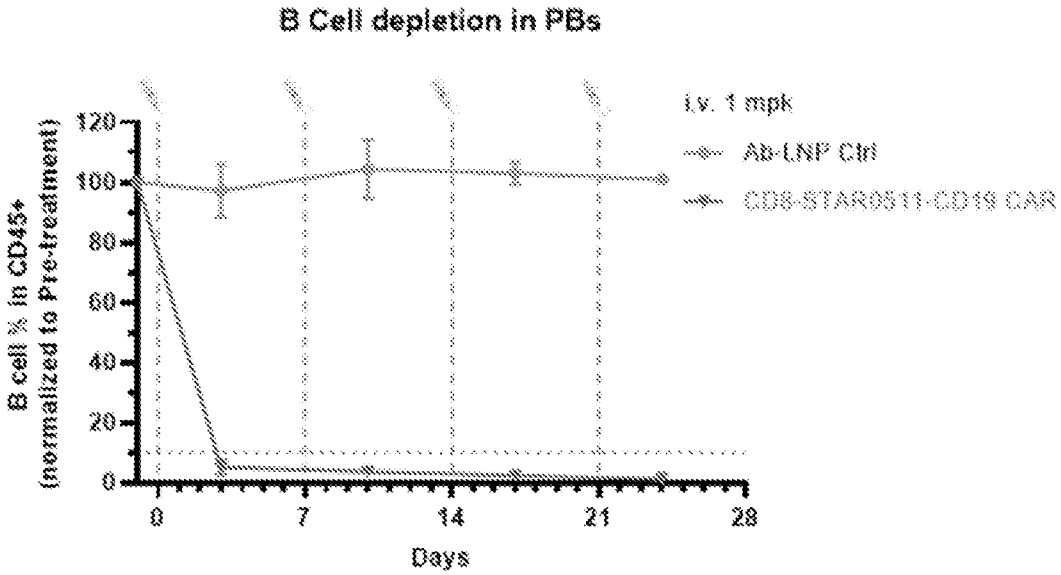


Figure 17a

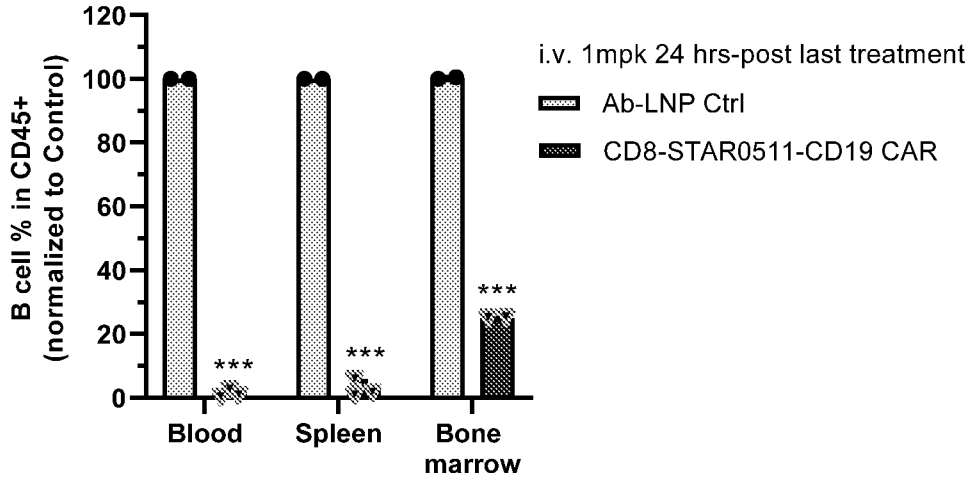


Figure 17b

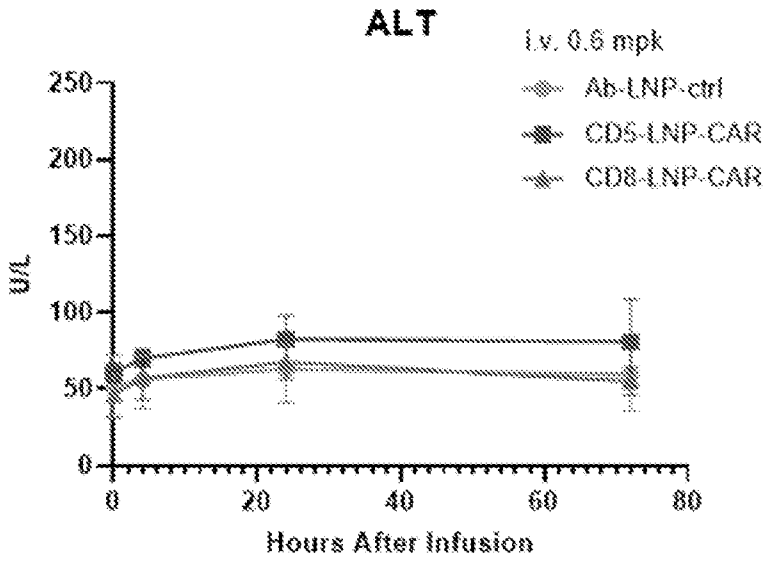


Figure 18a

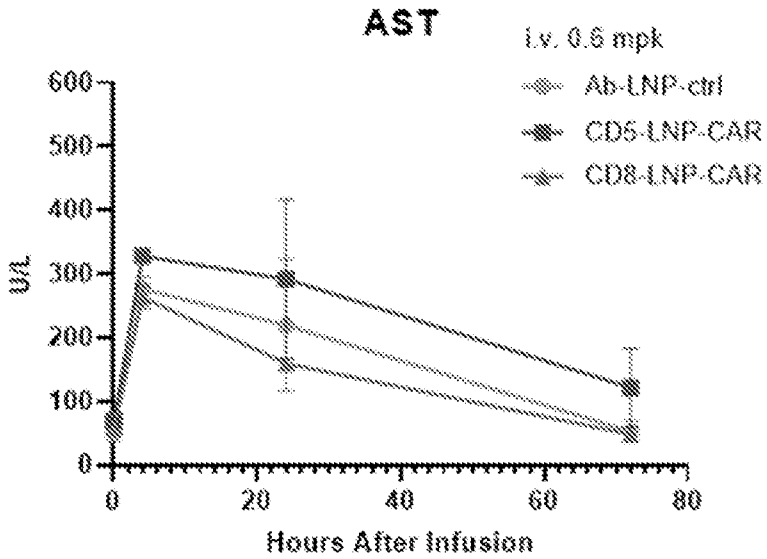


Figure 18b

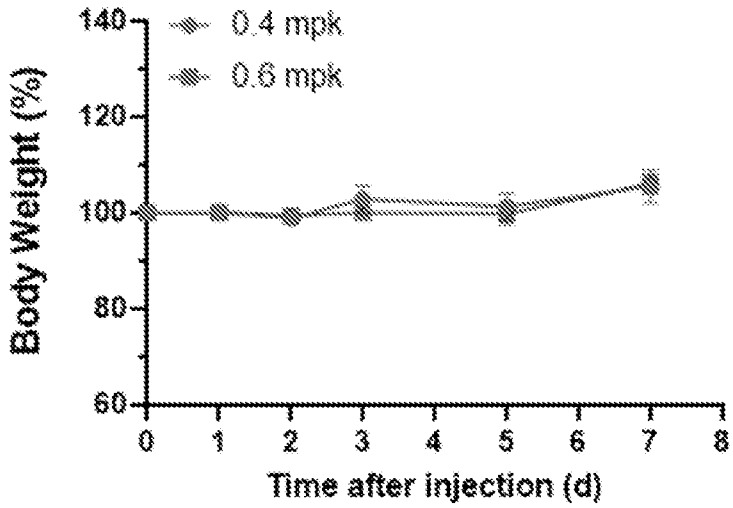


Figure 18c

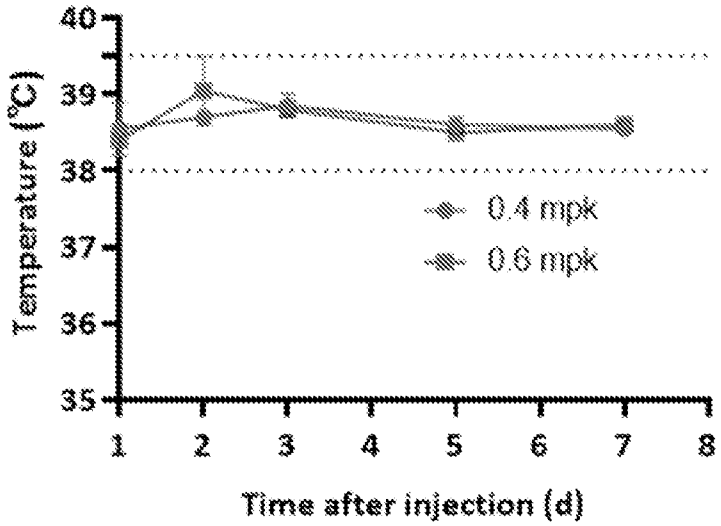


Figure 18d

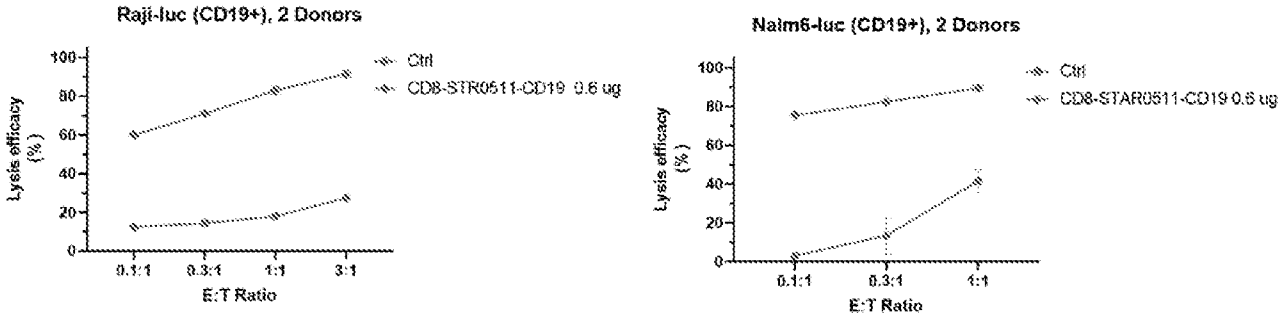


Figure 19

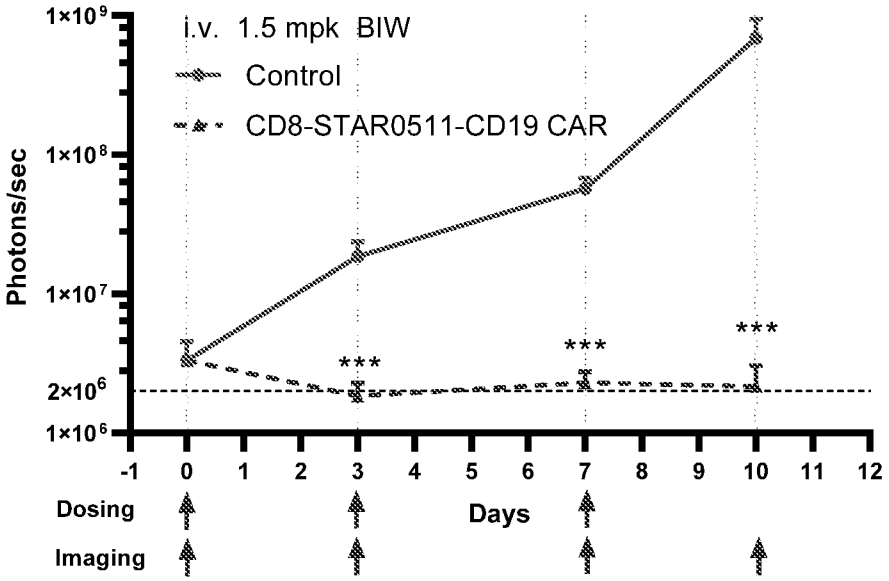

i.v. 1.5 mpk BIW
Control
CD8-STAR0511-CD19 CAR
Photons/sec
1×10⁹
1×10⁸
1×10⁷
2×10⁶
1×10⁶
***
***
***
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
Dosing
Imaging
Days

Figure 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2024/143635

A. CLASSIFICATION OF SUBJECT MATTER

A61K39/00(2006.01)i; C07K14/725(2006.01)i; C07K16/28(2006.01)i; C12N5/10(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC:A61K,C07K,C12N.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

