

EXHIBIT 1

US 20250051444A1

(19) **United States**(12) **Patent Application Publication****LIN et al.**(10) **Pub. No.: US 2025/0051444 A1**(43) **Pub. Date: Feb. 13, 2025**(54) **MULTI-SPECIFIC T CELL ENGAGERS
COMPRISING LRRC15 ANTIGEN-BINDING
DOMAIN***A61K 47/68* (2006.01)*A61P 35/00* (2006.01)(52) **U.S. CL.**CPC *C07K 16/2809* (2013.01); *A61K 47/6851*
(2017.08); *A61P 35/00* (2018.01); *C07K*
16/2818 (2013.01); *C07K 16/2875* (2013.01);
C07K 16/2878 (2013.01); *A61K 2039/505*
(2013.01); *C07K 2317/31* (2013.01); *C07K*
2317/55 (2013.01); *C07K 2317/565* (2013.01);
C07K 2317/569 (2013.01); *C07K 2317/622*
(2013.01); *C07K 2317/73* (2013.01); *C07K*
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ZHANG**, Shanghai (CN); **Tongcheng
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Shanghai (CN); **Binhua LV**, Shanghai
(CN); **Zelin SHENG**, Shanghai (CN)(21) Appl. No.: **18/717,574**(22) PCT Filed: **Dec. 12, 2022**(86) PCT No.: **PCT/CN2022/138466**

§ 371 (c)(1),

(2) Date: **Jun. 7, 2024**(30) **Foreign Application Priority Data**

Dec. 10, 2021 (CN) 2021115096596

Publication Classification(51) **Int. Cl.***C07K 16/28* (2006.01)*A61K 39/00* (2006.01)

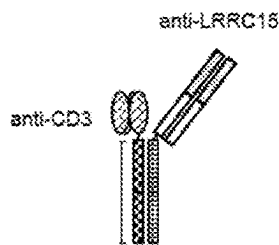
(57)

ABSTRACT

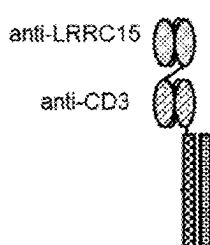
The present invention relates to multi-specific T cell engagers and the use thereof. Specifically, the present invention provides the multi-specific T cell engager, comprising a first targeting domain targeting leucine-rich repeat containing protein 15 (LRRC15) molecules highly expressed on the surface of a tumor cell, and further comprising a second targeting domain and/or a third targeting domain bound to the CD molecules of T cell surface. The LRRC15 related multi-specific T cell engagers of the present invention can effectively activate T cells and reduce the tumor stroma impact, and can be used as an effective therapeutic agent for treatment of tumors, especially tumors with a high expression level of LRRC15.

Specification includes a Sequence Listing.

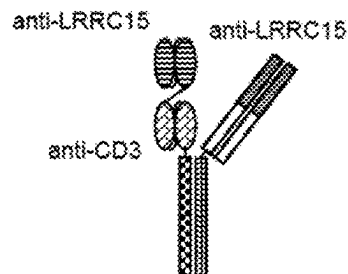
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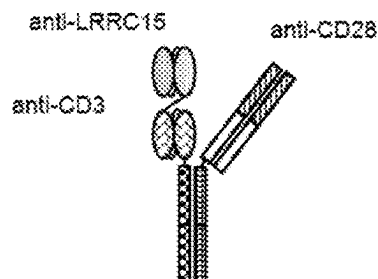
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c



d



e

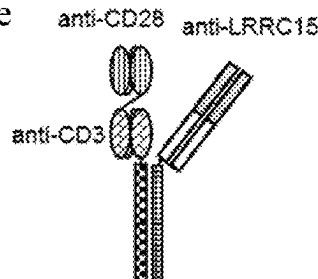




FIG. 1E

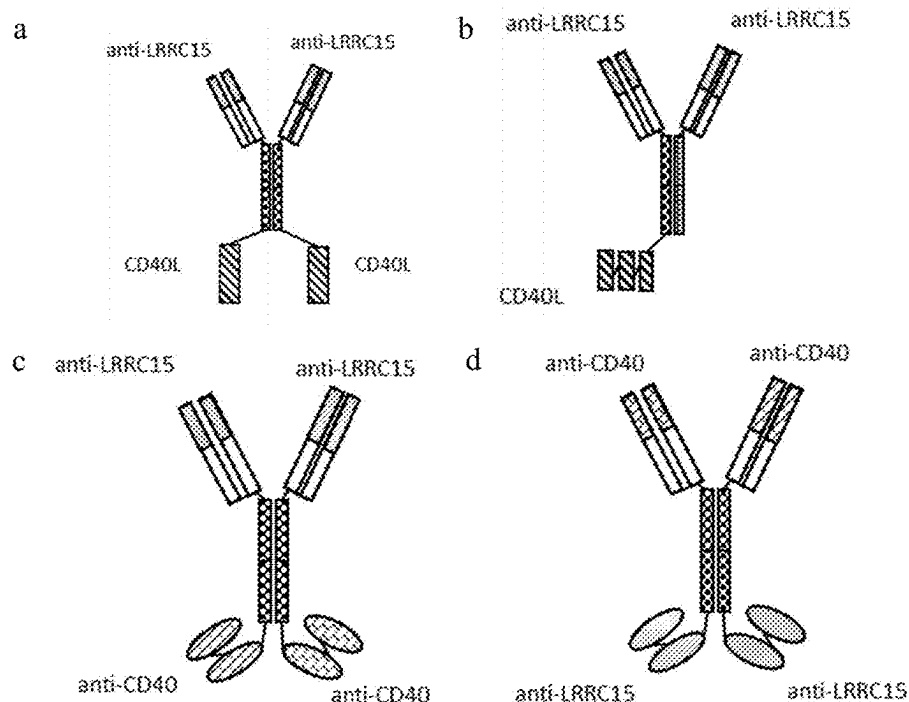


FIG. 2A

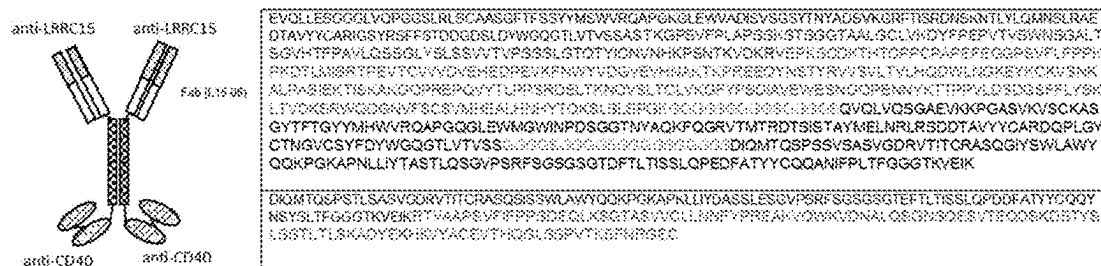


FIG. 2B

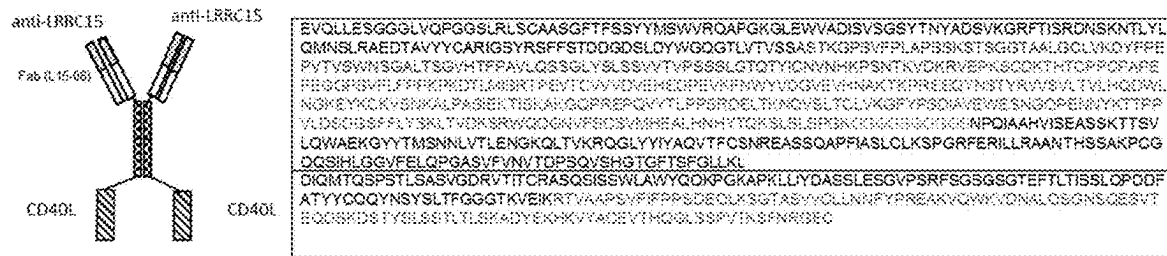


FIG. 2C



FIG. 2D



FIG. 2E

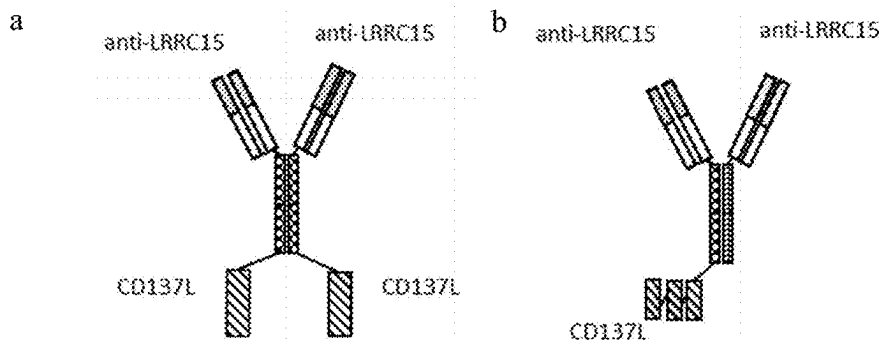


FIG. 3A

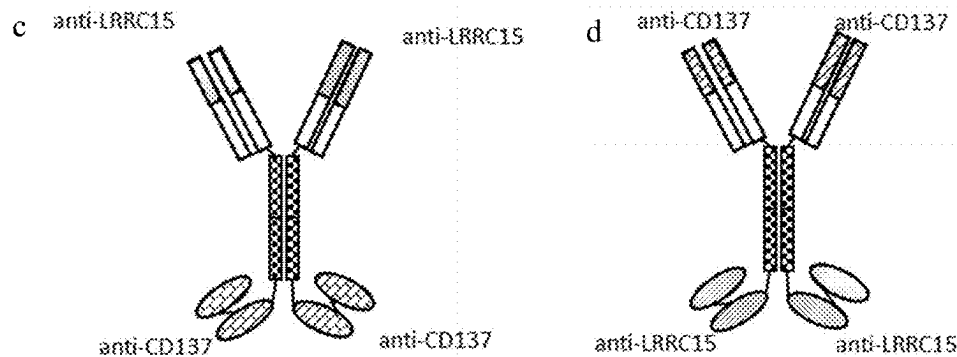
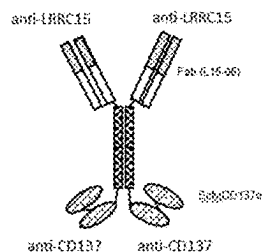


FIG. 3A (Continued)

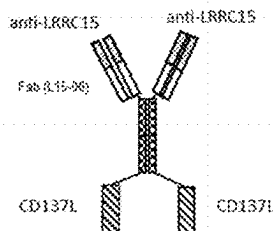


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FIG. 3B

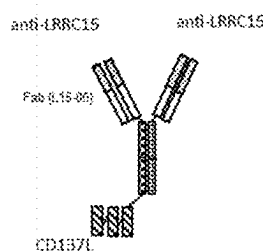


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FIG. 3C



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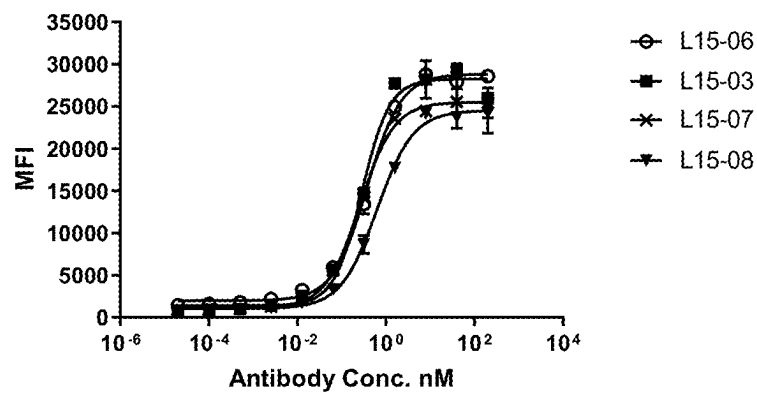
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FIG. 3D



FIG. 3E

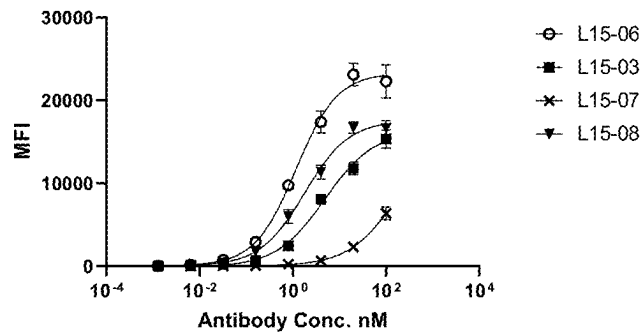
Anti-LRRC15 binding to huLRRC15 expressing CHO cells



	L15-06	L15-03	L15-07	L15-08
EC50	0.3764	0.2855	0.2594	0.6328

FIG. 4A

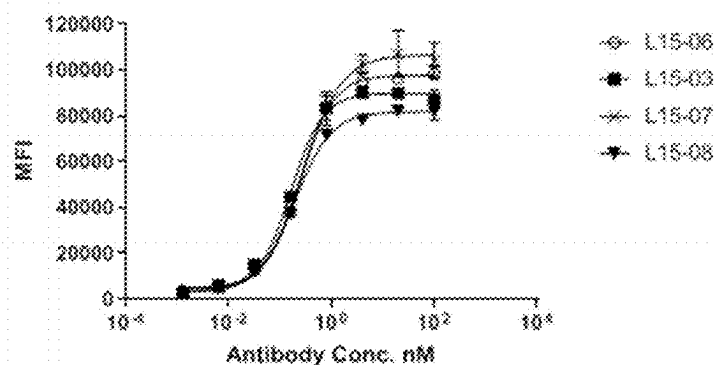
Anti-LRRC15 binding to mLRRC15 expressing CHO cells



	L15-06	L15-03	L15-07	L15-08
EC50	1.169	4.887	185.5	1.781

FIG. 4B

Anti-LRRC15 binding to cynoLRRC15 expressing CHO cells



	L15-06	L15-03	L15-07	L15-08
EC50	0.2392	0.1652	0.2789	0.1695

FIG. 4C

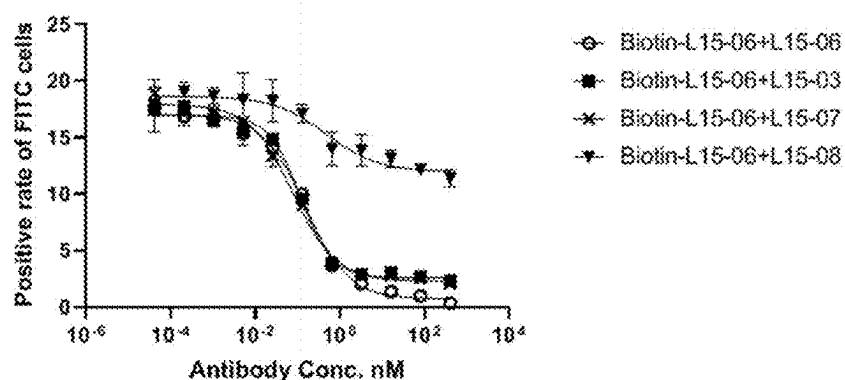
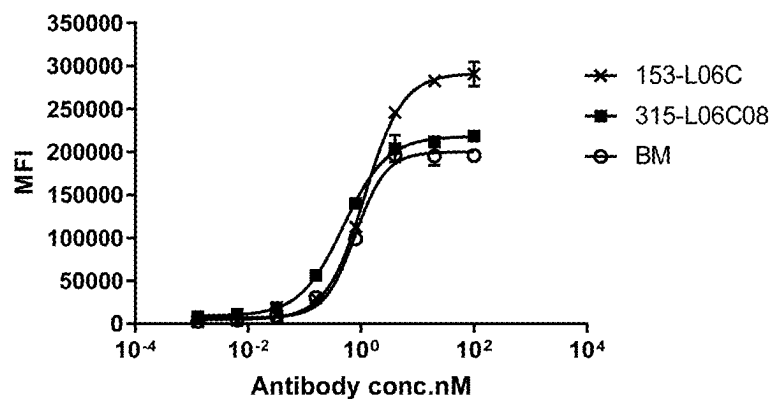


FIG. 4D

Anti-LRRC15 binding to huLRRC15 expressing CHO cells



	153-L06C	315-L06C08	BM
EC50	1.154	0.4883	0.7946

FIG. 5A

Anti-LRRC15 binding to cynoLRRC15 expressing CHO cells

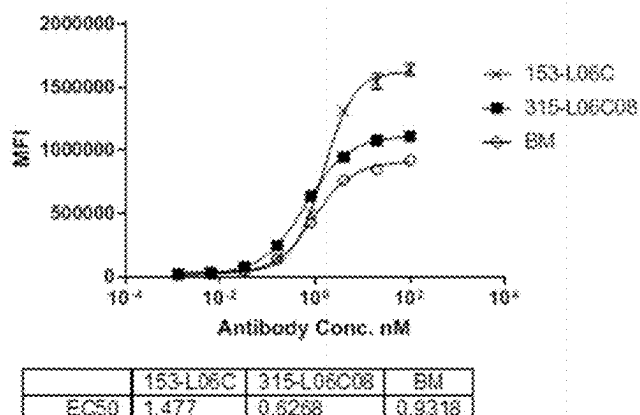


FIG. 5B

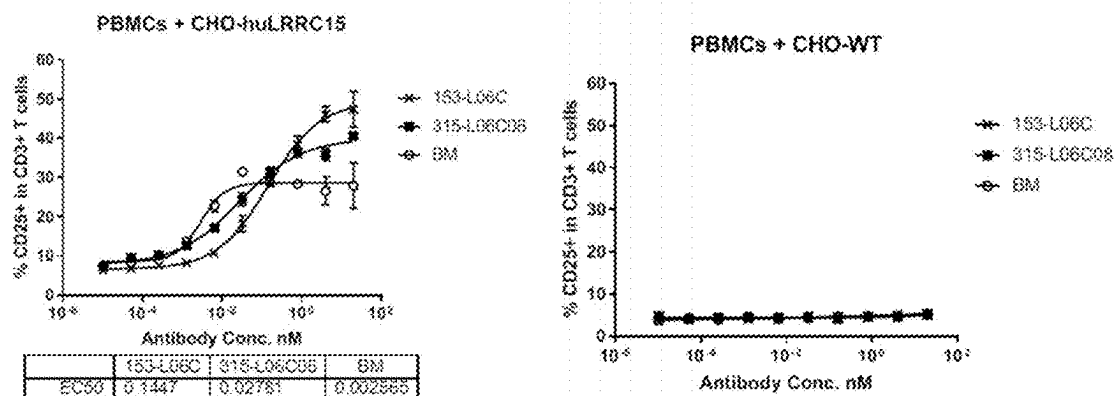


FIG. 6

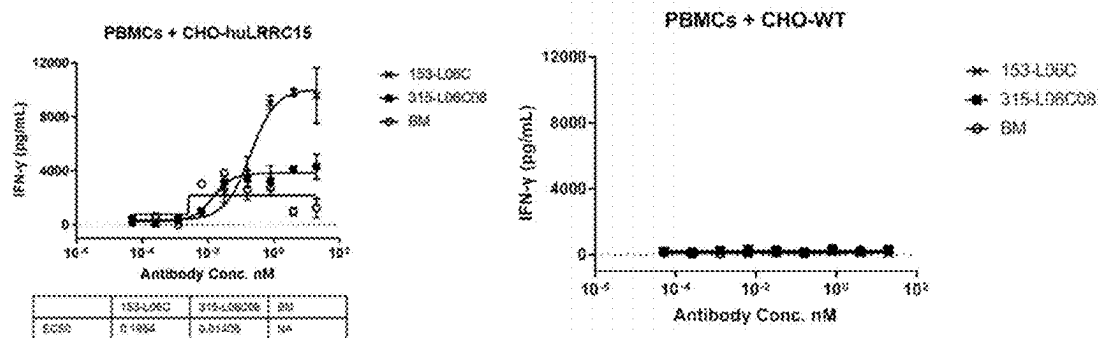


FIG. 7

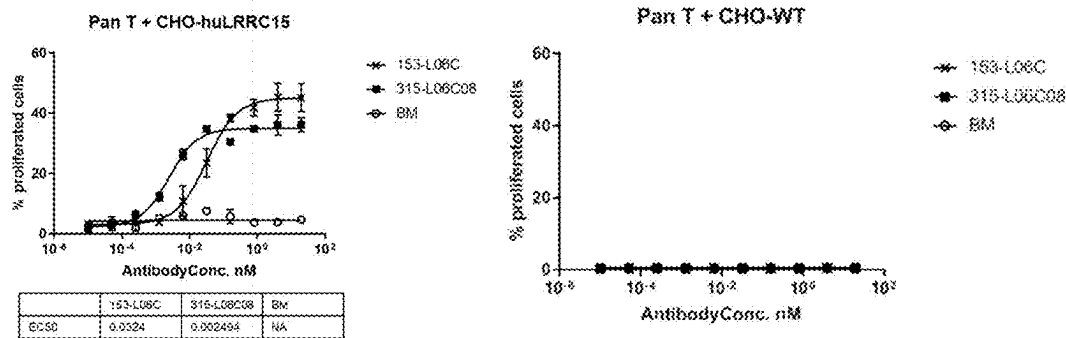


FIG. 8

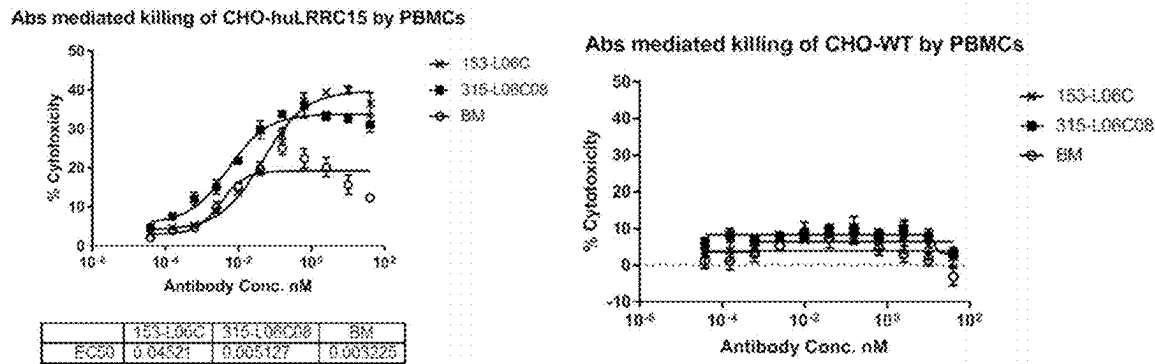


FIG. 9

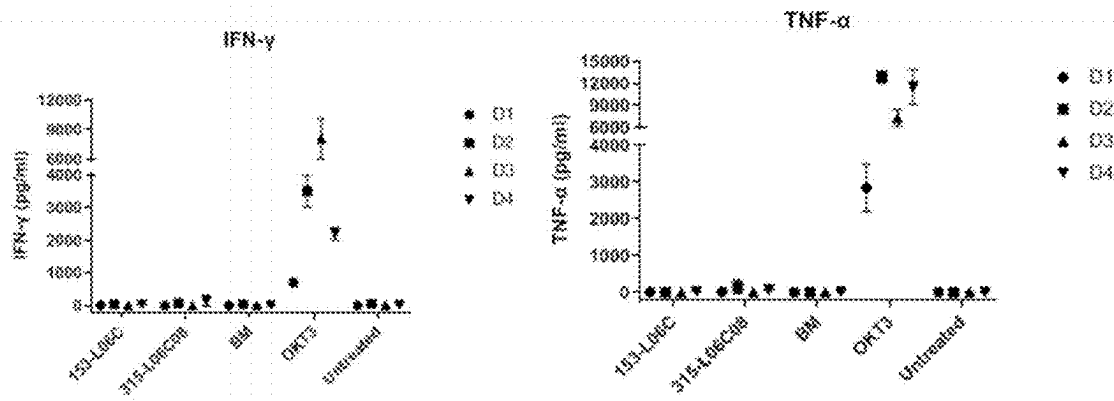


FIG. 10

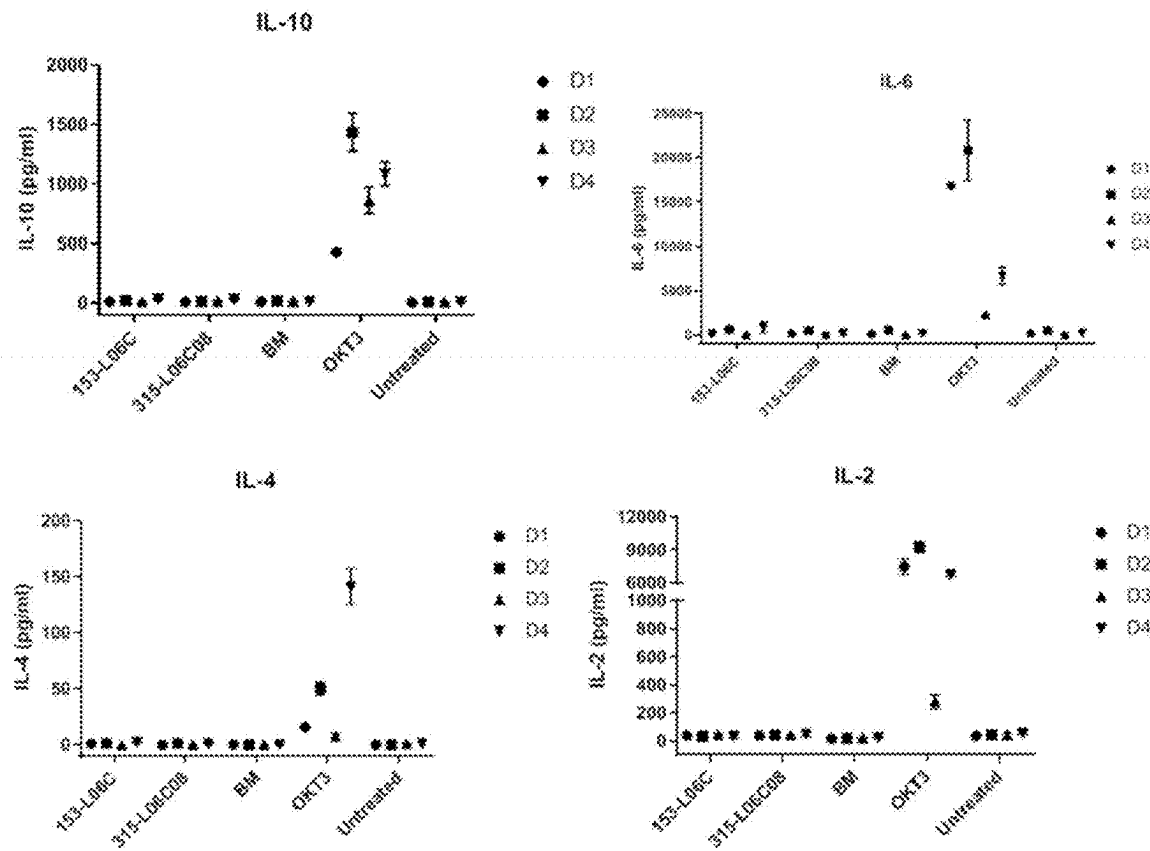


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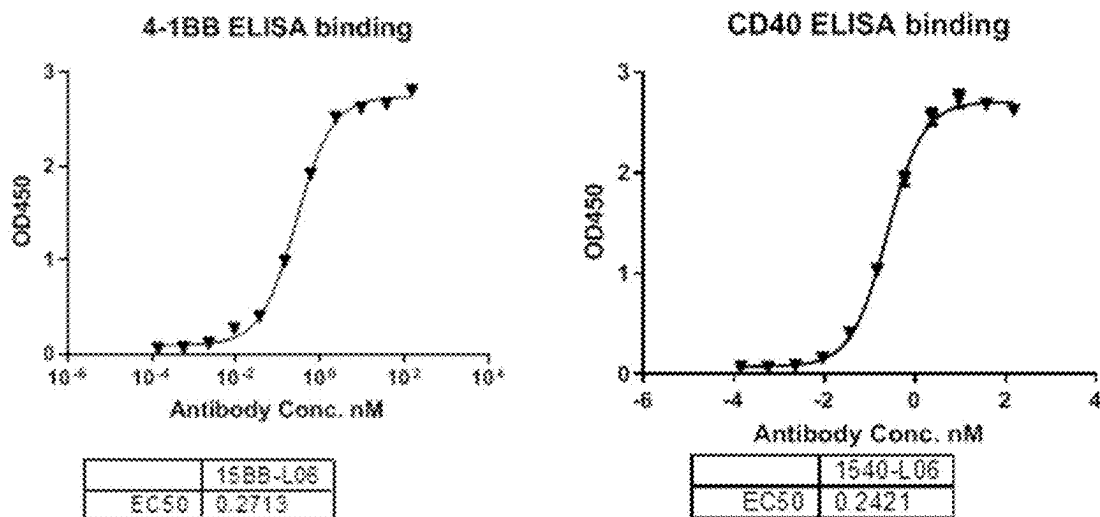


FIG. 11

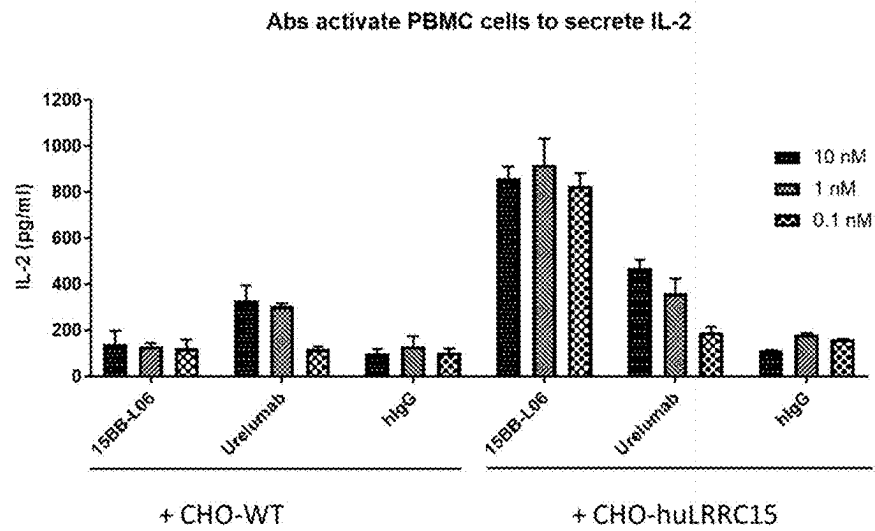


FIG. 12

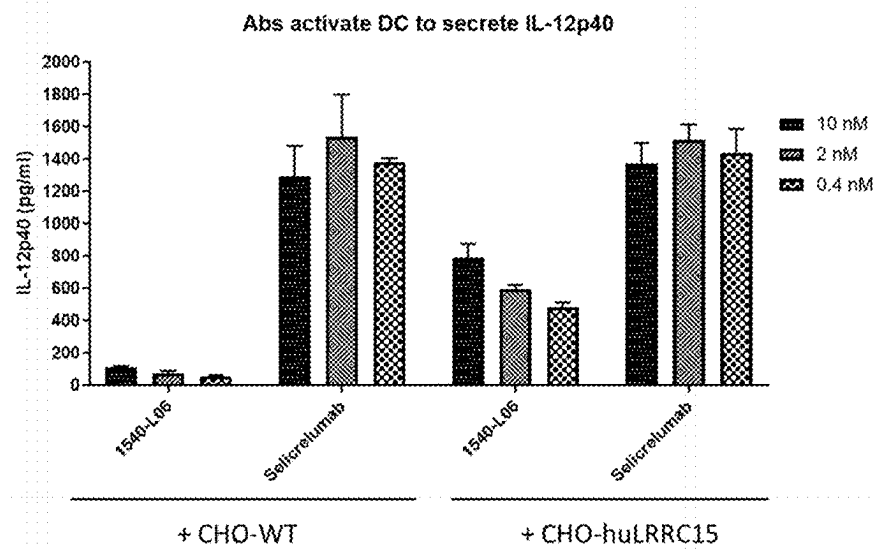


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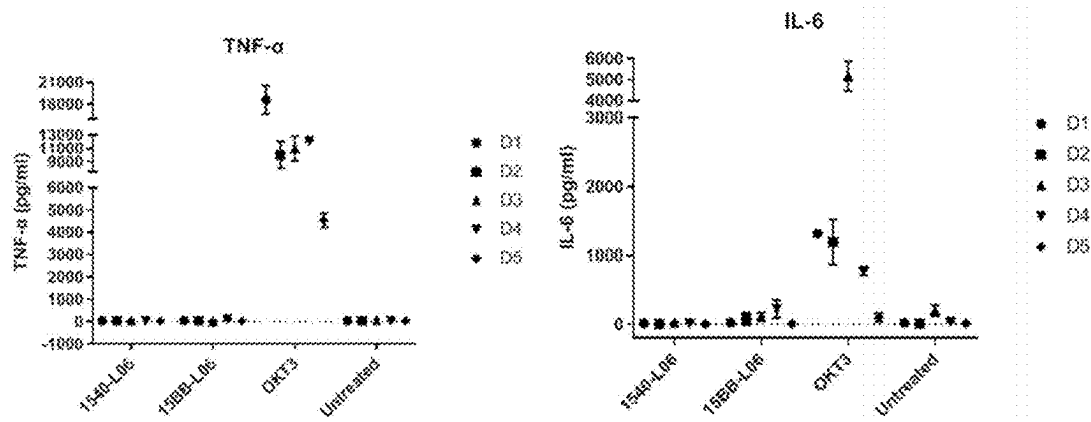


FIG. 14

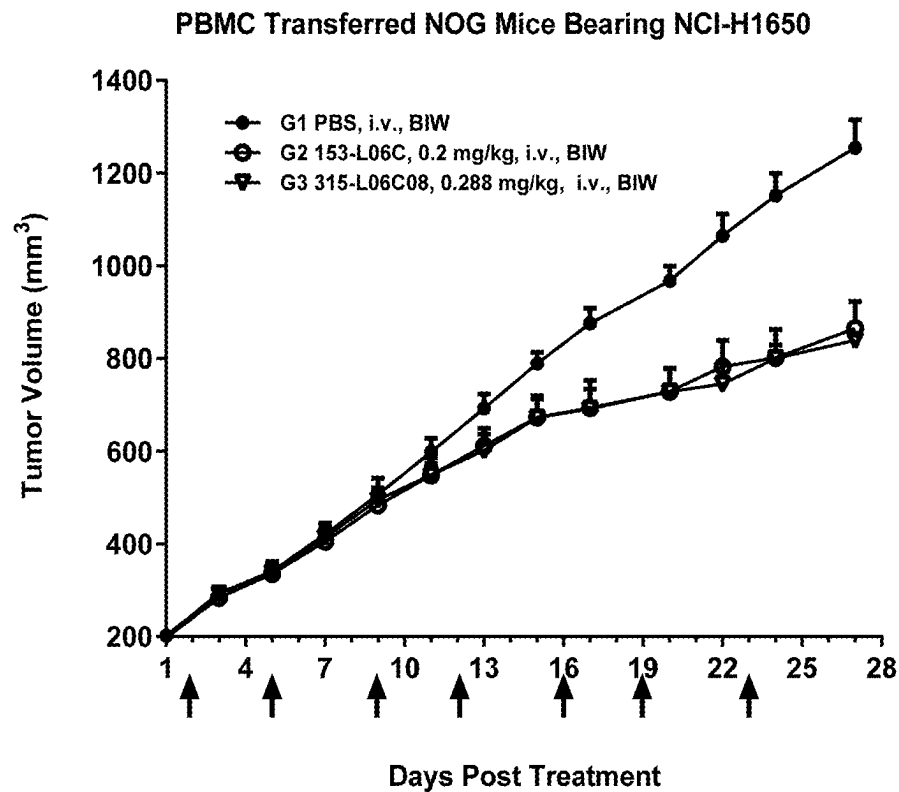


FIG. 15A

PBMC Transferred NOG Mice Bearing NCI-H1650

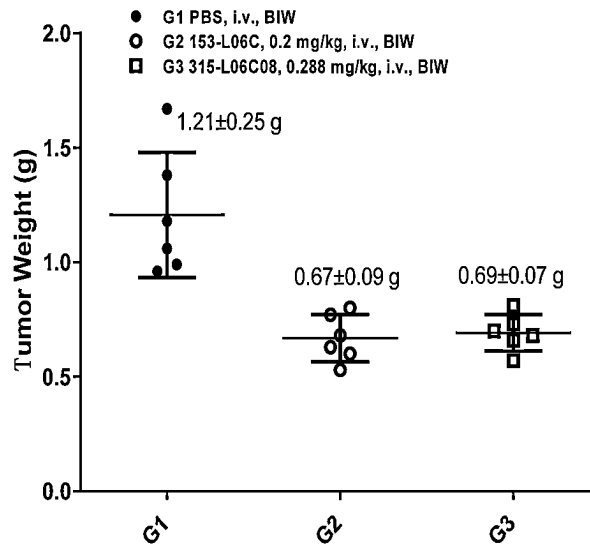


FIG. 15B

C57BL/6-hCD3e/h4-1BB Mice Bearing MC38-huLRRC15

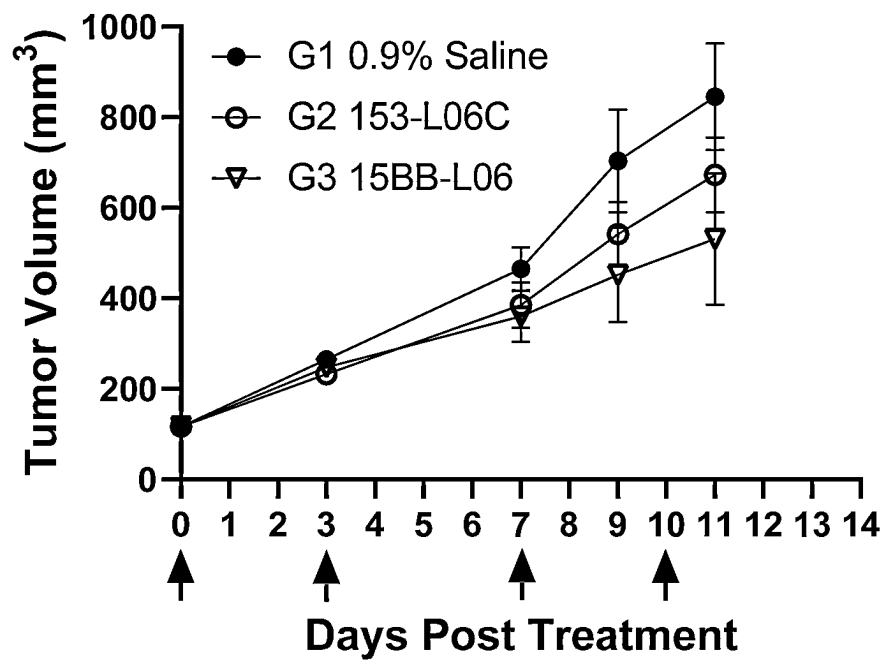


FIG. 15C

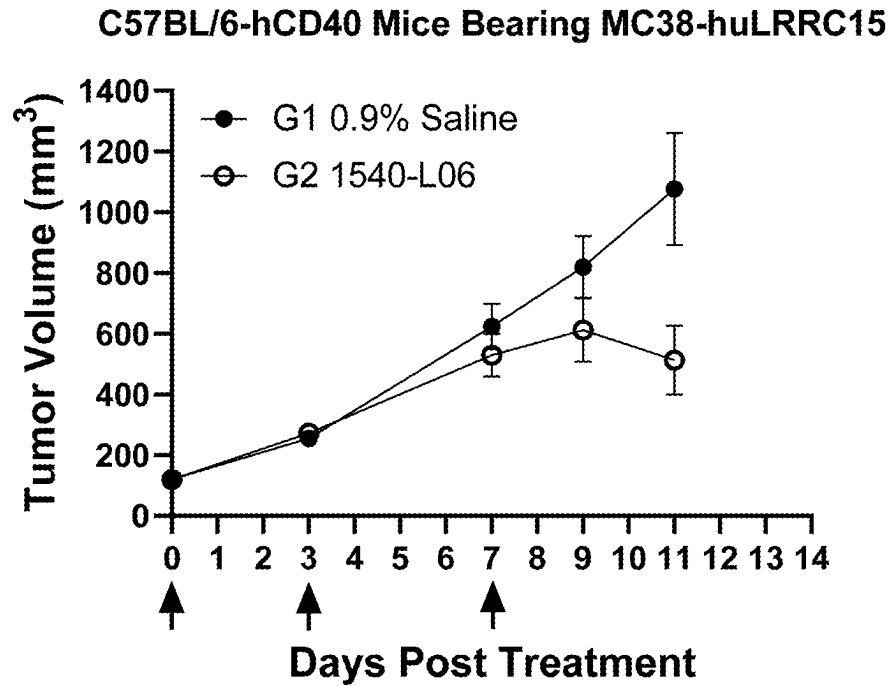


FIG. 15D

Abs mediated killing of CHO-huLRR15 by PBMCs

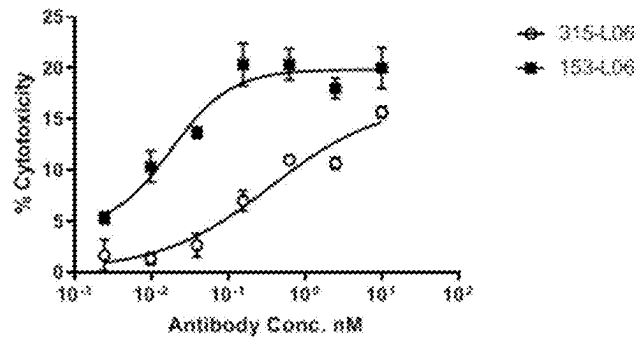
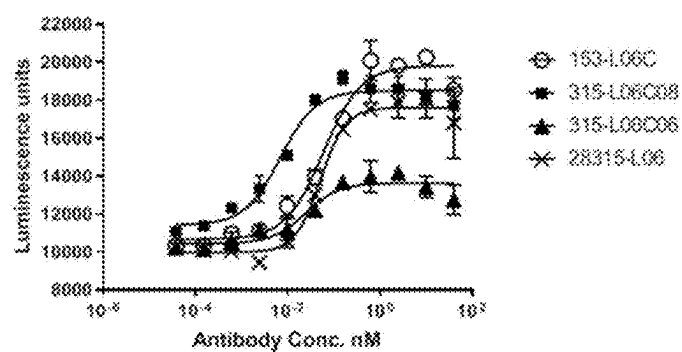


FIG. 16

Ab mediated killing of CHO-huLRRc15 by PBMCs



	153-L06C	315-L06C08	315-L06C06	28315-L06
EC50	0.05921	0.006934	0.03405	0.05098

FIG. 17

US 2025/0051444 A1

Feb. 13, 2025

1

MULTI-SPECIFIC T CELL ENGAGERS COMPRISING LRRC15 ANTIGEN-BINDING DOMAIN

TECHNICAL FIELD

[0001] The present invention relates to the technical field of cancer therapy, in particular, to multi-specific T cell engagers comprising anti-LRCC15 antibody or immunoreactive fragments thereof for cancer treatment.

BACKGROUND

[0002] Cancer is generally defined as a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Conventional cancer treatments are aimed at eliminating cancer tissue and preventing its spread. Such treatment options include surgery, chemotherapy, radiotherapy, hormone therapy, targeted therapy, and palliative care. Treatment is usually based on the type, location, and stage of the cancer, as well as the patient's health and preferences. However, these therapies have limitations, as they may be ineffective, especially when the cancer has already metastasized. In addition, chemotherapy and radiotherapy have a range of side effects associated with their cytotoxicity.

[0003] Currently, promising areas of cancer treatment include the use of antibodies for targeted therapy. Another promising area is the development of therapies that utilize the immune system to attack and kill tumor cells.

[0004] Leucine-rich repeat containing 15 (LRRC15) is a member of the leucine-rich repeat (LRR) superfamily. LRRC15 is highly expressed on tumor cells, including breast cancer, brain tumors, pancreatic cancer, lung cancer, melanoma, highly invasive sarcomas, and breast cancer bone metastasis, while its expression level in normal tissues is relatively low. In addition, it is also highly expressed on stromal fibroblasts in many solid tumors (e.g., breast, head and neck, lung, pancreas). Therefore, LRRC15 is a candidate for targeted therapies such as antibody drug conjugates, T cell engagers, and CAR-T cells.

[0005] CD3, CD28, and CD137 are receptors that are present on T cells. T cells can be activated by antigen-presenting cells through CD3, CD28, and CD137, which utilize MHC Class I/II, CD80 and CD86, and 4-1BBL, respectively. CD3 is part of the T cell receptor (TCR) and is the signaling component of the receptor. CD3 has 3 subunits, epsilon, delta, and gamma. Epsilon is associated with both delta and zeta, which together are responsible for signal transduction. CD3 signaling is considered to be signal 1 required for T cell activation. The co-receptors CD28 and CD137 are considered to be signal 2. Both signal 1 and signal 2 are necessary for full activation, proliferation, and survival of T cells.

[0006] CD40 ligand (CD40L) is mainly expressed by activated T cells. CD40L binds to CD40 on antigen-presenting cells (APCs), thereby inducing APC activation. In turn, APCs induce cytotoxic T lymphocytes (CTLs). CD40 and CD40L are costimulatory molecules that link innate and adaptive immunity.

[0007] Despite the increasing number of therapeutic monoclonal antibodies approved for the treatment of various cancers, resistance to these antibodies is frequently observed, given that cancer growth and metastasis development have many different molecular pathways. Although the immune system is the main mechanism for preventing cancer, cancer cells evade immune surveillance. Therefore, there is an urgent need in the field for improved therapeutic combinations of antagonists or antibodies and methods of using these agents to treat cancer.