VEN,CNABS, PubMed, DWPI, CNTXT, WOTXT, USTXT, EPTXT, JPTXT, CNKI, Web of Science, Patentics, Genbank,EBI ,STNext:applicant,inventors,antibody,CAR, CD19,CD2,CD3,CD5,CD7,CD8, LNP,mRNA,SL990,Ab-LNP,sequences.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2024249954 A1 (CAPSTAN THERAPEUTICS, INC.) 05 December 2024 (2024-12-05) claims 1–54, description paragraphs 6–19, 54–70, 197–198, 248–269	1,8-11,13-20
X	WO 2010145895 A1 (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE (INSERM),et al) 23 December 2010 (2010-12-23) claims 1–4, SEQ ID NO: 1–2	4
X	WO 2014159940 A1 (MACROGENICS, INC.) 02 October 2014 (2014-10-02) description pages 38–39	6
X	CN 109306012 A (SHANGHAI HRAIN BIOTECHNOLOGY CO., LTD.) 05 February 2019 (2019-02-05) claims 1–10, description pages 2–3	12
Y	WO 2024249954 A1 (CAPSTAN THERAPEUTICS, INC.) 05 December 2024 (2024-12-05) claims 1–54, description paragraphs 6–19, 54–70, 197–198, 248–269	2-7
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☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2025	**25 September 2025**