SUMMARY OF THE INVENTION

[0008] The present invention provides bispecific/trispecific T cell engagers comprising an LRRC15 antigen binding domain. The bispecific/trispecific T cell engager molecule can bind to LRRC15 and activate CD (cluster of differentiation) molecules (e.g., CD3, CD28, CD137, and CD40). Methods for treating diseases such as cancer using the antibodies and antibody conjugates of the present invention, pharmaceutical compositions and products thereof are also provided.

[0009] In the first aspect of the present invention, it provides a multi-specific T cell engager, which comprises:

[0010] A first targeting domain comprising one or more LRRC15 antigen binding domains;

[0011] A second targeting domain, wherein the second targeting domain binds to a target protein selected from the group consisting of: CD3, CD28, CD40, and CD137; and/or

[0012] optionally, a third targeting domain, wherein the third targeting domain binds to a target protein selected from the group consisting of: CD3, CD28, CD40, and CD137.

[0013] In another preferred embodiment, the targeting domain is in the form of a single domain antibody (sdAb), a fragment variable (Fv) heterodimer, a single chain Fv (scFv), a Fab fragment, a TriFab, or a combination thereof.

[0014] In another preferred embodiment, the LRRC15 antigen binding domain comprises the following three heavy chain variable region CDRs:

[0015] HCDR1 having an amino acid sequence as shown in SEQ ID NO: 32, 35, 38, or 41;

[0016] HCDR2 having an amino acid sequence as shown in SEQ ID NO: 33, 36, 39, or 42; and

[0017] HCDR3 having an amino acid sequence as shown in SEQ ID NO: 34, 37, 40, or 43;

[0018] And, the following three light chain variable region CDRs:

[0019] LCDR1 having an amino acid sequence as shown in SEQ ID NO: 44 or 49;

[0020] LCDR2 having an amino acid sequence as shown in SEQ ID NO: 45, 47, or 50; and

[0021] LCDR3 having an amino acid sequence as shown in SEQ ID NO: 46, 48, 51, or 52.

[0022] In another preferred embodiment, the LRRC15 antigen binding domain comprises three heavy chain variable region CDRs (HCDRs), and three light chain variable region CDRs (LCDRs) selected from the group consisting of:

Antibody Name	SEQ ID NO of HCDR1	SEQ ID NO of HCDR2	SEQ ID NO of HCDR3	SEQ ID NO of LCDR1	SEQ ID NO of LCDR2	SEQ ID NO of LCDR3
L15-06/L15-06C	32	33	34	44	45	46
L15-08	35	36	37	44	47	48
L15-03	38	39	40	49	50	51
L15-07	41	42	43	49	50	52

[0023] In another preferred embodiment, the LRRC15 antigen binding domain comprises the following three heavy chain variable region CDRs:

[0024] HCDR1 having an amino acid sequence as shown in SEQ ID NO: 32;

US 2025/0051444 A1

Feb. 13, 2025

2

[0025] HCDR2 having an amino acid sequence as shown in SEQ ID NO: 33; and

[0026] HCDR3 having an amino acid sequence as shown in SEQ ID NO: 34;

[0027] And, the following three light chain variable region CDRs:

[0028] LCDR1 having an amino acid sequence as shown in SEQ ID NO: 44;

[0029] LCDR2 having an amino acid sequence as shown in SEQ ID NO: 45; and

[0030] LCDR3 having an amino acid sequence as shown in SEQ ID NO: 46.

[0031] In another preferred embodiment, the LRRC15 antigen binding domain comprises the following three heavy chain variable region CDRs:

[0032] HCDR1 having an amino acid sequence as shown in SEQ ID NO: 35;

[0033] HCDR2 having an amino acid sequence as shown in SEQ ID NO: 36; and

[0034] HCDR3 having an amino acid sequence as shown in SEQ ID NO: 37;

[0035] And, the following three light chain variable region CDRs:

[0036] LCDR1 having an amino acid sequence as shown in SEQ ID NO: 44;

[0037] LCDR2 having an amino acid sequence as shown in SEQ ID NO: 47; and

[0038] LCDR3 having an amino acid sequence as shown in SEQ ID NO: 48.

[0039] In another preferred embodiment, the LRRC15 antigen binding domain comprises a heavy chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 1, 2, 3, or 4, and a light chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 5, 6, 7, or 8.

[0040] In another preferred embodiment, the LRRC15 antigen binding domain comprises a heavy chain variable region as shown in SEQ ID NO: 1, and a light chain variable region as shown in SEQ ID NO: 5.

[0041] In another preferred embodiment, the LRRC15 antigen binding domain comprises a heavy chain variable region as shown in SEQ ID NO: 2, and a light chain variable region as shown in SEQ ID NO: 6.

[0042] In another preferred embodiment, the LRRC15 antigen binding domain comprises a heavy chain variable region as shown in SEQ ID NO: 3, and a light chain variable region as shown in SEQ ID NO: 7.

[0043] In another preferred embodiment, the LRRC15 antigen binding domain comprises a heavy chain variable region as shown in SEQ ID NO: 4, and a light chain variable region as shown in SEQ ID NO: 8.

[0044] In another preferred embodiment, the LRRC15 antigen binding domain comprises a heavy chain variable region as shown in SEQ ID NO: 53, and a light chain variable region as shown in SEQ ID NO: 54.

[0045] In another preferred embodiment, the LRRC15 antigen binding domain is selected from the group consisting of: scFv, Fab, and a combination thereof.

[0046] In another preferred embodiment, the LRRC15 antigen binding domain is scFv.

[0047] In another preferred embodiment, the LRRC15 antigen binding domain is Fab, which comprises a heavy

chain variable region as shown in SEQ ID NO: 1, 2, 3, 4 or 53, and a light chain variable region as shown in SEQ ID NO: 5, 6, 7, 8 or 54; and

[0048] A heavy chain constant region CH1 as shown in SEQ ID NO: 26 or 28, and a light chain constant region CL as shown in SEQ ID NO: 30 or 31.

[0049] In another preferred embodiment, the second targeting domain is selected from the group consisting of: CD3 antigen binding domain, CD28 antigen binding domain, CD40 antigen binding domain, CD137 antigen binding domain, CD40L sequence, and CD137L sequence.

[0050] In another preferred embodiment, the third targeting domain is selected from the group consisting of: CD3 antigen binding domain, CD28 antigen binding domain, CD40 antigen binding domain, CD137 antigen binding domain, CD40L sequence, and CD137L sequence.

[0051] In another preferred embodiment, the multi-specific T cell engager further comprises an Fc fragment.

[0052] In another preferred embodiment, the Fc fragment is derived from IgG1 or IgG4.

[0053] In another preferred embodiment, the Fc fragment is an Fc fragment derived from IgG1, which has an amino acid sequence as shown in SEQ ID NO: 27.

[0054] In another preferred embodiment, the Fc fragment derived from IgG1 has mutations selected from the group consisting of:

[0055] N297A,

[0056] L234F/L235E/P331S,

[0057] Y349C/K370E/K409D/K439E,

[0058] S354C/D356K/E357K/D399K,

[0059] S354C/T366W,

[0060] Y349C/T366S/L368A/Y407V,

[0061] L234F/L235E/P331S/Y349C/K370E/K409D/K439E,

[0062] L234F/L235E/P331S/S354C/D356K/E357K/D399K,

[0063] L234F/L235E/P331S/S354C/T366W,

[0064] L234F/L235E/P331S/Y349C/T366S/L368A/Y407V,

[0065] N297A/Y349C/K370E/K409D/K439E,

[0066] N297A/S354C/D356K/E357K/D399K,

[0067] N297A/S354C/T366W,

[0068] N297A/Y349C/T366S/L368A/Y407V; and/or

[0069] S267E, S267E/G236D, S267E/S239D, S267E/L328F.

[0070] In another preferred embodiment, the Fc fragment has an amino acid sequence as shown in any one of SEQ ID NOs: 57-60.

[0071] In another preferred embodiment, the Fc fragment is an Fc fragment derived from IgG4, which has an amino acid sequence as shown in SEQ ID NO: 29.

[0072] In another preferred embodiment, the Fc fragment derived from IgG4 has mutations selected from the group consisting of:

[0073] Y349C/K370E/R409D/K439E,

[0074] S354C/E356K/E357K/D399K,

[0075] S354C/T366W,

[0076] Y349C/T366S/L368A/Y407V; and/or

[0077] S228P/S267E,

[0078] S228P/S267E/G236D,

[0079] S228P/S267E/S239D,

[0080] S228P/S267E/L328F.

[0081] In another preferred embodiment, the multi-specific T cell engager is a bispecific/trispecific T cell engager.

Exhibit 1

Page 47

US 2025/0051444 A1

Feb. 13, 2025

3

[0082] In another preferred embodiment, the multi-specific T cell engager is a bispecific T cell engager.

[0083] In another preferred embodiment, the bispecific T cell engager comprises: A first targeting domain comprising one or more LRRC15 antigen binding domains; and

[0084] A second targeting domain, wherein the second targeting domain is a CD3 antigen binding domain.

[0085] In another preferred embodiment, the bispecific T cell engager comprises: A first targeting domain comprising one or more LRRC15 antigen binding domains; and

[0086] A second targeting domain, wherein the second targeting domain is a CD40 antigen binding domain.

[0087] In another preferred embodiment, the bispecific T cell engager comprises:

[0088] A first targeting domain comprising one or more LRRC15 antigen binding domains; and

[0089] A second targeting domain, wherein the second targeting domain is a CD137 antigen binding domain.

[0090] In another preferred embodiment, the bispecific T cell engager comprises:

[0091] A first targeting domain comprising one or more LRRC15 antigen binding domains; and

[0092] A second targeting domain, wherein the second targeting domain is one or more CD40L sequences.

[0093] In another preferred embodiment, the bispecific T cell engager comprises:

[0094] A first targeting domain comprising one or more LRRC15 antigen binding domains; and

[0095] A second targeting domain, wherein the second targeting domain is one or more CD137L sequences.

[0096] In another preferred embodiment, the multi-specific T cell engager is a trispecific T cell engager.

[0097] In another preferred embodiment, the trispecific T cell engager comprises:

[0098] A first targeting domain comprising one or more LRRC15 antigen binding domains;

[0099] A second targeting domain, wherein the second targeting domain is a CD3 antigen binding domain; and

[0100] A third targeting domain, wherein the third targeting domain is a CD28 antigen binding domain.

[0101] In another preferred embodiment, the multi-specific T cell engager is a trispecific T cell engager.

[0102] In another preferred embodiment, the trispecific T cell engager comprises:

[0103] A first targeting domain comprising one or more LRRC15 antigen binding domains;

[0104] A second targeting domain, wherein the second targeting domain is a CD3 antigen binding domain; and

[0105] A third targeting domain, wherein the third targeting domain is a CD40 antigen binding domain.

[0106] In another preferred embodiment, the multi-specific T cell engager is a trispecific T cell engager.

[0107] In another preferred embodiment, the trispecific T cell engager comprises:

[0108] A first targeting domain comprising one or more LRRC15 antigen binding domains;

[0109] A second targeting domain, wherein the second targeting domain is a CD3 antigen binding domain; and

[0110] A third targeting domain, wherein the third targeting domain is a CD137 antigen binding domain.

[0111] In another preferred embodiment, the multi-specific T cell engager is a trispecific T cell engager.

[0112] In another preferred embodiment, the trispecific T cell engager comprises:

[0113] A first targeting domain comprising one or more LRRC15 antigen binding domains;

[0114] A second targeting domain, wherein the second targeting domain is a CD3 antigen binding domain; and

[0115] A third targeting domain, wherein the third targeting domain is a CD40L sequence.

[0116] In another preferred embodiment, the trispecific T cell engager comprises:

[0117] A first targeting domain comprising one or more LRRC15 antigen binding domains;

[0118] A second targeting domain, wherein the second targeting domain is a CD3 antigen binding domain; and

[0119] A third targeting domain, wherein the third targeting domain is a CD137L sequence.

[0120] In another preferred embodiment, the CD3 antigen binding domain comprises a heavy chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 11, 9, 10, or 12, and a light chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 13.

[0121] In another preferred embodiment, the CD3 antigen binding domain is selected from the group consisting of: scFv, Fab, and a combination thereof.

[0122] In another preferred embodiment, the CD28 antigen binding domain comprises a heavy chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 14, and a light chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 15.

[0123] In another preferred embodiment, the CD28 antigen binding domain is selected from the group consisting of: scFv, Fab, and a combination thereof.

[0124] In another preferred embodiment, the CD40 antigen binding domain comprises a heavy chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 16, and a light chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 17.

[0125] In another preferred embodiment, the CD40 antigen binding domain comprises a heavy chain variable region with the amino acid sequence shown in SEQ ID NO: 55, and a light chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 56.

[0126] In another preferred embodiment, the CD40 antigen binding domain is selected from the group consisting of: scFv, Fab, and a combination thereof.

[0127] In another preferred embodiment, the CD137 antigen binding domain comprises a heavy chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 18 or 20, and a light chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 19 or 21.

[0128] In another preferred embodiment, the CD137 antigen binding domain is selected from the group consisting of: scFv, Fab, and a combination thereof.

[0129] In another preferred embodiment, the CD40L sequence is the RBD receptor binding domain sequence of CD40L, which has the amino acid sequence shown in SEQ ID NO: 23.

[0130] In another preferred embodiment, the CD137L sequence is the RBD receptor binding domain sequence of CD137L, which has the amino acid sequence shown in SEQ ID NO: 25.

US 2025/0051444 A1

Feb. 13, 2025

4

[0131] In another preferred embodiment, the multi-specific T cell engager has a structure as shown in Formula I:



[0132] wherein “-” each independently represents a peptide bond or a linker peptide; “||” represents a linking bond between the polypeptide chains.

[0133] scFv1 is the first targeting domain, and the scFv1 is an anti-LRRC15 scFv;

[0134] scFv2 is the second targeting domain, and the scFv2 is an anti-CD3 scFv;

[0135] Fc1 and Fc2 each independently represent an Fc fragment.

[0136] In another preferred embodiment, the anti-LRRC15 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 1, 2, 3, 4 or 53, and a light chain variable region as shown in SEQ ID NO: 5, 6, 7, 8 or 54.

[0137] In another preferred embodiment, the LRRC15 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 53, and a light chain variable region as shown in SEQ ID NO: 54.

[0138] In another preferred embodiment, the anti-CD3 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 11, 9, 10, or 12, and a light chain variable region as shown in SEQ ID NO: 13.

[0139] In another preferred embodiment, the anti-CD3 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 11, and a light chain variable region as shown in SEQ ID NO: 13.

[0140] In another preferred embodiment, the Fc fragment is derived from IgG1 or IgG4.

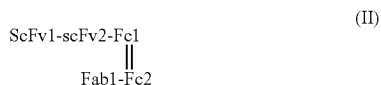
[0141] In another preferred embodiment, the Fc fragment is derived from IgG1.

[0142] In another preferred embodiment, the Fc fragment has an amino acid sequence as shown in SEQ ID NO: 57 or 58.

[0143] In another preferred embodiment, the Fc1 has an amino acid sequence as shown in SEQ ID NO: 57, and the Fc2 has an amino acid sequence as shown in SEQ ID NO: 58.

[0144] In another preferred embodiment, the amino acid sequence of “scFv1-scFv2-Fc1” is as shown in SEQ ID NO: 64; the amino acid sequence of “Fc2” is as shown in SEQ ID NO: 58.

[0145] In another preferred embodiment, the multi-specific T cell engager has a structure as shown in Formula II (FIG. 1A-c):



[0146] wherein “-” each independently represents a peptide bond or a linker peptide; “||” represents a linking bond between the polypeptide chains.

[0147] scFv1 and Fab1 are the first targeting domains, the scFv1 is an anti-LRRC15 scFv, and the Fab1 is an anti-LRRC15 Fab;

[0148] scFv2 is the second targeting domain, and the scFv2 is an anti-CD3 scFv; Fc1 and Fc2 each independently represent an Fc fragment.

[0149] In another preferred embodiment, the anti-LRRC15 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 1, 2, 3, 4 or 53, and a light chain variable region as shown in SEQ ID NO: 5, 6, 7, 8 or 54.

[0150] In another preferred embodiment, the anti-LRRC15 Fab comprises a heavy chain variable region as shown in SEQ ID NO: 1, 2, 3, 4 or 53, and a light chain variable region as shown in SEQ ID NO: 5, 6, 7, 8 or 54.

[0151] In another preferred embodiment, the LRRC15 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 53, and a light chain variable region as shown in SEQ ID NO: 54.

[0152] In another preferred embodiment, the anti-LRRC15 Fab comprises a heavy chain variable region as shown in SEQ ID NO: 2, and a light chain variable region as shown in SEQ ID NO: 6.

[0153] In another preferred embodiment, the anti-CD3 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 11, 9, 10, or 12, and a light chain variable region as shown in SEQ ID NO: 13.

[0154] In another preferred embodiment, the anti-CD3 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 11, and a light chain variable region as shown in SEQ ID NO: 13.

[0155] In another preferred embodiment, the Fc fragment is derived from IgG1 or IgG4.

[0156] In another preferred embodiment, the Fc fragment is derived from IgG1.

[0157] In another preferred embodiment, the Fc fragment has an amino acid sequence as shown in SEQ ID NO: 57 or 58.

[0158] In another preferred embodiment, the Fc1 has an amino acid sequence as shown in SEQ ID NO: 57, and the Fc2 has an amino acid sequence as shown in SEQ ID NO: 58.

[0159] In another preferred embodiment, the amino acid sequence of “scFv1-scFv2-Fc1” is as shown in SEQ ID NO: 64.

[0160] In another preferred embodiment, in the “Fab1-Fc2”, the amino acid sequence of “HC1 (heavy chain)-Fc2” is as shown in SEQ ID NO: 81, and the amino acid sequence of “LC1 (light chain)” is as shown in SEQ ID NO: 84.

[0161] In another preferred embodiment, the multi-specific T cell engager has a structure as shown in Formula III (FIG. 2A-c):



[0162] wherein “-” each independently represents a peptide bond or a linker peptide; “||” represents a linking bond between the polypeptide chains.

[0163] Fab1 is the first targeting domain, and the Fab1 is an anti-LRRC15 Fab;

[0164] scFv3 is the second targeting domain, and the scFv3 is an anti-CD40 scFv;

[0165] Fc3 is an Fc fragment.

US 2025/0051444 A1

Feb. 13, 2025

5

[0166] In another preferred embodiment, the “|” is a disulfide bond.

[0167] In another preferred embodiment, the anti-LRRC15 Fab comprises a heavy chain variable region as shown in SEQ ID NO: 1, 2, 3, 4 or 53, and a light chain variable region as shown in SEQ ID NO: 5, 6, 7, 8 or 54.

[0168] In another preferred embodiment, the LRRC15 Fab comprises a heavy chain variable region as shown in SEQ ID NO: 1, and a light chain variable region as shown in SEQ ID NO: 5.

[0169] In another preferred embodiment, the anti-CD40 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 16, and a light chain variable region as shown in SEQ ID NO: 17.

[0170] In another preferred embodiment, the Fc fragment is derived from IgG1 or IgG4.

[0171] In another preferred embodiment, the Fc fragment is derived from IgG1.

[0172] In another preferred embodiment, the Fc fragment has an amino acid sequence as shown in SEQ ID NO: 59 or 60.

[0173] In another preferred embodiment, in the “Fab1-Fc3-scFv3”, the amino acid sequence of “HC1 (heavy chain)-Fc3-scFv3” is as shown in SEQ ID NO: 70, and the amino acid sequence of “LC1 (light chain)” is as shown in SEQ ID NO: 83.

[0174] In another preferred embodiment, the multi-specific T cell engager has a structure as shown in Formula IV (FIG. 3A-c):



[0175] wherein “.” each independently represents a peptide bond or a linker peptide; “|” represents a linking bond between the polypeptide chains.

[0176] Fab1 is the first targeting domain, and the Fab1 is an anti-LRRC15 Fab;

[0177] scFv4 is the second targeting domain, and the scFv4 is an anti-CD137 scFv;

[0178] Fc3 is an Fc fragment.

[0179] In another preferred embodiment, the “|” is a disulfide bond.

[0180] In another preferred embodiment, the anti-LRRC15 Fab comprises a heavy chain variable region as shown in SEQ ID NO: 1, 2, 3, 4 or 53, and a light chain variable region as shown in SEQ ID NO: 5, 6, 7, 8 or 54.

[0181] In another preferred embodiment, the LRRC15 Fab comprises a heavy chain variable region as shown in SEQ ID NO: 1, and a light chain variable region as shown in SEQ ID NO: 5.

[0182] In another preferred embodiment, the anti-CD137 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 18 or 20, and a light chain variable region as shown in SEQ ID NO: 19 or 21.

[0183] In another preferred embodiment, the anti-CD137 scFv comprises a heavy chain variable region as shown in SEQ ID NO: 20, and a light chain variable region as shown in SEQ ID NO: 21.

[0184] In another preferred embodiment, the Fc fragment is derived from IgG1 or IgG4.

[0185] In another preferred embodiment, the Fc fragment is derived from IgG1.

[0186] In another preferred embodiment, the Fc fragment has an amino acid sequence as shown in SEQ ID NO: 59 or 60.

[0187] In another preferred embodiment, in the “Fab1-Fc3-scFv4”, the amino acid sequence of “HC1 (heavy chain)-Fc3-scFv4” is as shown in SEQ ID NO: 77, and the amino acid sequence of “LC1 (light chain)” is as shown in SEQ ID NO: 83.

[0188] In the second aspect of the present invention, it provides an antibody or antigen-binding fragment thereof against LRRC15, wherein the antibody comprises the following three heavy chain variable region CDRs:

[0189] HCDR1 having an amino acid sequence as shown in SEQ ID NO: 32, 35, 38, or 41;

[0190] HCDR2 having an amino acid sequence as shown in SEQ ID NO: 33, 36, 39, or 42; and

[0191] HCDR3 having an amino acid sequence as shown in SEQ ID NO: 34, 37, 40, or 43;

[0192] and, the following three light chain variable region CDRs:

[0193] LCDR1 having an amino acid sequence as shown in SEQ ID NO: 44 or 49;

[0194] LCDR2 having an amino acid sequence as shown in SEQ ID NO: 45, 47, or 50; and

[0195] LCDR3 having an amino acid sequence as shown in SEQ ID NO: 46, 48, 51, or 52.

[0196] In another preferred embodiment, the antibody or antigen-binding fragment thereof against LRRC15 comprises three heavy chain variable region CDRs (HCDRs) and three light chain variable region CDRs (LCDRs) selected from the group consisting of:

Antibody Name	SEQ ID NO of HCDR1	SEQ ID NO of HCDR2	SEQ ID NO of HCDR3	SEQ ID NO of LCDR1	SEQ ID NO of LCDR2	SEQ ID NO of LCDR3
L15-06/L15-06C	32	33	34	44	45	46
L15-08	35	36	37	44	47	48
L15-03	38	39	40	49	50	51
L15-07	41	42	43	49	50	52

[0197] In another preferred embodiment, the antibody or antigen-binding fragment thereof against LRRC15 comprises a heavy chain variable region having at least 80% sequence identity to the amino acid sequence as shown in SEQ ID NO: 1, 2, 3, or 4, and a light chain variable region having at least 80% sequence identity to the amino acid sequence as shown in SEQ ID NO: 5, 6, 7, or 8.

[0198] In another preferred embodiment, the antibody or antigen-binding fragment thereof against LRRC15 comprises a heavy chain variable region as shown in SEQ ID NO: 1, and a light chain variable region as shown in SEQ ID NO: 5.

[0199] In another preferred embodiment, the antibody or antigen-binding fragment thereof against LRRC15 comprises a heavy chain variable region as shown in SEQ ID NO: 2, and a light chain variable region as shown in SEQ ID NO: 6.

[0200] In another preferred embodiment, the antibody or antigen-binding fragment thereof against LRRC15 com-

US 2025/0051444 A1

Feb. 13, 2025

6

prises a heavy chain variable region as shown in SEQ ID NO: 3, and a light chain variable region as shown in SEQ ID NO: 7.

[0201] In another preferred embodiment, the antibody or antigen-binding fragment thereof against LRRC15 comprises a heavy chain variable region as shown in SEQ ID NO: 4, and a light chain variable region as shown in SEQ ID NO: 8.

[0202] In another preferred embodiment, the antibody or antigen-binding fragment thereof against LRRC15 comprises a heavy chain variable region as shown in SEQ ID NO: 53, and a light chain variable region as shown in SEQ ID NO: 54.

[0203] In another preferred embodiment, the antibody or antigen-binding fragment thereof is a human, mouse, humanized or chimeric antibody.

[0204] In another preferred embodiment, the antibody or antigen-binding fragment thereof is a human antibody.

[0205] In the third aspect of the present invention, it provides a polynucleotide encoding the multi-specific T cell engager according to the first aspect of the present invention or the antibody according to the second aspect of the present invention.

[0206] In the fourth aspect of the present invention, it provides an expression vector comprising the polynucleotide according to the third aspect of the present invention.

[0207] In another preferred embodiment, the expression vector includes a prokaryotic expression vector and a eukaryotic expression vector.

[0208] In the fifth aspect of the present invention, it provides a host cell comprising the expression vector according to the fourth aspect of the present invention, or the polynucleotide according to the third aspect of the present invention integrated into the genome thereof.

[0209] In another preferred embodiment, the host cell includes a prokaryotic cell or a eukaryotic cell.

[0210] In another preferred embodiment, the host cell is selected from the group consisting of: *Escherichia coli*, yeast cells, HEK 293T cells, CHO cells.

[0211] In the sixth aspect of the present invention, it provides a use of the multi-specific T cell engager according to the first aspect of the present invention for preparing a medicament for treating cancer/tumor.

[0212] In another preferred embodiment, the cancer/tumor is a cancer/tumor with high LRRC15 expression.

[0213] In another preferred embodiment, the cancer/tumor includes solid tumors and hematological tumors.

[0214] In another preferred embodiment, the cancer/tumor is a solid tumor.

[0215] In another preferred embodiment, the cancer/tumor is selected from the group consisting of: breast cancer, head and neck tumors, lung cancer, pancreatic cancer, ovarian cancer, melanoma, malignant glioma, sarcoma, bone cancer, and a combination thereof.

[0216] In the seventh aspect of the present invention, it provides an immunoconjugate comprising:

[0217] (i) the multi-specific T cell engager according to the first aspect of the present invention, or the antibody or antigen-binding fragment thereof according to the second aspect of the present invention; and

[0218] (ii) a coupling moiety selected from the group consisting of: a detectable label, a drug, a toxin, a cytokine, a radionuclide, and an enzyme.

[0219] In another preferred embodiment, the conjugate is selected from: fluorescent or luminescent labels, radioactive labels, MRI (magnetic resonance imaging) or CT (computed tomography) contrast agents, or enzymes capable of generating detectable products, radionuclides, biological toxins, cytokines (such as IL-2, etc.), antibodies, antibody Fc fragments, antibody scFv fragments, gold nanoparticles/nanorods, virus particles, liposomes, magnetic nanoparticles, prodrug activating enzymes (e.g., DT-diaphorase (DTD) or biphenyl hydrolase-like protein (BPHL)), chemotherapeutic agents (e.g., cisplatin) or any form of nanoparticles, etc.

[0220] In the eighth aspect of the present invention, it provides a pharmaceutical composition comprising: (a) the multi-specific T cell engager according to the first aspect of the present invention, or the antibody or antigen-binding fragment thereof according to the second aspect of the present invention; and (b) a pharmaceutically acceptable carrier.

[0221] In another preferred embodiment, the pharmaceutical composition is an injection.

[0222] In the ninth aspect of the present invention, it provides a method for treating cancer/tumor, which comprises a step of administering the multi-specific T cell engager according to the first aspect of the present invention to a subject in need thereof.

[0223] In another preferred embodiment, the subject in need thereof is a human or a non-human mammal.

[0224] In another preferred embodiment, the cancer/tumor is a cancer/tumor with high LRRC15 expression.

[0225] In another preferred embodiment, the cancer/tumor includes solid tumors and hematological tumors.

[0226] In another preferred embodiment, the cancer/tumor is a solid tumor.

[0227] In another preferred embodiment, the cancer/tumor is selected from the group consisting of: breast cancer, head and neck tumors, lung cancer, pancreatic cancer, ovarian cancer, melanoma, malignant glioma, sarcoma, bone cancer, and a combination thereof.

[0228] It should be understood that within the scope of the present invention, the above technical features of the present invention and the technical features specifically described below (such as embodiments) can be combined with each other to form new or preferred technical solutions. Due to space limitations, they are not listed here one by one.

DESCRIPTION OF DRAWINGS

[0229] FIG. 1A shows three structural forms of bispecific antibodies composed of LRRC15 antigen binding domain and CD3 antigen binding domain (a-c); and two structural forms of trispecific antibodies composed of LRRC15 antigen binding domain, CD3 antigen binding domain, and CD28 antigen binding domain (d-e);

[0230] Among them, the antibody structure a is composed of anti-CD3 scFv and anti-LRRC15 Fab (CH1-VH+CK-VL), wherein IgG1 Fc forms a heterodimer through charge pairs or knob and hole mutations; the antibody structure b is formed by anti-LRRC15 scFv and anti-CD3 scFv (scFv-scFv-FcxFc); wherein IgG1 Fc forms a heterodimer through charge pairs or knob and hole mutations; the molecular structure c is formed by anti-LRRC15 scFv, anti-CD3 scFv and anti-LRRC15 Fab (scFv-scFv-FcxFab-Fc), wherein IgG1 Fc forms a heterodimer through charge pairs or knob and hole mutations; the molecular structure d is formed by anti-LRRC15 scFv, anti-CD3 scFv and anti-CD28 Fab

US 2025/0051444 A1

Feb. 13, 2025

7

(scFv-scFv-Fc $\times$ Fab-Fc), wherein IgG1 Fc forms a heterodimer through charge pairs or knob and hole mutations; the molecular structure e is formed by anti-CD28 scFv, anti-CD3 scFv and anti-LRRC15 Fab (scFv-scFv-Fc $\times$ Fab-Fc), wherein IgG1 Fc forms a heterodimer through charge pairs or knob and hole mutations.

[0231] FIG. 1B shows an exemplary bispecific antibody of structure b in FIG. 1A, named 153-L06C.

[0232] FIG. 1C shows an exemplary bispecific antibody of structure c in FIG. 1A, named 315-L06C08.

[0233] FIG. 1D shows an exemplary bispecific antibody of structure a in FIG. 1A, named 315-L06.

[0234] FIG. 1E shows an exemplary trispecific antibody of structure e in FIG. 1A, named 28315-L06.

[0235] FIG. 2A shows four structural forms of bispecific antibodies composed of LRRC15 antigen binding domain and CD40 antigen binding domain;

[0236] Among them, the molecular structure a is formed by anti-LRRC15 antibody and CD40L (IgG-RBD $\times$ IgG-RBD); the molecular structure b is formed by anti-LRRC15 antibody and CD40L (IgG-trimeric RBD $\times$ Fab-Fc); the molecular structure c is formed by anti-LRRC15 antibody and anti-CD40 scFv (IgG-scFv $\times$ IgG-scFv); the molecular structure d is formed by anti-CD40 antibody and anti-LRRC15 scFv (IgG-scFv $\times$ IgG-scFv).

[0237] FIG. 2B shows an exemplary bispecific antibody of structure c in FIG. 2A, named 1540-L06.

[0238] FIG. 2C shows an exemplary bispecific antibody of structure a in FIG. 2A, named 1540L-L06.

[0239] FIG. 2D shows an exemplary bispecific antibody of structure b in FIG. 2A, named 1540TL-L06.

[0240] FIG. 2E shows an exemplary bispecific antibody of structure d in FIG. 2A, named 4015-L06.

[0241] FIG. 3A shows four structural forms of bispecific antibodies composed of LRRC15 antigen binding domain and CD137 antigen binding domain;

[0242] Among them, the molecular structure a is formed by anti-LRRC15 antibody and CD137L (IgG-RBD $\times$ IgG-RBD); the molecular structure b is formed by anti-LRRC15 antibody and CD137L (IgG-trimeric RBD $\times$ Fab-Fc); the molecular structure c is formed by anti-LRRC15 antibody and anti-CD137 scFv (IgG-scFv $\times$ IgG-scFv); the molecular structure d is formed by anti-CD137 antibody and anti-LRRC15 scFv (IgG-scFv $\times$ IgG-scFv).

[0243] FIG. 3B shows an exemplary bispecific antibody of structure c in FIG. 3A, named 15BB-L06.

[0244] FIG. 3C shows an exemplary bispecific antibody of structure a in FIG. 3A, named 15BBL-L06.

[0245] FIG. 3D shows an exemplary bispecific antibody of structure b in FIG. 3A, named 15BBTL-L06.

[0246] FIG. 3E shows an exemplary bispecific antibody of structure d in FIG. 3A, named BB15-L06.

[0247] FIG. 4A shows FACS detection of the binding of anti-LRRC15 antibody to a cell line expressing human LRRC15.

[0248] FIG. 4B shows FACS detection of the binding of anti-LRRC15 antibody to a cell line expressing mouse LRRC15.

[0249] FIG. 4C shows FACS detection of the binding of anti-LRRC15 antibody to a cell line expressing cynomolgus LRRC15.

[0250] FIG. 4D shows FACS detection of the competitive binding of LRRC15 antibodies to a cell line expressing human LRRC15.

[0251] FIG. 5A shows FACS detection of the binding of bispecific antibodies targeting LRRC15 and CD3 (153-L06C and 315-L06C08) to a cell line expressing human LRRC15.

[0252] FIG. 5B shows FACS detection of the binding of bispecific antibodies targeting LRRC15 and CD3 (153-L06C and 315-L06C08) to a cell line expressing cynomolgus LRRC15.

[0253] FIG. 6 shows the curve of the variation of the proportion of T cells expressing CD25 after stimulation of PBMC with bispecific antibodies (153-L06C; 315-L06C08) according to different antibody concentrations.

[0254] FIG. 7 shows the curve of the variation of the IFN- γ released by PBMC after stimulation of PBMC with bispecific antibodies (153-L06C; 315-L06C08) according to different antibody concentrations.

[0255] FIG. 8 shows the curve of the variation of the proportion of proliferating T cells after stimulation of T cells with bispecific antibodies (153-L06C; 315-L06C08) according to different antibody concentrations.

[0256] FIG. 9 shows the curve of the variation of the killing ratio of target cells after stimulation of PBMC with bispecific antibodies (153-L06C; 315-L06C08) according to different antibody concentrations.

[0257] FIG. 10 shows the results of the stimulation of PBMC with bispecific antibodies (153-L06C; 315-L06C08) to release cytokines.

[0258] FIG. 11 shows the binding curve of the bispecific antibody targeting LRRC15 and CD137 (15BB-L06) to 4-1BB protein, and the binding curve of the bispecific antibody targeting LRRC15 and CD40 (1540-L06) to CD40 protein detected by ELISA.

[0259] FIG. 12 shows the results of the stimulation of PBMC with bispecific antibody (15BB-L06) to release IL-2.

[0260] FIG. 13 shows the results of the stimulation of DC with bispecific antibody (1540-L06) to release IL-12p40.

[0261] FIG. 14 shows the results of the stimulation of PBMC with bispecific antibodies (15BB-L06; 1540-L06) to release cytokines (TNF- α and IL-6).

[0262] FIG. 15A shows the changes in tumor volume in NCI-H1650 PBMC mice during administration.

[0263] FIG. 15B shows the comparison of tumor weights between groups after administration in NCI-H1650 PBMC mice.

[0264] FIG. 15C shows the antitumor effect of 153-L06C or 15BB-L06 in MC38 model mice.

[0265] FIG. 15D shows the antitumor effect of 1540-L06 in MC38 model mice.

[0266] FIG. 16 shows a comparison of the killing effects of 153-L06 and 315-L06.

[0267] FIG. 17 shows a comparison of the killing effects of 153-L06C, 315-L06C08, 315-L08C06 and 28315-L06.

DETAILED DESCRIPTION

[0268] After extensive and in-depth research, the inventors of the present invention unexpectedly developed for the first time a class of multi-specific T cell engagers comprising LRRC15 antigen binding domains. The multi-specific T cell engager comprises a first targeting domain that targets the leucine-rich repeat-containing protein 15 (LRRC15) molecules highly expressed on the surface of tumor cells, wherein the first targeting domain is an LRRC15 antibody or antigen-binding fragment thereof, and further comprises a second targeting domain and/or a third targeting domain that

Exhibit 1

Page 52

US 2025/0051444 A1

Feb. 13, 2025

8

binds to the CD molecule on the surface of T cells. The multi-specific T cell engager of the present invention can simultaneously bind to tumor cells and T cells, and can be used as an effective therapeutic agent for tumor treatment, especially for tumors with high LRRC15 expression.

[0269] On this basis, the present invention is completed.

[0270] As used herein, the term “leucine-rich repeat-containing protein 15” or “LRRC15” refers to the protein encoded by the LRRC15 gene in humans. The leucine-rich repeat (LRR) domain is evolutionarily conserved in proteins associated with innate immunity. LRRC15 is expressed on stromal fibroblasts in many solid tumors (e.g., breast, head and neck, lung, pancreas) and directly on a subset of cancer cells of mesenchymal origin (e.g., sarcoma, melanoma, glioma), while most normal tissues show low or no expression.

[0271] As used herein, the terms “CD”, “cluster of differentiation”, or “cluster of differentiation molecules” refer to cell surface markers that can be used to identify and characterize leukocytes, such as CD3, CD28, and CD40. CD3 is the signaling component of the T cell receptor (TCR) complex. Since T cell activation requires CD3, drugs (usually monoclonal antibodies) that target it are being studied as immunostimulants for the treatment of cancer.

[0272] CD28 is the major costimulatory molecule required for the generation of a T cell-mediated immune response. Upon interaction with its ligands CD80 and CD86, CD28 transduces an activating signal, leading to the expression of anti-apoptotic proteins and enhanced synthesis of multiple cytokines, including IL-2. The CD28 costimulatory receptor is present on all T cells. Agonist antibodies targeting CD28 have led to severe adverse events clinically, compared to antibodies targeting other CD28 family members including CTLA-4, PD-1 or their ligands, the latter as checkpoint inhibitors, overcome tumor immune tolerance and can be used for cancer immunotherapy.

[0273] CD40 is a cell surface receptor that belongs to the tumor necrosis factor-R (TNF-R) family and is constitutively expressed on APCs such as B cells and dendritic cells. CD40L (CD154) is a type II transmembrane glycoprotein that can be induced and is restricted to cells of the hematopoietic system, such as platelets, granulocytes, and activated T/B/NK cells. CD40L binding to CD40 activates APCs, leading to upregulation of CD80, CD86 and other costimulatory molecules to stimulate CD8+ antigen-specific T cell responses.

[0274] The term “Fc fragment” or “Fc” refers to the portion of an antibody that does not have antigen binding activity but was originally observed to be easily crystallized, and hence was named Fc fragment (for fragment crystallizable). This fragment corresponds to the paired CH2 and CH3 domains, and is the portion of the antibody molecule that interacts with effector molecules and cells. The Fc fragments described herein can be derived from IgG1, IgG2, and IgG4 antibodies. For specific purposes, a particular IgG subclass may be preferred. For example, IgG1 is more effective than IgG2 and IgG4 in mediating ADCC and CDC. Therefore, IgG2 Fc may be preferred when effector function is not desired. However, molecules containing IgG2 Fc are generally more difficult to prepare and may be less stable than molecules containing IgG1 Fc. On the other hand, cross-binding to FcγRIIB is critical for enhancing costimulatory signals from CD40 or CD137, and IgG4 has an affinity about 2-fold higher than IgG1 and 10-fold higher than IgG2.

Mutations such as S267E and L328F can further enhance the cross-binding of IgG Fc to FcγRIIB. Additionally, the effector function of an antibody can be increased or decreased by introducing one or more mutations into the Fc (see, e.g., Strohl, *Curr. Opin. Biotech.*, 20:685-691, 2009).

[0275] As used herein, the term “antibody” or “immunoglobulin” refers to an approximately 150,000 dalton heterotetrameric protein with the same structural features, which is composed of two identical light chains (L) and two identical heavy chains (H). Each light chain is covalently linked to a heavy chain by a disulfide bond, and the number of disulfide bonds between the heavy chains of different immunoglobulin isotypes varies. Each heavy chain and light chain also has intra-chain disulfide bonds at regular intervals. One end of each heavy chain has a variable region (VH), followed by multiple constant regions. Each light chain has a variable region (VL) at one end and a constant region at the other end; the constant region of the light chain is opposite the first constant region of the heavy chain, and the variable region of the light chain is opposite the variable region of the heavy chain. Specific amino acid residues form an interface between the variable regions of the light and heavy chains.

[0276] As used herein, the term “variable” means that certain portions of the variable region of an antibody differ in sequence, and it gives rise to the binding and specificity of various specific antibodies for their specific antigens. However, variability is not evenly distributed throughout the antibody variable region. It is concentrated in three segments within the variable regions of the light and heavy chains called complementarity determining regions (CDRs) or hypervariable regions. The more conserved portions of the variable region are called framework regions (FRs). The variable regions of the natural heavy and light chains respectively contain four FR regions, which generally have a β-sheet configuration and are connected by three CDRs that form a connecting loop, forming partial β-sheet structures in some cases. The CDRs in each chain are closely clustered together through the FR regions and together with the CDRs of the other chain, forming the antigen-binding site of the antibody (see Kabat et al., *NIH Publ. No. 91-3242*, Vol. I, pages 647-669 (1991)). The constant regions do not directly participate in the binding of the antibody to the antigen, but they exhibit different effector functions, such as participating in antibody-dependent cytotoxicity of the antibody.

[0277] The antibodies of the present application can include, but are not limited to, polyclonal, monoclonal, monospecific, multispecific, bispecific, human, humanized, primatized, chimeric and single-chain antibodies. The antibodies disclosed herein can be from any animal origin, including birds and mammals. Preferably, the antibodies are human, mouse, donkey, rabbit, goat, guinea pig, camel, llama, horse or chicken antibodies.

[0278] The term “antibody fragment” or “antigen binding fragment” refers to a portion of an antibody, such as F(ab')₂, F(ab)₂, Fab', Fab, Fv, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments containing VL or VH domains, fragments produced by Fab expression libraries, and anti-idiotypic (anti-Id) antibodies. Regardless of structure, antibody fragments bind to the same antigen as the intact antibody recognizes. The term “antibody fragment” includes DARTs and diabodies. The term “antibody fragment” also includes any synthetic or genetically engineered protein that comprises an immunoglobulin

US 2025/0051444 A1

Feb. 13, 2025

9

variable region and acts like an antibody by forming a complex with a specific antigen. A “single-chain variable fragment” or “scFv” is a fusion protein of the variable regions of the heavy chain (VH) and light chain (VL) of an immunoglobulin. In some embodiments, the domain regions are connected by a short linker peptide of 10 to about 25 amino acids. The linker can be glycine-rich for flexibility and serine-rich or threonine-rich for solubility, and can be linked to the N-terminus of VH or the C-terminus of VL, or vice versa. Despite the removal of the constant region and the introduction of the linker, this protein still retains the specificity of the original immunoglobulin. For IgG, standard immunoglobulin molecules contain two identical light chain polypeptides of approximately 23,000 daltons and two identical heavy chain polypeptides of 53,000-70,000 daltons. The four chains are typically linked by disulfide bonds in a “Y” configuration, with the light chains connecting (bracketing) the heavy chains from the mouth of the “Y” and extending through the variable region.

[0279] As described above, the variable region allows the antibody to selectively recognize and specifically bind to an epitope on the antigen. That is, the VL domain and VH domain of the antibody or a subset of the complementarity determining regions (CDRs) of the antibody combine to form the variable region that defines the three-dimensional antigen binding site. This tetrameric antibody structure forms the antigen binding site present at the end of each arm of the Y configuration. More specifically, the antigen binding site is defined by the three CDRs (i.e., HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3) on each of the VH and VL chains. In some cases, for example, certain immunoglobulin molecules are derived from camelid animal species or engineered based on camelid immunoglobulin. Alternatively, immunoglobulin molecules may be composed of only heavy chains without light chains or only light chains without heavy chains.

[0280] In naturally occurring antibodies, the six CDRs present in each antigen binding domain are short, non-contiguous amino acid sequences that are specifically positioned to form the “antigen binding domain” due to the three-dimensional configuration of the antibody in an aqueous environment. The remaining amino acids in the antigen binding domain, called “framework” region domains, exhibit less intermolecular variability. The framework regions adopt a predominantly β -sheet conformation, and the CDRs form loops that are connected and form a part of the β -sheet structure in some cases. Thus, the framework region acts as a scaffold that positions the CDRs in the correct orientation through interchain non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface that is complementary to the epitope on the immunoreactive antigen. This complementary surface promotes non-covalent binding of the antibody to its cognate epitope. Since they have been precisely defined, the ordinary skilled person in the art of domain structures can readily identify the amino acids comprising the CDRs and framework regions, respectively, for any given heavy or light chain variable region.

[0281] As used herein, the term “light chain constant region (CL)” encompasses the amino acid sequence CL (SEQ ID NO: 30 or 31) derived from an antibody light chain. Preferably, the light chain constant region comprises at least one of the constant kappa domain or the constant lambda domain.

[0282] As used herein, the term “heavy chain constant region (CH)” encompasses the amino acid sequence derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain constant region comprises at least one of the following: a CH1 domain (SEQ ID NO: 26 or 28), a hinge region (e.g., an upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. It is understood that the heavy chain constant regions can be modified such that their amino acid sequences differ from those of naturally occurring immunoglobulin molecules.