Name and mailing address of the ISA/CN	Authorized officer
CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China**	**LI,XuYing** Telephone No. **(+86) 010-53961960**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2014159940 A1 (MACROGENICS, INC.) 02 October 2014 (2014-10-02) description pages 38–39	2-7
X	WO 2024077232 A2 (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 11 April 2024 (2024-04-11) claims 1-61	8-20
X	CN 111542338 A (TAKEDA PHARMACEUTICAL COMPANY LIMITED) 14 August 2020 (2020-08-14) claims 1-34	8-20
X	BILLINGSLEY, M. M.et al. "In Vivo mRNA CAR T Cell Engineering via Targeted Ionizable Lipid Nanoparticles with Extrahepatic Tropism" *SMALL*, Vol. 20, 10 December 2023 (2023-12-10), Introduction,Figure 1-2	1-20
X	WANG,S.et al. "Unleashing the Potential: Designing Antibody-Targeted Lipid Nanoparticles for Industrial Applications with CMC Considerations and Clinical Outlook" *MOLECULAR PHARMACEUTICS*, Vol. 21, 20 December 2023 (2023-12-20), ABSTRACT,Figure 2-3	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2024/143635

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2024/143635

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **17-18**
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 17-18 relate to methods of using certain products for treating or preventing diseases and therefore do not warrant an international search under PCT Rule 39.1(iv). Nevertheless, a search has been conducted based on the use of said products in the manufacture of a medicament.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2024/143635

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2024/143635

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				AU	2018397910	B2	13 March 2025
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				EA	202091566	A1	18 September 2020
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