[0283] As used herein, a “variant” of an antibody, antibody fragment, or antibody domain refers to an antibody, antibody fragment, or antibody domain that: (1) has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the original antibody, antibody fragment, or antibody domain, and (2) specifically binds to the same target as the original antibody, antibody fragment, or antibody domain specifically binds. It should be understood that in the case of the sequence identity being expressed in the form of “at least x % identical” or “at least x % identity”, such embodiments include any and all numerical percentages equal to or greater than the lower limit. Further, it should be understood that in the presence of amino acid sequences in the present application, they should be construed as additionally disclosing or encompassing those having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to said amino acid sequence.

[0284] The scope of the multi-specific molecules of the present invention includes a variety of compositions and methods, including: asymmetric IgG-like antibodies (e.g., trifunctional monoclonal antibodies (triomabs)/quadromas); knobs-into-holes antibodies; Cross monoclonal antibodies (Cross MAb); electrostatically matched antibodies; LUZ-Y; chain-exchange engineered domain (SEED) bodies; Fab-exchanged antibodies, symmetric IgG class antibodies; two-in-one antibodies; cross-linked monoclonal antibodies, mAb2; Cov X-body; dual variable domain (DVD)-Ig fusion proteins; IgG-like bispecific antibodies; Ts2Ab; BsAb; scFv/Fc fusions; dual (scFv) 2-Fabs; F(ab)2 fusion proteins; bifunctional or Bis-Fab; Dock-and-Lock (DNL); Fab-Fv; scFv-based antibodies and bispecific antibody-based antibodies, such as bispecific T cell engagers (BiTEs); tandem bispecific antibodies (TandAb); DARTs; single-chain bispecific antibodies; TCR-like antibodies; human serum albumin scFv fusion proteins, COMBODIES and IgG/non-IgG fusion proteins.

[0285] As used herein, the phrase “multi-specific T cell engager” refers to a molecule comprising at least two targeting domains having different binding specificities, wherein at least one targeting domain specifically binds to a T cell surface antigen. In some embodiments, the multi-specific inhibitor is a polypeptide comprising a scaffold and two or more immunoglobulin antigen binding domains targeting different antigens or epitopes. In some embodiments, the multi-specific T cell engager is a bispecific antibody. In some other embodiments, the multi-specific T cell engager is a trispecific antibody.

[0286] As used herein, the phrase “bispecific” refers to a molecule comprising at least two targeting domains having different binding specificities. Each targeting domain is capable of specifically binding to a target molecule and, upon binding to the target molecule, inhibits the biological

US 2025/0051444 A1

Feb. 13, 2025

10

function of the target molecule. In some embodiments, the bispecific T cell engager is a polymeric molecule having two or more peptides. In some embodiments, the targeting domain comprises the antigen binding domain or CDRs of an antibody. In some embodiments, the targeting domain comprises a ligand or fragment thereof that specifically binds to a target protein. In some embodiments, the bispecific inhibitor is a bispecific antibody.

[0287] The terms “bispecific antibody” and “bispecific T cell engager” can be used interchangeably herein to refer to an antibody that can specifically bind to two different antigens (or epitopes). In some embodiments, the bispecific antibody is a full-length antibody that binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC) and binds to a different antigen (or epitope) on its second arm (the other pair of HC/LC). In these embodiments, the bispecific antibody has two different antigen binding arms (both in terms of specificity and CDR sequence) and is monovalent for each antigen it binds to.

[0288] In some other embodiments, the bispecific antibody is a full-length antibody that can bind two different antigens (or epitopes) in each of its two binding arms (two pairs of HC/LC). In these embodiments, the bispecific antibody has two identical antigen binding arms, with the same specificity and the same CDR sequence, and is bivalent for each antigen it binds to.

[0289] The terms “trispecific antibody” and “trispecific T cell engager” can be used interchangeably herein to refer to a molecule comprising three targeting domains having three different binding specificities. Each targeting domain is capable of specifically binding to a target molecule and, upon binding to the target molecule, inhibits the biological function of the target molecule. In some embodiments, the trispecific antagonist is a polymeric molecule having two or more peptides. In some embodiments, the targeting domain comprises the antigen binding domain or CDRs of an antibody. In some embodiments, the targeting domain comprises a ligand or fragment thereof that specifically binds to a target protein.

[0290] In a preferred embodiment of the present invention, a bispecific antibody targeting LRRC15 and CD3 is constructed, having the structure shown in FIG. 1A a-c. An exemplary molecule of structure a is shown in FIG. 1D, wherein the anti-CD3 scFv can be an scFv constructed from the VH and VL of any anti-CD3 antibody described in the present invention; the anti-LRRC15 Fab can be a Fab constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention. An exemplary molecule of structure b is shown in FIG. 1B, wherein the anti-LRRC15 scFv can be an scFv constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention; the anti-CD3 scFv can be an scFv constructed from the VH and VL of any anti-CD3 antibody described in the present invention. An exemplary molecule of structure c is shown in FIG. 1C, wherein the anti-LRRC15 scFv can be an scFv constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention; the anti-CD3 scFv can be an scFv constructed from the VH and VL of any anti-CD3 antibody described in the present invention; the anti-LRRC15 Fab can be a Fab constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention.

[0291] In another preferred embodiment of the present invention, a bispecific antibody targeting LRRC15, CD3 and

CD28 is constructed, having the structure shown in FIG. 1A d-e. An exemplary molecule of structure e is shown in FIG. 1E, wherein the anti-CD28 scFv can be an scFv constructed from the VH and VL of any anti-CD28 antibody described in the present invention; the anti-CD3 scFv can be an scFv constructed from the VH and VL of any anti-CD3 antibody described in the present invention; the anti-LRRC15 Fab can be a Fab constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention.

[0292] In another preferred embodiment of the present invention, a bispecific antibody targeting LRRC15 and CD40 is constructed, having the structure shown in FIG. 2A a-d. An exemplary molecule of structure a is shown in FIG. 2C, wherein the anti-LRRC15 Fab can be a Fab constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention; CD40L can be any CD40L described in the present invention. An exemplary molecule of structure b is shown in FIG. 2D, wherein the anti-LRRC15 Fab can be a Fab constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention; CD40L can be any CD40L described in the present invention. An exemplary molecule of structure c is shown in FIG. 2B, wherein the anti-LRRC15 Fab can be a Fab constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention; the anti-CD40 scFv can be an scFv constructed from the VH and VL of any anti-CD40 antibody described in the present invention. An exemplary molecule of structure d is shown in FIG. 2E, wherein the anti-CD40 Fab can be a Fab constructed from the VH and VL of any anti-CD40 antibody described in the present invention; the anti-LRRC15 scFv can be an scFv constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention.

[0293] In another preferred embodiment of the present invention, a bispecific antibody targeting LRRC15 and CD137 is constructed, having the structure shown in FIG. 3A a-d. An exemplary molecule of structure a is shown in FIG. 3C, wherein the anti-LRRC15 Fab can be a Fab constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention; CD137L can be any CD137L described in the present invention. An exemplary molecule of structure b is shown in FIG. 3D, wherein the anti-LRRC15 Fab can be a Fab constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention; CD137L can be any CD137L described in the present invention. An exemplary molecule of structure c is shown in FIG. 3B, wherein the anti-LRRC15 Fab can be a Fab constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention; the anti-CD137 scFv can be an scFv constructed from the VH and VL of any anti-CD137 antibody described in the present invention. An exemplary molecule of structure d is shown in FIG. 3E, wherein the anti-CD137 Fab can be a Fab constructed from the VH and VL of any anti-CD137 antibody described in the present invention; the anti-LRRC15 scFv can be an scFv constructed from the VH and VL of any anti-LRRC15 antibody described in the present invention.

[0294] The present invention also provides polynucleotide molecules encoding the aforementioned antibodies or fragments thereof. The polynucleotides of the present invention can be in DNA or RNA form. DNA forms include cDNA, genomic DNA, or synthetic DNA. DNA can be single-stranded or double-stranded. DNA can be the coding strand or the non-coding strand. The coding region sequence

US 2025/0051444 A1

Feb. 13, 2025

11

encoding the mature polypeptide can be the same as or a degenerate variant of the coding region sequence of the antibody of the present invention. As used herein, “degenerate variant” in the present invention refers to a nucleic acid sequence that encodes a polypeptide having the same amino acid sequence as the polypeptide of the present invention, but has a different coding region sequence.

[0295] Polynucleotides encoding the mature polypeptides of the present invention include: coding sequences encoding only the mature polypeptide; coding sequences for the mature polypeptide and various additional coding sequences; coding sequences for the mature polypeptide (and optionally additional coding sequences) and non-coding sequences.

[0296] The term “polynucleotide encoding a polypeptide” can be a polynucleotide that includes the coding sequence for this polypeptide, or it can be a polynucleotide that further includes additional coding and/or non-coding sequences.

[0297] The present invention also relates to polynucleotides that hybridize to the above sequences and have at least 50%, preferably at least 70%, more preferably at least 80% identity between the two sequences. The present invention particularly relates to polynucleotides that can hybridize to the polynucleotides described in the present invention under stringent conditions. In the present invention, “stringent conditions” refers to: (1) hybridization and washing at low ionic strength and high temperature, such as 0.2×SSC, 0.1% SDS, 60° C.; or (2) hybridization with a denaturant, such as 50% (v/v) formamide, 0.1% calf serum/0.1% Ficoll, 42° C., etc.; or (3) hybridization only occurs when the identity between the two sequences is at least 90%, preferably above 95%. Moreover, the polypeptides encoded by the polynucleotides that can hybridize have the same biological function and activity as the mature polypeptides shown in SEQ ID NO.4 and SEQ ID NO.9.

[0298] The full-length nucleotide sequence of the antibody of the present invention or a fragment thereof can generally be obtained by PCR amplification, recombination or artificial synthesis. A feasible method is to use artificial synthesis to synthesize the related sequences, especially when the fragment length is shorter. Usually, a very long fragment can be obtained by first synthesizing multiple small fragments and then ligating them. In addition, the coding sequence of the heavy chain can be fused with an expression tag (such as 6His) to form a fusion protein.

[0299] Once the relevant sequence is obtained, the relevant sequence can be obtained in large quantities by recombination. This is usually done by cloning it into a vector, transforming it into a cell, and then isolating the relevant sequence from the proliferated host cells by conventional methods. The biological molecules (nucleic acids, proteins, etc.) involved in the present invention include biological molecules that exist in an isolated form.

[0300] At present, it is possible to obtain the DNA sequence encoding the protein of the present invention (or its fragment, or its derivative) completely by chemical synthesis. The DNA sequence can then be introduced into various existing DNA molecules (or vectors) and cells known in the art. In addition, mutations can be introduced into the protein sequence of the present invention by chemical synthesis.

[0301] The present invention also relates to vectors comprising the appropriate DNA sequences described above and

appropriate promoter or control sequences. These vectors can be used to transform appropriate host cells to enable them to express proteins.

[0302] The host cell can be a prokaryotic cell, such as a bacterial cell; or a lower eukaryotic cell, such as a yeast cell; or a higher eukaryotic cell, such as a mammalian cell. Representative examples include: *Escherichia coli*, *Streptomyces*; bacterial cells of *Salmonella typhimurium*; fungal cells such as yeast; insect cells of *Drosophila* S2 or Sf9; animal cells of CHO, COS7, 293 cells, etc.

[0303] Transformation of host cells with recombinant DNA can be carried out using conventional techniques well known to those skilled in the art. When the host is a prokaryotic organism such as *Escherichia coli*, competent cells capable of taking up DNA can be harvested after the exponential growth phase and treated with the CaCl₂ method, the steps of which are well known in the art. Another method is to use MgCl₂. If necessary, transformation can also be carried out by electroporation. When the host is a eukaryote, the following DNA transfection methods can be selected: calcium phosphate coprecipitation method, conventional mechanical methods such as microinjection, electroporation, liposome packaging, etc.

[0304] The obtained transformants can be cultured by conventional methods to express the polypeptide encoded by the gene of the present invention. Depending on the host cell used, the culture medium used in the culture can be selected from various conventional culture media. The culture is carried out under conditions suitable for the growth of the host cell. When the host cells grow to an appropriate cell density, the selected promoter is induced by an appropriate method (such as temperature shift or chemical induction), and the cells are cultured for a further period of time.

[0305] In the above methods, the recombinant polypeptide can be expressed intracellularly, or on the cell membrane, or secreted extracellularly. If necessary, the recombinant protein can be separated and purified by various separation methods using its physical, chemical and other properties. These methods are well known to those skilled in the art. Examples of these methods include but are not limited to: conventional renaturation treatment, treatment with protein precipitants (salting out method), centrifugation, osmotic lysis, ultrafiltration, ultracentrifugation, molecular sieve chromatography (gel filtration), adsorption chromatography, ion exchange chromatography, high performance liquid chromatography (HPLC) and other various liquid chromatography techniques and combinations of these methods.

[0306] The antibodies of the present invention can be used alone or in combination or conjugated with detectable labels (for diagnostic purposes), therapeutic agents, PK (protein kinase) modification moieties, or any combination of the above substances.

[0307] Detectable labels for diagnostic purposes include but are not limited to: fluorescent or luminescent labels, radioactive labels, MRI (magnetic resonance imaging) or CT (computed tomography) contrast agents, or enzymes capable of producing detectable products.

[0308] Couplable therapeutic agents include but are not limited to: insulin, IL-2, interferon, calcitonin, GHRH peptide, enterostatin analogs, albumin, antibody fragments, cytokines, and hormones.

[0309] The present invention also provides a composition. In a preferred embodiment, the composition is a pharmaceutical composition, which comprises the aforementioned

US 2025/0051444 A1

Feb. 13, 2025

antibody or active fragment or fusion protein thereof, and a pharmaceutically acceptable carrier. Typically, these substances can be formulated in a non-toxic, inert and pharmaceutically acceptable aqueous carrier medium, the pH of which is usually about 5-8, preferably about 6-8, although the pH value may vary depending on the nature of the substance being formulated and the disease to be treated. The prepared pharmaceutical composition can be administered by conventional routes, including but not limited to: oral, respiratory, intra-tumoral, intraperitoneal, intravenous, or topical administration.

[0310] The pharmaceutical composition of the present invention can be used for the treatment of cancer/tumors, especially solid tumors, especially solid tumors with high LCRR15 expression.

[0311] The pharmaceutical composition of the present invention comprises a safe and effective amount (such as 0.001-99 wt %, preferably 0.01-90 wt %, more preferably 0.1-80 wt %) of the aforementioned monoclonal antibody (or conjugate thereof) of the present invention and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to: saline, buffers, glucose, water, glycerol, ethanol, and a combination thereof. The pharmaceutical formulation should be compatible with the route of administration. The pharmaceutical composition of the present invention can be prepared in the form of an injection, for example, by conventional methods using a physiological saline solution or an aqueous solution containing glucose and other excipients. The pharmaceutical composition, such as an injection or solution, should be manufactured under aseptic conditions. The dosage of the active ingredient is a therapeutically effective amount, for example about 1 microgram/kg body weight to about 10 milligrams/kg body weight per day. In addition, the pharmaceutical composition of the present invention can also be used together with other therapeutic agents.

[0312] When using the pharmaceutical composition, a safe and effective amount of the immunoconjugate is administered to a mammal, wherein the safe and effective amount is usually at least about 10 micrograms/kg body weight and in most cases does not exceed about 8 milligrams/kg body weight, preferably the dose is about 10 micrograms/kg body weight to about 1 milligram/kg body weight. Of course, the specific dosage should also consider the route of administration, the patient's health status, etc., which are all within the scope of the skills of a skilled physician.

[0313] The main advantages of the present invention include:

[0314] (1) Cancer-associated fibroblasts (CAFs) are an important component of the microenvironment of most solid tumors. These cells promote cancer progression by supporting the growth of tumor cells, participating in the remodeling of the tumor extracellular matrix, promoting angiogenesis, and regulating tumor-induced inflammation. CAFs coexist with other cells such as endothelial cells and immune cells expressing CD40 in the cancer stroma. Thus, since LRRC15 is specifically expressed on CAFs, bispecific/trispecific antibodies can be designed for this target to bring immune activation signals into the tumor stroma, thereby helping to the transform of cold tumors into hot tumors in the treatment of most solid tumors.

[0315] (2) Compared with ordinary tumor-associated antigen (TAA) targets, the expression of LRRC15 on multiple solid tumors and CAFs makes the bispecific/trispecific antibodies containing the LRRC15 antigen binding domain in the present invention more widely used.

[0316] (3) Due to the barrier effect of the tumor stroma, it is difficult for ordinary TAA targeting bispecific antibodies to directly bring immune regulators to the location of tumor cells, while bispecific antibodies that target LRRC15 expressed on CAFs at one end can easily induce immune cell activation by binding and activating regulatory targets (such as targeting TNFRSF) at the other end, thereby playing a role in the tumor stroma.

[0317] (4) The LRRC15-related bispecific/trispecific antibodies of the present invention can effectively activate T cells and reduce the matrix effects of tumors.

[0318] The present invention is further explained below in conjunction with specific examples. It should be understood that these examples are only for illustration of the present invention and not for limiting the scope of the present invention. The experimental methods not specifically mentioned in the following examples are generally carried out under conventional conditions, such as those described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989), or according to the conditions recommended by the manufacturer. Unless otherwise specified, percentages and parts are percentages by weight and parts by weight.

[0319] Sequences used to prepare the multi-specific T cell engager of the present invention

Sequences of LRRC15 antibodies			
	HCDR1	HCDR2	HCDR3
L15-06H/ L15-06CH	GFTFSSYYMS (SEQ ID NO: 32)	DISVSGSYTNYADSV KG (SEQ ID NO: 33)	IGSYRSFFSTDDGDSL DY (SEQ ID NO: 34)
L15-08H	GFTFSDYYMS (SEQ ID NO: 35)	DISVSSSYTNYADSV KG (SEQ ID NO: 36)	LGGYRSFFSTDDGDSL DY (SEQ ID NO: 37)
L15-03H	GGTFSSYTIS (SEQ ID NO: 38)	GIIPMFGTANYAQKF QG (SEQ ID NO: 39)	GVALLATDFDY (SEQ ID NO: 40)
L15-07H	GGTFSTYAIS (SEQ ID NO: 41)	GITPMFGTPNYAQKF QG (SEQ ID NO: 42)	GDWYDYHFDY (SEQ ID NO: 43)

US 2025/0051444 A1

Feb. 13, 2025

13

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Sequences of LRR15 antibodies			
	LCDR1	LCDR2	LCDR3
L15-06L/ L15-06CL	RASQSISSWLA (SEQ ID NO: 44)	DASSLES (SEQ ID NO: 45)	QQYNSYSLT (SEQ ID NO: 46)
L15-08L	RASQSISSWLA (SEQ ID NO: 44)	DAFSLES (SEQ ID NO: 47)	QQYNSYSIT (SEQ ID NO: 48)
L15-03L	TGTSSDVGGYNY VS (SEQ ID NO: 49)	EVSNRPS (SEQ ID NO: 50)	QSWTHDNSVV (SEQ ID NO: 51)
L15-07L	TGTSSDVGGYNY VS (SEQ ID NO: 49)	EVSNRPS (SEQ ID NO: 50)	QSYANRDNFVV (SEQ ID NO: 52)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYYMSWVRQAPG KGLEWVADISVSGSYTNYADSVKGRFTISRDNKNTLYLQMN SLRAEDTAVYYCARIGSYRSPFSTDDGDSLQYWGQGLVTVSS	VH; L15-06H (SEQ ID NO: 1)		
QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPG KGLEWVSDISVSSSYTNYADSVKGRFTISRDNKNTLYLQMN SLRAEDTAVYYCARLGGYRSPFSTDDGDSLQYWGQGLVTVSS	VH; L15-08H (SEQ ID NO: 2)		
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYTISWVRQAPG QGLEWMGGIIPMFGTANYAQKFGQGRVTITADESTSTAYMELS SLRSEDVAVYYCARGVALLATDFDYWGQGLVTVSS	VH; L15-03H (SEQ ID NO: 3)		
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSTYAIWVRQAPG QGLEWMGGITPMFGTPNYAQKFGQGRVTITADESTSTAYMELS SLRSEDVAVYYCARGDWYDHFQYWGQGLVTVSS	VH; L15-07H (SEQ ID NO: 4)		
DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKA PKLLIYDASSLESQVPSRFGSGSGTEFTLTISSLQPDGFATYYC QQYNSYSLTFGGGTKVEIK	VL; L15-06L (SEQ ID NO: 5)		
DIQLTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKA PKLLIYDAFLESQVPSRFGSGSGTEFTLTISSLQPDGFATYYC QQYNSYSITFGGGTKVEIK	VL; L15-08L (SEQ ID NO: 6)		
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPG KAPKLMIEVSNRPSGVSNRFGSGSGNTASLTISGLQAEDEA DYQCQSWTHDNSVVFVGGGKLTVL	VL; L15-03L (SEQ ID NO: 7)		
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPG KAPKLMIEVSNRPSGVSNRFGSGSGNTASLTISGLQAEDEA DYQCQSYANRDNFVVFVGGGKLTVL	VL; L15-07L (SEQ ID NO: 8)		
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYYMSWVRQAPG KCLEWVADISVSGSYTNYADSVKGRFTISRDNKNTLYLQMN SLRAEDTAVYYCARIGSYRSPFSTDDGDSLQYWGQGLVTVSS	VH; L15-06CH (SEQ ID NO: 53)		
DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKA PKLLIYDASSLESQVPSRFGSGSGTEFTLTISSLQPDGFATYYC QQYNSYSLTFGGCGTKVEIK	VL; L15-06CL (SEQ ID NO: 54)		

Sequences of CD3 antibodies

EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWRQAPG KGLEWVGRIRSKANNYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGLVTVSS	VH; CD3vB (SEQ ID NO: 9)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWRQAPG KGLEWVGRIRSKSNYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCVRHGNFGDSYVSWFAYWGQGLVTVSS	VH; CD3vG (SEQ ID NO: 10)

US 2025/0051444 A1

Feb. 13, 2025

14

-continued

Sequences of CD3 antibodies	
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCVRHGNFGDSYVSWFEYWGQGLTVTVSS	VH; CD3vI (SEQ ID NO: 11)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPG KGLEWVGRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYLQ MNSLRAEDTAVYYCVRHGNFGDSYVSWFDYWGQGLTVTVSS	VH; CD3vJ (SEQ ID NO: 12)
QAVVTQEPSTLTVSPGGTVTLTCGSSTGAVTTSNYANWVQQKP GKSPRGLIGGTNKRAPGVPARFSGSLLGGAALTISGAQPEDE ADYYCALWYSNHWVFGGKTLTVL	VL; CD3 (SEQ ID NO: 13)
Sequences of CD28 antibody	
QVQLVQSGAEVKKPGASVKVSCASGYTFTSYIHWVRQAP GQGLEWIGSIYPGNVNTNYNEKFKDRATLTVDTSISTAYMELS RLRSDDTAVYFCTRSHYGLDNFVWGQGTITVTVSS	VH; CD28 (C50S) (SEQ ID NO: 14)
DIQMTQSPSSLSASVGDRTVITCHASQNIYVWLNWYQQKPGK APKLLIYKASNLHTGVPSRFSGSGSDFTLTISLQPEDFATY YCQQGQTYPTFGGKTKVEIK	VL; CD28 (SEQ ID NO: 15)
Sequences of CD40 antibodies	
QVQLVQSGAEVKKPGASVKVSCASGYTFTGYIHWVRQAP PGQGLEWVGWINPDSSGNTYAQKFQGRVTMTDRDTSISTAYM ELNRLRSDDTAVYYCARDQPLGYCTNGVCSYFDYWGQGLTV TVSS	VH; CD40 (SEQ ID NO: 16)
DIQMTQSPSSVSASVGDRTVITCRASQGIYSWLAWYQQKPGK APNLLIYTASTLQSGVPSRFSGSGSDFTLTISLQPEDFATY YCQQANIFPLTFGGKTKVEIK	VL; CD40 (SEQ ID NO: 17)
EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYIHWVRQAPG KGLEWVARVIPNAGGTSYNQKFKGRFTLSVDNSKNTAYLQM NSLRAEDTAVYYCAREGIYWWGQGLTVTVSS	VH; CD40d (SEQ ID NO: 55)
DIQMTQSPSSLSASVGDRTVITCRSSQSLVHNSGNTFLHWYQQ KPGKAPKLLIYTVSNRFGVPSRFSGSGSDFTLTISLQPEDF ATYFCSQTTHVPWTFGQGTKEIK	VL; CD40d (SEQ ID NO: 56)
Sequences of CD137 antibodies	
QVQLQQWGAGLLKPSETLSLTCVYGGSFSGYYWSWIRQSPE KGLEWIGEINHGGYVTYNPSLESRTISVDTSKNQFSLKLSSV TAADTAVYYCARDYGPNGYDWYFDLWGRGLTVTVSS	VH; CD137e (SEQ ID NO: 18)
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQA PRLLIYDASNRTGIPARFSGSGSDFTLTISLQPEDFAVYY CQQRSNWPPALTFGGKTKVEIK	VL; CD137e (SEQ ID NO: 19)
EVQLVQSGAEVKKPGESLRISCKGSGYSFSTYISWVRQMPG KGLEWMGKIYPGDSYTNYSFPGQVTTISADKSISTAYLQWSS LKASDTAMYYCARGYGFIDYWGQGLTVTVSS	VH; CD137o (SEQ ID NO: 20)
SYELTQPPSVSVSPGQTASITCSGDNIGDQYAHWYQQKPGQSP VLVIYQDKNRPSGIPERFSGSGNSGNTATLTISGTQAMDEADYY CATYTGFGSLAVFGGKTLTVL	VL; CD137o (SEQ ID NO: 21)

US 2025/0051444 A1

Feb. 13, 2025

15

Sequences of CD40 ligand (CD40L)
 Variants of CD40LRBD can use one of the following segments: 112-261 segment, 113-261 segment, 116-261 segment, 117-261 segment, 119-261 segment, and 121-261 segment in the CD40 ligand sequence.

HRRLDKIEDERNLHEDFVFMKTIQRCNTGERSLSLLNCEEIKS	CD40LECD
QFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASS	(47-261)
KTTSVLQWAEKGYTMSNNLVTLENGKQLTVKRQGLYYIYA	(SEQ ID NO: 22)
QVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKP	
CGQQSIHLGGVFELQPGASVFVNVTDPQSQVSHGTGFTSFGLLK	
L	

NPQIAAHVISEASSKTTSVLQWAEKGYTMSNNLVTLENGKQ	CD40LRBD
LTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERI	(119-261)
LLRAANTHSSAKPCGQSIHLGGVFELQPGASVFVNVTDPQSQ	(SEQ ID NO: 23)
VSHGTGFTSFGLLKL	

Sequences of CD137 ligand (CD137L)
 Variants of CD137LRBD can use one of the following segments: 52-248 segment, 58-248 segment, 71-248 segment, 80-248 segment, 85-248 segment, 52-254 segment, 58-254 segment, 71-254 segment, 80-254 segment, and 85-254 segment in the CD137 ligand sequence.

ACPWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGM	CD137LECD
FAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL	(50-254)
VVAKAGVYVFFQLELRVVGEGSGSVSLALHLQPLRSAA	(SEQ ID NO: 24)
GAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHL	
HTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSE	

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSD	CD137LRBD
PGLAGVSLTGGLSYKEDTKELVVAKAGVYVFFQLELRV	(71-254)
AGEGSGSVSLALHLQPLRSAAAGAAALALTVDLPPASSEARN	(SEQ ID NO: 25)
AFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLG	
LFRVTPEIPAGLPSRSE	

Sequence of IgG1 CH

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNS	CH1-3 IgG1
GALTSGVHTFPAVLQSSGLYSLSSVTVPPSSSLGTQTYICNVN	CH1: (SEQ
HKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPP	ID NO: 26)
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV	Fc: (SEQ ID
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKV	NO: 27)
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV	
LTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSF	
LYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLS	
PGK	

[0320] The bold part is the Fc region.

[0321] The Fc region can contain the following mutations:

Mutations to knock out FcγR function and/or the KIH or charged pairs mutations	Mutations to increase the FcγRIIb binidng
N297A, L234F/L235E/P331S, Y349C/K370E/K409D/K439E, S354C/D356K/E357K/D399K, S354C/T366W, Y349C/T366S/L368A/Y407V, L234F/L235E/P331S/Y349C/K370E/K409D/K439E, L234F/L235E/P331S/S354C/D356K/E357K/D399K, L234F/L235E/P331S/S354C/T366W, L234F/L235E/P331S/Y349C/T366S/L368A/Y407V, N297A/Y349C/K370E/K409D/K439E, N297A/S354C/D356K/E357K/D399K, N297A/S354C/T366W, N297A/Y349C/T366S/L368A/Y407V.	S267E, S267E/G236D, S267E/S239D, S267E/L328F

US 2025/0051444 A1

Feb. 13, 2025

16

[0322] In a preferred embodiment of the present invention, the Fc region has the following sequences:

EPKSSDKTHTCPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPCRKLTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc (C220S/L234F/L235E/P331S/S354C/D356K/E357K/D399K) (SEQ ID NO: 57)
EPKSSDKTHTCPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVCTLPSPRDELTKNQVSLTCLVEGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSDLTVDKSRWQQGNVFSVCSVMHEALHNHYTQESLSLSPGK	Fc (L234F/L235E/P331S/Y349C/K370E/K409D/K439E) (SEQ ID NO: 58)
EPKSSDKTHTCPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVEHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc (L234F/L235E/S267E/P331S) (SEQ ID NO: 59)
EPKSSDKTHTCPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSLSPGK	Fc (L234F/L235E/P331S) (SEQ ID NO: 60)

Sequence of IgG4 CH

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTITYTCNVDHKPSNTKVDKRV ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI ISKAKGQPREPQVYTLPPSQKMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCVMHEALHNHYTQKSLSL SLGK	CH1-3 IgG4 (S228P) CH1: (SEQ ID NO: 28) Fc: (SEQ ID NO: 29)
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[0323] The bold part is the Fc region.

[0324] The Fc region can contain the following mutations:

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Mutations of the KIH or the charged pairs	Mutations to increase the Fcγ RIIB binidng	Mutations of the KIH or the charged pairs	Mutations to increase the Fcγ RIIB binidng
Y349C/K370E/R409D/K439E, S354C/E356K/E357K/D399K,	S228P/S267E, S228P/S267E/G236D,	S354C/T366W, Y349C/T366S/L368A/Y407V	S228P/S267E/S239D, S228P/S267E/L328F

Sequences of CL

RTVAAPSVMFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	CL; Kappa (SEQ ID NO: 30)
GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKNKYAASSYLSTPEQWKSRSYSCQVTHEGSTVEKTVAPTECS	CL; Lambda (SEQ ID NO: 31)

US 2025/0051444 A1

Feb. 13, 2025

17

Optional linker sequences
G
GS
GSS
GSSSG
GGGGS
GGGSGGGGS
GGGSGGGSGGGGS
GGGSGGGSGGGSGGGGS
GGGSGGGSGGGSGGGSGGGGS
PGGGGSP
PGGGSGPGGGSGPGGGGSP
GEPGSGE
GEPGSGEGEPGSGE
GEPGSGEGEPGSGEGEPGSGE
EGEPGSGEGEPGSGEGEPGSGEGEPGSGE
GKPGS
GKPGSGKPGS
GKPGSGKPGSGKPGS
GKPGSGKPGSGKPGSGKPGS
GKPGSGKPGSGKPGSGKPGSGKPGS
SSSSG
SSSSGSSSSG
SSSSGSSSSGSSSSG
SSSSGSSSSGSSSSGSSSSG
GRPGSGPGSGRPGSGRPGS
GRPGSGPGSGRPGSGRPGSGRPGS
GKPGSGRPGSGKPGSGRPGS

Example 1

Preparation and Verification of Anti-LRRC15 Antibodies

[0325] The LRRC15 antibodies of the present invention were obtained by phage library screening. The phage screening technology is referenced in, for example, Kay, B. Winter, J. & McCafferty, J. (eds.) (1996) Phage Display of Polypeptides and Proteins: A Laboratory Manual (Academic Press). During the screening, specific binding molecules were screened for the extracellular domain of LRRC15 by ELISA and FACS methods, and the sequences were confirmed by sequencing. Finally, four candidate antibodies were obtained: L15-06, L15-08, L15-03 and L15-07.

[0326] Furthermore, the binding ability of the LRRC15 antibodies of the present invention to CHO cells expressing LRRC15 was tested. Therein, CHO cells expressing

LRRC15 were prepared by the following method: the full-length sequence gene fragments of human, mouse and monkey LRRC15 were obtained by whole gene synthesis, and then these fragments were inserted into the backbone of the stable expression vector to obtain the expression plasmid vectors. These three plasmids were transfected into CHO cells, and monoclonal cell lines were screened under the pressure of puromycin, which are the flow cytometry cell lines used in this example.

[0327] CHO cells expressing LRRC15 were washed with FACS buffer (0.5% BSA in PBS) and resuspended at 2E6 cells/ml with the gradient diluted LRRC15 antibodies. The resuspension was incubated on ice for 30 minutes, then washed twice with FACS buffer and incubated with the second antibody on ice for 30 minutes. After washing the resuspended cells with FACS buffer again, the cells were detected by flow cytometry. Based on the measured mean fluorescence intensity, the half maximal binding concentration (EC50) of each antibody was determined.

[0328] The results of the analysis are shown in FIG. 4A-C. The binding abilities of the four antibodies to cells expressing human LRRC15 were similar, but the binding abilities to cells expressing mouse LRRC15 were quite different. Among them, L15-06 had the strongest binding ability, followed by L15-08, L15-03 was weaker, and L15-07 was the weakest. In addition, all four antibodies have strong monkey cross-binding ability.

[0329] In addition, competitive experiments of antibody binding were also carried out: after the four monoclonal antibody molecules were bound to CHO cells expressing LRRC15, the biotin-labeled monoclonal antibody molecule was used to compete for binding, and then the signal intensity was detected by SA-FITC. The greater the decrease in signal, the stronger the competition. The results showed that L15-08 had almost no competitive effect on L15-06, indicating that L15-06 and L15-08 bind to different epitopes of LRRC15 (FIG. 4D).

Example 2

Preparation of Multi-Specific T Cell Engager Molecules of the Present Invention

[0330] According to the four different LRRC15 antibody sequences obtained in Example 1 and the antibody sequence derived from L15-06 (L15-06C), a variety of multi-specific antibody molecules were designed.

[0331] The molecular sequence fragments of the multi-specific antibodies were obtained by the whole gene synthesis method, and then the target sequence was inserted into the expression vector by conventional gene cloning methods (see for example, Lo. B.K.C methods in Molecular Biology. Volume 248, 2004. Antibody Engineering).

[0332] HEK293E cells were transfected with the vector carrying the multi-specific molecule. The cells were cultured in F17 medium (1 L F17+10 mL 10% PF68+30 mL 200 mM L-glutamine) at 37° C., 5% CO₂, for 7 days, then the corresponding molecules can be produced. After the expression was finished, the supernatant was collected and purified to obtain the multi-specific T cell engager molecule, which could be used for various experimental analysis.

[0333] Using the above method, the following multi-specific molecules were prepared:

[0334] (1) Bispecific antibodies composed of LRRC15 antigen binding domain and CD3 antigen binding domain,

US 2025/0051444 A1

Feb. 13, 2025

as shown in structures a-c in FIG. 1A; trispecific antibodies composed of LRRC15 antigen binding domain, CD3 antigen binding domain, and CD28 antigen binding domain, as shown in structures d-e in FIG. 1A.

[0335] A bispecific antibody of structure a in FIG. 1A is named 315-L06, and its structure is shown in FIG. 1D;

[0336] A bispecific antibody of structure b is named 153-L06C, and its structure is shown in FIG. 1B; another bispecific antibody of structure b is named 153-L07, which differs from 153-L06C in that the anti-LRRC15 scFv uses VH and VL of L15-07; another bispecific antibody of structure b is named 153-L06, which differs from 153-L06C in that the anti-LRRC15 scFv uses VH and VL of L15-06;

[0337] A bispecific antibody of structure c is named 315-L06C08, and its structure is shown in FIG. 1C; another bispecific antibody of structure c is named 315-L0608, which differs from 315-L06C08 in that the anti-LRRC15 scFv uses VH and VL of L15-06; another bispecific antibody of structure c is named 315-L0806, which differs from 315-L06C08 in that the anti-LRRC15 scFv uses VH and VL of L15-08, and the anti-LRRC15 Fab uses VH and VL of L15-06; another bispecific antibody of structure c is named 315-L08C06, which differs from 315-L06C08 in that the anti-LRRC15 scFv uses VH and VL of L15-08C, and the anti-LRRC15 Fab uses VH and VL of L15-06;

[0338] A trispecific antibody of structure e is named 28315-L06, and its structure is shown in FIG. 1E.

[0339] (2) Bispecific antibodies composed of LRRC15 antigen binding domain and CD40 antigen binding domain, as shown in a-d of FIG. 2A.

[0340] A bispecific antibody of structure a in FIG. 2A is named 1540L-L06, and its structure is shown in FIG. 2C;

[0341] A bispecific antibody of structure b is named 1540TL-L06, and its structure is shown in FIG. 2D;

[0342] A bispecific antibody of structure c is named 1540-L06, and its structure is shown in FIG. 2B;

[0343] A bispecific antibody of structure d is named 4015-L06, and its structure is shown in FIG. 2E; another bispecific antibody of structure d is named 40D15-L06C, which differs from 4015-L06 in that the anti-CD40 IgG uses the VH and VL of CD40d, and the anti-LRRC15 scFv uses the VH and VL of L15-06C; another bispecific antibody of structure d is named 40D15-L06CS, which differs from 4015-L06 in that the anti-CD40 IgG uses the VH and VL of CD40d, the Fc fragment is replaced with the fragment shown in SEQ ID NO: 60, and the anti-LRRC15 scFv uses the VH and VL of L15-06C.

[0344] (3) Bispecific antibodies composed of LRRC15 antigen-binding domain and CD137 antigen-binding domain, as shown in a-d of FIG. 3A.

[0345] A bispecific antibody of structure a in FIG. 3A is named 15BBL-L06, and its structure is shown in FIG. 3C.

[0346] A bispecific antibody of structure b is named 15BBTL-L06, and its structure is shown in FIG. 3D.

[0347] A bispecific antibody of structure c is named 15BB-L06, and its structure is shown in FIG. 3B. Another bispecific antibody of structure c is named 15BBc-L06, which differs from 15BB-L06 in that the anti-CD137 scFv uses the VH and VL of CD137c.

[0348] A bispecific antibody of structure d is named BB15-L06, and its structure is shown in FIG. 3E. Another bispecific antibody of structure d is named BBe15-L06, which differs from BB15-L06 in that the anti-CD137 IgG uses the VH and VL of CD137c.

[0349] Exemplary molecules prepared in this example are shown in the following table:

Molecule Name	Chain 1 (SEQ ID NO:)	Chain 2 (SEQ ID NO:)	Chain 3 (SEQ ID NO:)
315-L06	61	80	83
153-L06	62	58	—
153-L07	63	58	—
153-L06C	64	58	—
315-L0608	62	81	84
315-L0806	65	80	83
315-L08C06	66	80	83
315-L06C08	64	81	84
28315-L06	67	80	83
1540L-L06	68	—	83
1540TL-L06	69	82	83
1540-L06	70	—	83
4015-L06	71	—	85
40D15-L06C	72	—	86
40D15-L06CS	73	—	86
15BBL-L06	74	—	83
15BBTL-L06	75	82	83
15BBc-L06	76	—	83
15BB-L06	77	—	83
BBe15-L06	78	—	87
BB15-L06	79	—	88

Example 3

Binding Verification of Bispecific Antibody Molecules Targeting LRRC15 and CD3 with Target Molecules

[0350] The binding of bispecific antibody molecules targeting LRRC15 and CD3 (153-L06C, with a structure shown in FIG. 1B; 315-L06C08, with a structure shown in FIG. 1C), a control sample BM bispecific molecule (its sequences refer to WO2021022304A2) with CHO cells expressing human LRRC15 and CHO cells expressing cynomolgus LRRC15 were detected, and the method was as described in Example 1.

[0351] The results showed that 315-L06C08 exhibited the lowest EC50 value for the CHO cells expressing human LRRC15, indicating the strongest binding affinity. Conversely, 153-L06C demonstrated the highest binding platform period, meaning it could bind more antibody molecules at saturation dose. (FIG. 5A).

[0352] For CHO cells expressing cynomolgus LRRC15, the binding trend of each molecule was similar to that of CHO cells expressing human LRRC15 (FIG. 5B).

Example 4

Characterization of Bispecific Antibody Molecules Targeting LRRC15 and CD3

4.1 T Cell Activation Assay of Bispecific Antibody Molecules Targeting LRRC15 and CD3

[0353] The T cell activation ability of bispecific antibody molecules targeting LRRC15 and CD3 (153-L06C and 315-L06C08) was measured, with the above BM molecule as a control sample. The specific method is as follows:

[0354] CHO cells expressing LRRC15 (wild-type CHO cells not expressing LRRC15 were used as a control) were washed with PBMC buffer (RPMI-1640+10% FBS+1% Penstrep) and resuspended with gradient diluted antibodies to be tested at 2E6 cells/ml. The prepared PBMC cells were added to the resuspension to make the E/T cell ratio of 10:1,

US 2025/0051444 A1

Feb. 13, 2025

19

and incubated at 37° C. for 48 h, then the cells were washed twice with FACS buffer and co-incubated with anti-CD3 and anti-CD25 secondary antibodies on ice for 30 min. After washing the resuspended cells with FACS buffer again, analysis was conducted by flow cytometry. Based on the measured mean fluorescence intensity, the half maximal activation concentration (EC50) of each antibody was calculated.

[0355] CD25 is an important marker of T cell activation. The results showed that the three bispecific molecules induced T cell activation only in the presence of target cells. None exhibited activation effects on T cells in the presence of cells lacking the target protein expression. Among them, BM had the lowest EC50 for T cell activation, but the lowest maximum proportion of activated cells. 153-L06C required the highest dose for activation but also achieved the largest maximum proportion of activated cells. 315-L06C08 fell in the middle of the other two molecules (FIG. 6).

4.2 IFN- γ Release Assay of Bispecific Antibody Molecules Targeting LRRC15 and CD3

[0356] The ability of bispecific antibody molecules targeting LRRC15 and CD3 (153-L06C and 315-L06C08) to stimulate PBMCs to release IFN- γ was measured, with the above BM molecule as a control sample. The specific method is as follows:

[0357] CHO cells expressing LRRC15 (wild-type CHO cells not expressing LRRC15 were used as a control) were washed with PBMC buffer (RPMI-1640+10% FBS+1% Penstrep) and resuspended with gradient diluted antibodies to be tested at 2E6 cells/ml. The prepared PBMC cells were added to the resuspension to make the E/T cell ratio of 10:1, and incubated at 37° C. for 48 h. The supernatant was taken to test the IFN- γ content using IFN- γ ELISA kit.

[0358] The results showed that the three bispecific molecules could stimulate PBMCs to release IFN- γ only in the presence of target cells. None induced any significant IFN- γ release in the absence of target cells. Among them, BM exhibited the weakest stimulatory effect. 153-L06C achieved the highest peak IFN- γ release, but there was a large gap compared to the EC50 value of 315-L06C08 for inducing IFN- γ release (FIG. 7).

4.3 T Cell Proliferation Assay Stimulated by Bispecific Antibody Molecules Targeting LRRC15 and CD3

[0359] The ability of bispecific antibody molecules targeting LRRC15 and CD3 (153-L06C and 315-L06C08) to stimulate T cell proliferation was measured, with the above BM molecule as a control sample. The specific method is as follows:

[0360] T cells were isolated from PBMCs using a Pan T Cell Isolation Kit (cat #130-096-535, Miltenyi) and labeled with CFSE. 50 μ l of labeled T cells (containing 5E4 cells) were mixed with 100 μ l of CHO cells expressing LRRC15 (containing 1E4 cells) (wild-type CHO cells not expressing LRRC15 were used as a control) in each well of a 96-well plate, and resuspended with 50 μ l of gradient diluted antibodies to be tested, then subjected to incubation at 37° C. for 5 days. Then the cells were washed twice with FACS buffer and analyzed by flow cytometry. Based on the proliferation of T cells under different conditions, the half-maximal

proliferation-promoting concentration (EC50) of each antibody was determined according to the corresponding CFSE staining signal value.

[0361] The results showed that 153-L06C and 315-L06C08 could promote cell proliferation only in the presence of target cells. None of the three bispecific molecules significantly promoted proliferation in the presence of cells lacking LRRC15 expression. Among them, BM had almost no effect on T cell proliferation. 153-L06C achieved the highest maximum proportion of proliferating T cells, while it exhibited a large gap compared to the EC50 value of 315-L06C08 for promoting proliferation (FIG. 8).

4.4 Cytotoxicity Assay of PBMCs on Target Cells Stimulated by Bispecific Antibody Molecules Targeting LRRC15 and CD3

[0362] The ability of bispecific antibody molecules targeting LRRC15 and CD3 (153-L06C and 315-L06C08) to stimulate PBMCs to kill target cells was measured, with the above BM molecule as a control sample. The specific method is as follows:

[0363] CHO cells expressing LRRC15 were washed with PBMC buffer (RPMI-1640+10% FBS+1% Penstrep) and resuspended with gradient diluted antibodies to be tested at 2E6 cells/ml. The prepared PBMC cells were added to the resuspension to make the E/T cell ratio of 10:1, then subjected to incubation at 37° C. for 48 h. CytoTox-Glo™ cytotoxicity assay reagent was added to the incubated 96-well plate. Based on the measured chemiluminescence intensity, the killing effect of each antibody on target cells was determined.

[0364] The results demonstrated that all three bispecific molecules could stimulate PBMCs to kill target cells, but only in their presence. None exhibited significant killing activity against cells lacking LRRC15 expression. BM displayed the weakest killing effect and exhibited a significant hook effect. 153-L06C achieved the highest maximum proportion of killing, while it showed a large gap compared to the EC50 value of 315-L06C08 for inducing killing (FIG. 9).

4.5 Cytokine Release Assay of PBMCs Stimulated by Bispecific Antibody Molecules Targeting LRRC15 and CD3

[0365] The ability of bispecific antibody molecules targeting LRRC15 and CD3 (153-L06C and 315-L06C08, with structures shown in FIGS. 1B and 1C, respectively) to stimulate PBMC to release cytokines was measured, with the above BM molecule as a control sample, and CD3 antibody (OKT3) as a positive control. The specific method is as follows:

[0366] PBMC cells from 4 different donors (D1, D2, D3, D4) were thawed and diluted to 10E7 cells/ml with PBMC buffer (RPMI-1640+10% FBS+1% Penstrep) in a 96-well plate, and the plate was placed in a 37° C. incubator for 48 hours. The antibody to be tested was diluted to 10 nM with PBMC buffer and added to the 96-well plate containing PBMC cells, then subjected to incubation at 37° C. for 24 hours. The BD™ Cytometric Bead Array (CBA) kit was used to capture and detect the cytokine content in the culture supernatant, which could show the cytokine release of different donor PBMCs stimulated by each antibody.

[0367] The results showed that the three bispecific molecules had no significant promoting effect on the release of 6 cytokines, while the CD3 antibody (OKT3) as a positive

US 2025/0051444 A1

Feb. 13, 2025

20

control had different degrees of cytokine release. Untreated was the group without addition of antibody, and as a negative control, there was no significant cytokine release (FIG. 10).

Example 5

Binding Verification of Bispecific Antibody Molecules Targeting LRRC15 and CD137/CD40 with Target Molecules

[0368] The binding of bispecific antibody molecules targeting LRRC15 and CD137 (15BB-L06, with a structure shown in FIG. 3B) to 4-1BB protein, and the binding of bispecific antibody molecules targeting LRRC15 and CD40 (1540-L06, with a structure shown in FIG. 2B) to CD40 protein were detected by ELISA method. The specific method is as follows:

[0369] A 96-well plate was coated with 4-1BB or CD40 antigen proteins overnight at 4° C., then the 96-well plate was washed three times with 0.05% PBST solution, and added with 200 μ l of 1% BSA to each well for blocking at room temperature for 1 h. The plate was washed three times with 0.05% PBST again. Gradient diluted antibody molecules (15BB-L06 or 1540-L06) were resuspended with 1% BSA and added to the 96-well plate in duplicate wells at 100 μ l per well. The plate was washed three times with 0.05% PBST, then added with anti-hIgG-HRP secondary antibody and incubated at room temperature for 0.5 h. The plate was washed three times with 0.05% PBST, then added with TMB reagent for color development and the value at 450 nm in an enzyme reader was read. Based on the measured mean OD450 value, the half maximal binding concentration (EC50) of each antibody was determined.

[0370] The results showed that 15BB-L06 could bind to 4-1BB, and 1540-L06 could bind to CD40 (FIG. 11). Moreover, the 4-1BB antibody or CD40 antibody part in the bispecific antibody molecule is in the form of scFv, which will not lead to over-activation after the antibody binds to the antigen compared with its IgG form, thereby reducing the risk of the bispecific antibody molecule during administration.

Example 6

Characterization of Bispecific Antibody Molecules Targeting LRRC15 and CD137/CD40

[0371] The ability of bispecific antibody targeting LRRC15 and CD137 (15BB-L06) to stimulate PBMCs to release IL-2, and the ability of bispecific antibody targeting LRRC15 and CD40 (1540-L06) to stimulate DCs to release IL-12p40 were measured.

[0372] 10 μ g/ml of CD3 antibody (OKT3, eBioscience) was added to each well of a 96-well plate, at 100 μ l per well, and incubated at 37° C. for 2 h. CHO cells expressing LRRC15 (wild-type CHO cells not expressing LRRC15 were used as a control) were washed with PBMC buffer (RPMI-1640+10% FBS+1% Penstrep) and resuspended with gradient diluted antibodies to be tested at 2E6 cells/ml. The prepared PBMC cells were added to the resuspension to make the E/T cell ratio of 10:1, then subjected to incubation at 37° C. for 48 h. The supernatant was taken and the IL-2 content was detected using the IL-2 ELISA kit.

[0373] The results showed that in the presence of target cells, 15BB-L06 stimulated higher IL-2 release than Urelumab (4-1BB monospecific antibody), but in the presence

of cells not expressing the target protein, IL-2 release was similar to that of control IgG, demonstrating the effectiveness of the antibody molecule's target specificity. In contrast, the control antibody Urelumab did not have this specificity (FIG. 12).

[0374] Monocytes were isolated from PBMCs using a monocyte separation kit (cat #130-096-537, Miltenyi). GM-CSF and IL-4 were added at 20 ng/ml to induce the differentiation of monocytes into DC cells, and DC cells were harvested after 7 days. CHO cells expressing LRRC15 (wild-type CHO cells not expressing LRRC15 were used as a control) were washed with PBMC buffer (RPMI-1640+10% FBS+1% Penstrep) and resuspended with gradient diluted antibodies to be tested at 2E6 cells/ml. The prepared DC cells were added to the resuspension to make the E/T cell ratio of 3:1, and subjected to incubation at 37° C. for 24 h. The supernatant was taken and the IL-12p40 content was detected using the IL-12p40 ELISA kit.

[0375] The results showed that although 1540-L06 stimulated slightly lower IL-12p40 release than Selicrelumab (CD40 monospecific antibody) in the presence of target cells, IL-12p40 release was significantly reduced in the presence of cells not expressing the target protein, demonstrating the effectiveness of the antibody molecule's target specificity. In contrast, the control antibody Selicrelumab did not have this specificity (FIG. 13).

[0376] In addition, the ability of the above bispecific molecules to stimulate PBMCs to release cytokines (TNF- α and IL-6) was measured, with CD3 antibody (OKT3) as a positive control. The specific method is as follows:

[0377] PBMC cells from 5 different donors were thawed and diluted to 10E7 cells/ml with PBMC buffer (RPMI-1640+10% FBS+1% Penstrep) in a 96-well plate, and the plate was placed in a 37° C. incubator for 48 h. The antibody to be tested was diluted to 10 nM with PBMC buffer and added to the 96-well plate containing PBMC cells, then subjected to incubation at 37° C. for 24 hours. TNF- α and IL-6 ELISA kits were used to capture and detect the cytokine content in the culture supernatant, respectively, which could show the cytokine release of different donor PBMCs stimulated by each antibody.

[0378] The results showed that the two bispecific molecules 1540-L06 and 15BB-L06 had no significant promoting effect on the release of the two cytokines TNF- α and IL-6 (FIG. 14).

Example 7

In Vivo Efficacy Study of Bispecific Antibody Molecules Targeting LRRC15

7.1 NCI-H1650 Model

[0379] NOG mice were inoculated with NCI-H1650 cells (1×10^7 cells/mouse), and PBMCs were inoculated 15 days later. When the average tumor volume grew to about 200 mm³, the mice were grouped and administered (the control group was administered with PBS, and the experimental groups were administered with 153-L06C and 315-L06C08, respectively). The administration was conducted by intravenous injection, twice a week, for 7 consecutive weeks. During this period, the tumor volume was measured (FIG. 15A) and the mice were observed for any abnormalities.

[0380] The results showed that at equimolar doses, 153-L06C (0.2 mg/kg) and 315-L06C08 (0.288 mg/kg) showed

US 2025/0051444 A1

Feb. 13, 2025

21

similar antitumor effects, with TGIs of 37% and 39%, respectively. The animals tolerated the drug well during the administration period. At the end of the experiment, the tumors were collected and weighed. The tumor weight results showed more significantly the antitumor effects of 153-L06C and 315-L06C08, with tumor weights of 55% and 57% of that of the control group, respectively (FIG. 15B).

7.2 MC38 Model

[0381] Humanized hCD3e/h4-1BB double-gene knock-in C57BL/6 mice were inoculated with MC38-huLRRC15 cells (2×10^6 cells/mouse), and when the average tumor volume grew to about 120 mm³, the mice were grouped and administered (the control group was administered with saline, and the experimental groups were administered with 153-L06C and 15BB-L06, respectively). The administration was conducted by intravenous injection, twice a week, for 4 consecutive weeks.

[0382] The results showed that 153-L06C (1 mg/kg) and 15BB-L06 (1 mg/kg) showed similar antitumor effects, with TGIs of 24% and 43%, respectively (FIG. 15C).

[0383] Humanized hCD40 gene knock-in C57BL/6 mice were inoculated with MC38-huLRRC15 cells (2×10^6 cells/mouse), and when the average tumor volume grew to about 120 mm³, the mice were grouped and administered (the control group was administered with saline, and the experimental group was administered with 1540-L06). The administration was conducted by intravenous injection, twice a week (the first two doses were 1 mg/kg, and the third dose was 6 mg/kg), for 3 consecutive weeks. After the dose was adjusted to 6 mg/kg, 1540-L06 showed a significant antitumor effect, with a TGI of 59% (FIG. 15D).

Comparison Example 1

Comparative Study of Multi-Specific T Cell Engager Molecules with Different Structures

[0384] The ability of the bispecific antibody molecules targeting LRRC15 and CD3 (153-L06 and 315-L06) prepared in Example 2 to stimulate PBMCs to kill target cells was measured. The specific method refers to Example 4.4.

[0385] The results showed that the killing effect of 153-L06 was significantly better than that of 315-L06, indicating that the molecule with the structure of 153-L06 has a better ability to stimulate target cell killing (FIG. 16).

[0386] The ability of the bispecific antibody molecules targeting LRRC15 and CD3 (153-L06C, 315-L06C08, 315-L08C06) and the trispecific antibody molecule targeting LRRC15, CD3 and CD28 (28315-L06) prepared in Example 2 to stimulate PBMCs to kill target cells was measured. The specific method refers to Example 4.4.

[0387] The results showed that the killing effect of 315-L06C08 was significantly better than that of other molecules, indicating that the molecule with the structure of 315-L06C08 has a better ability to stimulate target cell killing (FIG. 17).

[0388] All of the references mentioned in this invention are incorporated herein by reference as if each individual reference were specifically and individually incorporated by reference. It should also be understood that after reading the foregoing disclosure of the invention, those skilled in the art can make various changes or modifications to the invention, and these equivalent forms are also within the scope of the claims appended to this application.

SEQUENCE LISTING

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                 note = VH; L15-06H
                 organism = synthetic construct

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ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIG SYRSFFSTDD GDSL DYWGQG 120
TLVTVSS 127

SEQ ID NO: 2      moltype = AA  length = 127
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                 mol_type = protein
                 note = VH; L15-08H
                 organism = synthetic construct

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TLVTVSS 127

SEQ ID NO: 3      moltype = AA  length = 120
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source           1..120
                 mol_type = protein
                 note = VH; L15-03H
                 organism = synthetic construct

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SEQ ID NO: 4      moltype = AA  length = 119
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US 2025/0051444 A1

Feb. 13, 2025

22

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FEATURE                               Location/Qualifiers
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                                   note = VH; L15-07H
                                   organism = synthetic construct

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SEQ ID NO: 5                        moltype = AA length = 107
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source                               1..107
                                   mol_type = protein
                                   note = VL; L15-06L
                                   organism = synthetic construct

SEQUENCE: 5
DIQMTQSPST LSASVGDRVT ITCRASQSIG SWLAWYQQKP GKAPKLLIYD ASSLESGVPS 60
RFGSGSGSTE FTLTISSLQP DDFATYYCQQ YNSYSLTFGG GTKVEIK 107

SEQ ID NO: 6                        moltype = AA length = 107
FEATURE                               Location/Qualifiers
source                               1..107
                                   mol_type = protein
                                   note = VL; L15-08L
                                   organism = synthetic construct

SEQUENCE: 6
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RFGSGSGSTE FTLTISSLQP DDFATYYCQQ YNSYSITFGG GTKVEIK 107

SEQ ID NO: 7                        moltype = AA length = 110
FEATURE                               Location/Qualifiers
source                               1..110
                                   mol_type = protein
                                   note = VL; L15-03L
                                   organism = synthetic construct

SEQUENCE: 7
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SNRFGSGSKG NTASLTISGL QAEDEADYYC QSWTHDNSV VFGGTKLTVL 110

SEQ ID NO: 8                        moltype = AA length = 111
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source                               1..111
                                   mol_type = protein
                                   note = VL; L15-07L
                                   organism = synthetic construct

SEQUENCE: 8
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SNRFGSGSKG NTASLTISGL QAEDEADYYC QSYANRDNFV VFGGTKLTV L 111

SEQ ID NO: 9                        moltype = AA length = 125
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source                               1..125
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                                   note = VH; CD3vB
                                   organism = synthetic construct

SEQUENCE: 9
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YYADSVKGRF TISRDDSKNT LYLQMNSLRA EDTAVYYCVR HGNFGDSYVS WFAYWGQGTL 120
VTSS 125

SEQ ID NO: 10                       moltype = AA length = 125
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source                               1..125
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                                   note = VH; CD3vG
                                   organism = synthetic construct

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US 2025/0051444 A1

Feb. 13, 2025

23

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organism = synthetic construct

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SEQ ID NO: 12      moltype = AA length = 125
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                  mol_type = protein
                  note = VH; CD3vJ
                  organism = synthetic construct

SEQUENCE: 12
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YYADSVKGRF TISRDDSKNT LYLQMNSLRA EDTAVYYCVR HGNFGDSYVS WFDYWGQGT 120
LTVSS 125

SEQ ID NO: 13      moltype = AA length = 109
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                  mol_type = protein
                  note = VL; CD3
                  organism = synthetic construct

SEQUENCE: 13
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PARFSGSLLG GKAALTISGA QPEDEADYYC ALWYSNHWVF GGGTKLTVL 109

SEQ ID NO: 14      moltype = AA length = 120
FEATURE           Location/Qualifiers
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                  mol_type = protein
                  note = VH;CD28 (C50S)
                  organism = synthetic construct

SEQUENCE: 14
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NEKFKDRATL TVDTSISTAY MELSLRLSDD TAVYFCTRSY YGLDWNFPDVW GQGTITVTVSS 120

SEQ ID NO: 15      moltype = AA length = 107
FEATURE           Location/Qualifiers
source            1..107
                  mol_type = protein
                  note = VL; CD28
                  organism = synthetic construct

SEQUENCE: 15
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RFSGSGSGTD FTLTISSLQP EDFATYYCQQ GQTPYPTFGG GTKVEIK 107

SEQ ID NO: 16      moltype = AA length = 126
FEATURE           Location/Qualifiers
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                  mol_type = protein
                  note = VH; CD40
                  organism = synthetic construct

SEQUENCE: 16
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AQKFQGRVTM TRDTSISTAY MELNRLSDD TAVYYCARDQ PLGYCTNGVC SYFDYWGQGT 120
LTVTVSS 126

SEQ ID NO: 17      moltype = AA length = 107
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                  mol_type = protein
                  note = VL; CD40
                  organism = synthetic construct

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RFSGSGSGTD FTLTISSLQP EDFATYYCQQ ANIFPLTFGG GTKVEIK 107

SEQ ID NO: 18      moltype = AA length = 121
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                  mol_type = protein
                  note = VH; CD137e
                  organism = synthetic construct

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US 2025/0051444 A1

Feb. 13, 2025

24

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S 121

SEQ ID NO: 19 moltype = AA length = 109
FEATURE Location/Qualifiers
source 1..109
 mol_type = protein
 note = VL; CD137e
 organism = synthetic construct

SEQUENCE: 19
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SEQ ID NO: 20 moltype = AA length = 116
FEATURE Location/Qualifiers
source 1..116
 mol_type = protein
 note = VH; CD137o
 organism = synthetic construct

SEQUENCE: 20
EVQLVQSGAE VKKPGESLRI SCKGSGYSFS TYWISWVRQM PGKGLEWMGK IYPGDSYTNV 60
SPSFQGGVTI SADKSISTAY LQWSSLKASD TAMYYCARGY GIFDYWGQGT LVTVSS 116

SEQ ID NO: 21 moltype = AA length = 108
FEATURE Location/Qualifiers
source 1..108
 mol_type = protein
 note = VL; CD137o
 organism = synthetic construct

SEQUENCE: 21
SYELTQPPSV SVSPGQTASI TCSGDNIGDQ YAHWYQQKPG QSPVLVIYQD KNRPSGIPER 60
FSGSNSGNTA TLTISGTQAM DEADYYCATY TGFGSLAVFG GGTKLTVL 108

SEQ ID NO: 22 moltype = AA length = 215
FEATURE Location/Qualifiers
source 1..215
 mol_type = protein
 note = CD40LECD (47-261)
 organism = synthetic construct

SEQUENCE: 22
HRRLDKIEDE RNLHEDFVFM KTIQRCNTGE RSLSLLNCEE IKSQFEGFVK DIMLNKEETK 60
KENSFEMQKG DQNPQIAAHV ISEASSKTTS VLQWAEKGYT TMSNNLVTLE NGKQLTVKRQ 120
GLYYIYAQVT FCSNREASSQ APFIASLCLK SPGRFERILL RAANTHSSAK PCGQQSIHLG 180
GVFELQPGAS VFNVTDPDSQ VSHGTGFTSF GLLKL 215

SEQ ID NO: 23 moltype = AA length = 143
FEATURE Location/Qualifiers
source 1..143
 mol_type = protein
 note = CD40LRBD (119-261)
 organism = synthetic construct

SEQUENCE: 23
NPQIAAHVIS EASSKTTSVL QWAEKGYTM SNNLVTLENG KQLTVKRQGL YYIYAQVTFC 60
SNREASSQAP FIASLCLKSP GRFERILLRA ANTHSSAKPC GQQSIHLGGV FELQPGASVF 120
VNVTDPSQVS HGTGFTSFGL LKL 143

SEQ ID NO: 24 moltype = AA length = 205
FEATURE Location/Qualifiers
source 1..205
 mol_type = protein
 note = CD137LECD (50-254)
 organism = synthetic construct

SEQUENCE: 24
ACFWAVSGAR ASPGSAASPR LREGPELSPD DPAGLLDLRQ GMFAQLVAQN VLLIDGPLSW 60
YSDPGLAGVS LTGGLSYKED TKELVVAKAG VYYVFFQLEL RRVVAGEGSG SVSLALHLQP 120
LRSAGAAAL ALTVDLPAS SEARNSAFGF QGRLLHLSAG QRLGVHLHTE ARARHAWQLT 180
QGATVGLGFR VTPEIPAGLP SPRSE 205

SEQ ID NO: 25 moltype = AA length = 184
FEATURE Location/Qualifiers
source 1..184
 mol_type = protein
 note = CD137LRBD (71-254)
 organism = synthetic construct

SEQUENCE: 25
REGPELSPDD PAGLLDLRQG MFAQLVAQNV LLIDGPLSWY SDPGLAGVSL TGGLSYKEDT 60

US 2025/0051444 A1

Feb. 13, 2025

25

-continued

KELVVAKAGV YYVFFQLELR RVVAGEGSGS VSLALHLQPL RSAAGAAALA LTVDLPPASS 120
 EARNSAFGFQ GRLLHLSAGQ RLGVLHLTEA RARHAWQLTQ GATVLGLFRV TPEIPAGLPS 180
 PRSE 184

SEQ ID NO: 26 moltype = AA length = 98
 FEATURE Location/Qualifiers
 source 1..98
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 26
 ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60
 GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRV 98

SEQ ID NO: 27 moltype = AA length = 232
 FEATURE Location/Qualifiers
 source 1..232
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 27
 EPKSCDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF 60
 NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT 120
 ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTPP 180
 PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP GK 232

SEQ ID NO: 28 moltype = AA length = 98
 FEATURE Location/Qualifiers
 source 1..98
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 28
 ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS 60
 GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRV 98

SEQ ID NO: 29 moltype = AA length = 229
 FEATURE Location/Qualifiers
 source 1..229
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 29
 ESKYGPPCP CPAPAEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY 60
 VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK 120
 AKGQPREPQV YTLPPSQKMK TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTPPVVL 180
 KSDGSFFFLYS RLTVDKSRWQ EGNVFCSCVM HEALHNNHYTQ KSLSLSLGK 229

SEQ ID NO: 30 moltype = AA length = 107
 FEATURE Location/Qualifiers
 source 1..107
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 30
 RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD 60
 SKDSTYSLSS TLTLKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC 107

SEQ ID NO: 31 moltype = AA length = 106
 FEATURE Location/Qualifiers
 source 1..106
 mol_type = protein
 organism = Homo sapiens

SEQUENCE: 31
 GQPKAAPSVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV AWKADSSPVK AGVETTPPSK 60
 QSNKYAASS YLSLTPEQWK SHRSYSCQVT HEGSTVEKTV APTCS 106

SEQ ID NO: 32 moltype = AA length = 10
 FEATURE Location/Qualifiers
 source 1..10
 mol_type = protein
 note = HCDR1; L15-06H/ L15-06CH
 organism = synthetic construct

SEQUENCE: 32
 GFTFSSYYMS 10

SEQ ID NO: 33 moltype = AA length = 17
 FEATURE Location/Qualifiers
 source 1..17
 mol_type = protein
 note = HCDR2; L15-06H/ L15-06CH

US 2025/0051444 A1

Feb. 13, 2025

26

-continued

SEQUENCE: 33	organism = synthetic construct	
DISVSGSYTN YADSVKG		17
SEQ ID NO: 34	moltype = AA length = 18	
FEATURE	Location/Qualifiers	
source	1..18	
	mol_type = protein	
	note = HCDR3; L15-06H/ L15-06CH	
	organism = synthetic construct	
SEQUENCE: 34		
IGSYRSFFST DDGDSL DY		18
SEQ ID NO: 35	moltype = AA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = protein	
	note = HCDR1; L15-08H	
	organism = synthetic construct	
SEQUENCE: 35		
GFTFSDYMS		10
SEQ ID NO: 36	moltype = AA length = 17	
FEATURE	Location/Qualifiers	
source	1..17	
	mol_type = protein	
	note = HCDR2; L15-08H	
	organism = synthetic construct	
SEQUENCE: 36		
DISVSSSYTN YADSVKG		17
SEQ ID NO: 37	moltype = AA length = 18	
FEATURE	Location/Qualifiers	
source	1..18	
	mol_type = protein	
	note = HCDR3; L15-08H	
	organism = synthetic construct	
SEQUENCE: 37		
LGGYRSFFST DDGDSL DY		18
SEQ ID NO: 38	moltype = AA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = protein	
	note = HCDR1; L15-03H	
	organism = synthetic construct	
SEQUENCE: 38		
GGTFSSYTIS		10
SEQ ID NO: 39	moltype = AA length = 17	
FEATURE	Location/Qualifiers	
source	1..17	
	mol_type = protein	
	note = HCDR2; L15-03H	
	organism = synthetic construct	
SEQUENCE: 39		
GIIPMFGTAN YAQKFQG		17
SEQ ID NO: 40	moltype = AA length = 11	
FEATURE	Location/Qualifiers	
source	1..11	
	mol_type = protein	
	note = HCDR3; L15-03H	
	organism = synthetic construct	
SEQUENCE: 40		
GVALLATDFD Y		11
SEQ ID NO: 41	moltype = AA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = protein	
	note = HCDR1; L15-07H	
	organism = synthetic construct	
SEQUENCE: 41		
GGTFSTY AIS		10

US 2025/0051444 A1

Feb. 13, 2025

27

-continued

SEQ ID NO: 42	moltype = AA length = 17	
FEATURE	Location/Qualifiers	
source	1..17	
	mol_type = protein	
	note = HCDR2; L15-07H	
	organism = synthetic construct	
SEQUENCE: 42		
GITPMFGTPN YAQKFQG		17
SEQ ID NO: 43	moltype = AA length = 10	
FEATURE	Location/Qualifiers	
source	1..10	
	mol_type = protein	
	note = HCDR3; L15-07H	
	organism = synthetic construct	
SEQUENCE: 43		
GDWYDYHFDY		10
SEQ ID NO: 44	moltype = AA length = 11	
FEATURE	Location/Qualifiers	
source	1..11	
	mol_type = protein	
	note = LCDRL; L15-06L/ L15-06CL/L15-08L	
	organism = synthetic construct	
SEQUENCE: 44		
RASQSISSWL A		11
SEQ ID NO: 45	moltype = AA length = 7	
FEATURE	Location/Qualifiers	
source	1..7	
	mol_type = protein	
	note = LCDR2; L15-06L/ L15-06CL	
	organism = synthetic construct	
SEQUENCE: 45		
DASSLES		7
SEQ ID NO: 46	moltype = AA length = 9	
FEATURE	Location/Qualifiers	
source	1..9	
	mol_type = protein	
	note = LCDR3; L15-06L/ L15-06CL	
	organism = synthetic construct	
SEQUENCE: 46		
QQYNSYSLT		9
SEQ ID NO: 47	moltype = AA length = 7	
FEATURE	Location/Qualifiers	
source	1..7	
	mol_type = protein	
	note = LCDR2; L15-08L	
	organism = synthetic construct	
SEQUENCE: 47		
DAFSLES		7
SEQ ID NO: 48	moltype = AA length = 9	
FEATURE	Location/Qualifiers	
source	1..9	
	mol_type = protein	
	note = LCDR3; L15-08L	
	organism = synthetic construct	
SEQUENCE: 48		
QQYNSYSIT		9
SEQ ID NO: 49	moltype = AA length = 14	
FEATURE	Location/Qualifiers	
source	1..14	
	mol_type = protein	
	note = LCDRL; L15-03L/L15-07L	
	organism = synthetic construct	
SEQUENCE: 49		
TGTSSDVGGY NYVS		14
SEQ ID NO: 50	moltype = AA length = 7	
FEATURE	Location/Qualifiers	
source	1..7	
	mol_type = protein	

US 2025/0051444 A1

Feb. 13, 2025

28

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note = LCDR2; L15-03L/L15-07L
organism = synthetic construct

SEQUENCE: 50
EVSNRPS 7

SEQ ID NO: 51
FEATURE
source
moltype = AA length = 10
Location/Qualifiers
1..10
mol_type = protein
note = LCDR3; L15-03L
organism = synthetic construct

SEQUENCE: 51
QSWTHDNSVV 10

SEQ ID NO: 52
FEATURE
source
moltype = AA length = 11
Location/Qualifiers
1..11
mol_type = protein
note = LCDR3; L15-07L
organism = synthetic construct

SEQUENCE: 52
QSYANRDNFV V 11

SEQ ID NO: 53
FEATURE
source
moltype = AA length = 127
Location/Qualifiers
1..127
mol_type = protein
note = VH; L15-06CH
organism = synthetic construct

SEQUENCE: 53
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYMSWVRQA PGKCLEWVAD ISVSGSYTNY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIG SYRSFFSTDD GDSLDYWGQG 120
TLVTVSS 127

SEQ ID NO: 54
FEATURE
source
moltype = AA length = 107
Location/Qualifiers
1..107
mol_type = protein
note = VL; L15-06CL
organism = synthetic construct

SEQUENCE: 54
DIQMTQSPST LSASVGRVIT ITCRASQSI SWLAWYQQKP GKAPKLLIYD ASSLESGVPS 60
RFGSGSGSTE FTLTISSLQP DDFATYYCQQ YNSYSLTFGC GTKVEIK 107

SEQ ID NO: 55
FEATURE
source
moltype = AA length = 114
Location/Qualifiers
1..114
mol_type = protein
note = VH; CD40d
organism = synthetic construct

SEQUENCE: 55
EVQLVESGGG LVQPGGSLRL SCAASGYSTF GYIIHWVRQA PGKGLEWVAR VIPNAGGTSY 60
NQKFKGRFTL SVDNSKNTAY LQMNSLRAED TAVYYCAREG IYWWGQGTIV TVSS 114

SEQ ID NO: 56
FEATURE
source
moltype = AA length = 112
Location/Qualifiers
1..112
mol_type = protein
note = VL; CD40d
organism = synthetic construct

SEQUENCE: 56
DIQMTQSPSS LSASVGRVIT ITCRSSQSLV HSGNFTFLHW YQQKPGKAPK LLIYTVSNRF 60
SGVPSRFSGS GSGTDFTLTI SSLQPEDFAT YFCSQTHVWP WTFGQGTKVE IK 112

SEQ ID NO: 57
FEATURE
source
moltype = AA length = 232
Location/Qualifiers
1..232
mol_type = protein
note = Fc(C220S/L234F/L235E/P331S/S354C/D356K/E357K/D399K)
organism = synthetic construct

SEQUENCE: 57
EPKSSDKTHT CPPCPAPEFE GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF 60
NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPASIEKT 120
ISKAKGPPE PQVYTLPPCR KKLTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTT 180
PVLKSDGSFF LYSKLTVDKS RWQGNVFSC SVMHEALHNN YTQKSLSLSP GK 232

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US 2025/0051444 A1

Feb. 13, 2025

29

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SEQ ID NO: 58      multype = AA  length = 232
FEATURE            Location/Qualifiers
source             1..232
                  mol_type = protein
                  note = Fc(L234F/L235E/P331S/Y349C/K370E/K409D/K439E)
                  organism = synthetic construct
```

SEQUENCE: 58

EPKSCDTH	TPCPAPEFE	GGPSVFLFP	KPKDTLMISR	TPEVTCVVV	VSHEDPEVK	60
NWYVDGVEV	NAKTKPREE	YNSTRYRV	LTVLHQDWL	N	GKEYKCKV	120
ISKAGQPRE	PQVCTLP	PPSR	DELTKNQV	SL	TCCLVEGF	180
PVLSDSGS	FF	LYSDLTV	DKS	RWQQGNV	FS	232
				SVMHEAL	HNH	
				YTOESL	SLSP	
				GK		

```
SEQ ID NO: 59      moltype = AA  length = 232
FEATURE            Location/Qualifiers
source             1..232
                  mol_type = protein
                  note = Fc(L234F/L235E/S267E/P331S)
                  organism = synthetic construct
```

SEQUENCE: 59													
EPKSCDTH	TPCPAP	EPFEG	GGPSV	FLFP	KPKD	TLMISR	TPEV	TCVV	VD	VEHEDPEVKF	60		
NWYVDG	VEVH	NAKTK	PREEQ	YNSTR	YRVSV	LTVL	HQDW	LN	GKEY	KCKVSN	KALPASIEKT	120	
ISKAGQ	PRE	PQVY	TLPPSR	DELTK	QVSL	TC	LVKG	FYPS	DI	AVEWESNG	QENNYKTTP	180	
PVLDS	SGSFF	LQSK	LTVDKS	RWQQ	GNVFS	C	SVMH	EALH	NH	YTKS	LSLSLP	GK	232

```
SEQ ID NO: 60      moltype = AA  length = 232
FEATURE           Location/Qualifiers
source            1..232
                  mol_type = protein
                  note = Fc(L234F/L235E/P331S)
                  organism = synthetic construct
```

[illegible]

```
SEQ ID NO: 61      moltype = AA  length = 486
FEATURE            Location/Qualifiers
source             1..486
                  mol_type = protein
                  note = chain 1
                  organism = synthetic construct
```

SEQUENCE: 61									
EVQLVDSGGG	LTVPGGSLRL	SCAASGFTFS	TYAMNWVRQA	PGKGLEWVGR	IRSKYNNYAT	60			
YVADSVKGRF	LTRRDSKNT	LYLQMSLSRA	EDTAVYYCVR	HGMFGDSYVS	WFEYWGQGTL	120			
VTVSSGGGGS	GGGSGGGGS	GGGGSQAVVT	QEPSLTVSPG	GTVTLTGCGSS	TGAVTTSNYA	180			
NWVQKPGKS	PRGLIGPNTK	RAPGVPARFS	GSLLGGKAAI	LTSQAQPEDE	ADYICALWYS	240			
NHWVFGGKTK	LTVLEPKSD	KTHTCPPCPA	PEFEGGSPVF	TFPPKPKDIT	MISRTPEVTC	300			
VVVDVSHEDP	EVKFNWYVDG	VEVNHAKTKP	REEQYNSYTR	VVSVLTVLHQ	DWLNGKEYKC	360			
KVSNKALPAS	IEKTTISKAG	QPREPQVYTL	PPCRKLTKN	QVSLTCLVKG	FYPSPDIAEV	420			
ESNGQPPENNY	KTTPPVVLKSD	GSPFLYSKLT	VDKSRWQQGN	VFSCSVMHEA	LHNHYTKQSL	480			
SLSPGK						486			

```
SEQ ID NO: 62      moltype = AA length = 746
FEATURE            Location/Qualifiers
source             1..746
                  mol_type = protein
                  note = chain 1
                  organism = synthetic construct
```

SEQUENCE: 62						
EVQLVLESGGGG	LVPQGGSLRL	SCAASGFTFS	SYYMSWVRQA	PGKGLEWVAD	ISVSGSYTNY	60
ADVSKGRFTI	SRDNSKNTLY	LQMNSLRAED	TAVVYCARIQ	SYRSFSTD	GDSL DYWGQG	120
TLVTVSSGGG	GSGGGGSGGG	GSGGGGSDIQ	MTQSPSTLSA	SVGDRVITTC	RASQISSWL	180
AWYQQKPKGA	PKLLIYDASS	LESQVPSRFS	GSGSGTEFLT	TISSLQDPDF	ATYYCYQQYS	240
YSLTFGGGTGK	VEIKSGSRRGS	EVQLVESGGG	LVPQGGSLRL	SCAASGFTFS	TYAMNWRVA	300
PGKGLEWVGR	IRSKYNRYAT	YYADSVKGRF	TISRDSKNT	LYLQMNSLRA	EDTAVVYCVR	360
HGNFGDSYVS	WF EYWQQT L	VTVSSGKPGS	GKPGSGKPGS	GKPGSQAVVT	QEPSLT VSPG	420
GTVTLT CGSS	TGAVTTSYLA	NWVQQKPGKS	PRGLIGTGKN	RAPGVPARFS	PSELGGKAAL	480
TISGAGPEDE	ADYCALSNYA	NHWVFSGGTG	LTVLTPKPSD	KHTHCPPCPA	PFEGSGSVSF	540
LFPPPKPD TL	MISRTPEVTC	VVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	600
VVSVLTVLHQ	DWLNGKEYKC	KVSNKALPAS	IEKTSKAKG	QPREPVYT L	PPCRKLTKN	660
QVSLTCLVKG	FHNSDIAVEW	ESNGQPENNY	KTPPVLVKSD	GSFFLYSKLT	VDKSRWQQGN	720
VFSLCSVMHEA	LPHNHYTQKSL	SLSPGK				746

```
SEQ ID NO: 63          moltype = AA  length = 742
FEATURE                Location/Qualifiers
```

US 2025/0051444 A1

Feb. 13, 2025

30

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source                1..742
                      mol_type = protein
                      note = chain 1
                      organism = synthetic construct

SEQUENCE: 63
QVQLVQSGAE VKKPGSSVKV SCKASGGTFS TYAISWVRQA PGQGLEWMGG ITPMFGTPNY 60
AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCARGD WYDYHFDYWG QGTLVTVSSG 120
GGGSGGGGSG GGGSGGGGSG SALTQPASVS GSPGQSITIS CTGTSSDVGG YNYVSWYQQH 180
PGKAPKLMYI EVSNRPSGVS NRRFSGSKSGN TASLTISGLQ AEDEADYYCQ SYANRDNFV 240
FGGGTKLTVL SGGGSGSEVL VESGGGLVQP GGSRLRLSCAA SGFTFSTYAM NWVRQAPGKG 300
LEWVGRIRSK YNNYATYYAD SVKGRFTISR DDSKNTLYLQ MNSLRAEDTA VYYCVRHGNF 360
GDSYVSWFEY WQQGTLTVTS SGKPGSGKPG SGKPGSGKPG SQAVVTQEPS LTVSPGGTVT 420
LTCGSSTGAV TTSNYANWVQ QKPGKSPRGL IGGTNKRAPG VPARFSGSLG GKAAALTISG 480
AQPEDEADYY CALWYSNHWV FGGGTKLTVL EPKSSDKTHT CPPCPAPEFE GGPSVFLFPP 540
KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV 600
LTVLHQDWLN GKKEYCKVSN KALPASIEKT ISKAKGQPRE PQVYTLPPCR KKLTKNQVSL 660
TCLVKGFYPS DIAVEWESNG QPENNYKTTT PVLKSDGSFF LYSKLTVDKS RWQQGNVFSC 720
SVMHEALHNNH YTKSLSLSP GK 742

SEQ ID NO: 64         moltype = AA length = 746
FEATURE              Location/Qualifiers
source               1..746
                    mol_type = protein
                    note = chain 1
                    organism = synthetic construct

SEQUENCE: 64
EVQLLESGGG LVQPGGSLRL SCAASGFTFS SYMSWVRQA PGKCLEWVAD ISVSGSYTNY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIG SYRFFSTDD GDSLQVWGQ 120
TLVTVSSGGG GSGGGGSGGG GSGGGGSDIQ MTQSPSTLSA SVGDRVITTC RASQSISSWL 180
AWYQQKPGKA PKLLIYDASS LESGVPSRFS GSGSGTEFTL TISLQPDFF ATYYCQQYNS 240
YSITFGCGTK VEIKSGGGGS EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWRQA 300
PGKGLEWVGR IRSKYNNTAT YYADSVKGRF TISRDDSKNT LYLMNSLRA EDTAVYYCVR 360
HGNFGDSYVS WFEYWGQGTL VTVSSGKPGS GKPGSGKPGS GKPGSQAVVT QEPSTVSPG 420
GTVTLTCGSS TGAVTTSNYA NWVQQKPGKS PRGLIGGTNK RAPGVPARFS GSLLGGKAAL 480
TISGAQPEDE ADYYCALWYS NHWVFGGGTK LTVLEPKSSD KTHTCPPCPA PEFEGGPSVF 540
LPPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR 600
VVSVLTVLHQ DWLNGKEYKC KVSNKALPAS IEKTISKAKG QPREPQVYTL PPCRKKLTKN 660
QVSLTCLVKG FYPSTIAVEW ESNQGPENNY KTTTPVLKSD GSFFLYSKLT VDKSRWQQGN 720
VFSCSVMHEA LHNHYTQKSL SLSPGK 746

SEQ ID NO: 65         moltype = AA length = 746
FEATURE              Location/Qualifiers
source               1..746
                    mol_type = protein
                    note = chain 1
                    organism = synthetic construct

SEQUENCE: 65
QVQLVESGGG LVKPGGSLRL SCAASGFTFS DYMSWIRQA PGKGLEWVSD ISVSSSYTNY 60
ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARLG GYRFFSTDD GDSLQVWGQ 120
TLVTVSSGGG GSGGGGSGGG GSGGGGSDIQ LTQSPSTLSA SVGDRVITTC RASQSISSWL 180
AWYQQKPGKA PKLLIYDAFS LESGVPSRFS GSGSGTEFTL TISLQPDFF ATYYCQQYNS 240
YSITFGCGTK VEIKSGGGGS EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWRQA 300
PGKGLEWVGR IRSKYNNTAT YYADSVKGRF TISRDDSKNT LYLMNSLRA EDTAVYYCVR 360
HGNFGDSYVS WFEYWGQGTL VTVSSGKPGS GKPGSGKPGS GKPGSQAVVT QEPSTVSPG 420
GTVTLTCGSS TGAVTTSNYA NWVQQKPGKS PRGLIGGTNK RAPGVPARFS GSLLGGKAAL 480
TISGAQPEDE ADYYCALWYS NHWVFGGGTK LTVLEPKSSD KTHTCPPCPA PEFEGGPSVF 540
LPPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR 600
VVSVLTVLHQ DWLNGKEYKC KVSNKALPAS IEKTISKAKG QPREPQVYTL PPCRKKLTKN 660
QVSLTCLVKG FYPSTIAVEW ESNQGPENNY KTTTPVLKSD GSFFLYSKLT VDKSRWQQGN 720
VFSCSVMHEA LHNHYTQKSL SLSPGK 746

SEQ ID NO: 66         moltype = AA length = 746
FEATURE              Location/Qualifiers
source               1..746
                    mol_type = protein
                    note = chain 1
                    organism = synthetic construct

SEQUENCE: 66
QVQLVESGGG LVKPGGSLRL SCAASGFTFS DYMSWIRQA PGKCLEWVSD ISVSSSYTNY 60
ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARLG GYRFFSTDD GDSLQVWGQ 120
TLVTVSSGGG GSGGGGSGGG GSGGGGSDIQ LTQSPSTLSA SVGDRVITTC RASQSISSWL 180
AWYQQKPGKA PKLLIYDAFS LESGVPSRFS GSGSGTEFTL TISLQPDFF ATYYCQQYNS 240
YSITFGCGTK VEIKSGGGGS EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWRQA 300
PGKGLEWVGR IRSKYNNTAT YYADSVKGRF TISRDDSKNT LYLMNSLRA EDTAVYYCVR 360
HGNFGDSYVS WFEYWGQGTL VTVSSGKPGS GKPGSGKPGS GKPGSQAVVT QEPSTVSPG 420
GTVTLTCGSS TGAVTTSNYA NWVQQKPGKS PRGLIGGTNK RAPGVPARFS GSLLGGKAAL 480

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US 2025/0051444 A1

Feb. 13, 2025

31

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TISGAQPEDE	ADYYCALWYS	NHWVFGGK	LTVLEPKSSD	KHTHCPPCA	PEFEGGPSVF	540
LFPPKPKDTL	MISRTPEVTC	VVVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	600
VVSVLTVLHQ	DWLNKEYKC	KVSNKALPAS	IEKTISKAKG	QPREPQVYTL	PPCRKKLTKN	660
QVSLTCLVK	FYPSPDIWEV	ESNGQPENNY	KTPPVVLKSD	GSFFLYSKLT	VDKSRWQQGN	720
VFSCSVMHEA	LHNHYTQKSL	SLSPGK				746

SEQ ID NO: 67 moltype = AA length = 739
 FEATURE Location/Qualifiers
 source 1..739
 mol_type = protein
 note = chain 1
 organism = synthetic construct

SEQUENCE: 67

QVQLVQSGAE	VKKPGASVKV	SKASGYTFT	SYIHWRQA	PGGLEWIGS	IYPGNVNTNY	60
NEKFKDRATL	TVDTSISTAY	MELSRLRSD	TAVYFCTRSH	YGLDWNFDVW	GQGTTVTVSS	120
GGGGSGGGGS	GGGGSGGGGS	DIQMTQSPSS	LSASVGRVIT	ITCHASQNIY	VWLNWYQOKP	180
GKAPKLLIYK	ASNLTHTGVP	RFGSGSGGTD	FTLTISLQOP	EDFATYYCQQ	GQTYPTTFGG	240
GTKVEIKSGG	GGSEVQLVES	GGGLVQPGGS	LRLSCAASGF	TFSTYAMNWV	RQAPGKGLEW	300
VGRIRSKYNN	YATYYADSVK	GRFTISRDDS	KNTLYLQMNS	LRAEDTAVYY	CVRHGNFGDS	360
YVSWFEYWQ	GTLTVTSSGK	PGSGKPGSGK	PGSGKPGSQA	VVTQEPSLTV	SPGGTVTLTC	420
GSSTGAVTTS	NYANWVQKPK	GKSPRGLIGG	TNKRAPGVPA	RFGSLLGGK	AALTISGAQP	480
EDEADYYCAL	WYSNHWVFGG	GTLTVLEPK	SSDKTHTCPP	CPAPEFEGGP	SVFLFPPKPK	540
DTLMISRTPE	VTCVVVDVSH	EDPEVKFNWY	VDGVEVHNAK	TKPREEQYNS	TYRVSVSLTV	600
LHQDWLNGKE	YKCKVSNKAL	PASIEKTISK	AKGQPREPQV	YTLPPCRKKL	TKNQVSLTCL	660
VKGFYPSDIA	VEWESNGQPE	NNYKTPPVVL	KSDGSFFLYS	KLTVDKSRWQ	QGNVVFSCSVM	720
HEALHNYHTQ	KSLSLSPGK					739

SEQ ID NO: 68 moltype = AA length = 610
 FEATURE Location/Qualifiers
 source 1..610
 mol_type = protein
 note = chain 1
 organism = synthetic construct

SEQUENCE: 68

EVQLLESGGG	LVQPGGSLRL	SCAASGFTFS	SYMSWVRQA	PGKLEWVAD	ISVSGSYTNY	60
ADSVKGRFTI	SRDNSKNTLY	LQMNSLRAED	TAVYYCARIG	SYRFFFTDD	GDSLQYWGQG	120
TLVTVSSAST	KGPSVFPLAP	SSKSTSGGTA	ALGCLVKDYF	PEPVTVSWNS	GALTSGVHTF	180
PAVLQSSGLY	SLSSVVTGPS	SSLGTQTYIC	NVNHKPSNTK	VDKRVEPKSC	DKTHTCPPCP	240
APEFEGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVEHED	PEVKFNWYVD	GVEVHNAKTK	300
PREEQYNSTY	RVVSVLTVLH	QDWLNKEYK	CKVSNKALPA	SIEKTISKAK	GQPREPQVYT	360
LPPSRDELTK	NQVSLTCLVK	GFYPSPDIAE	WESNGQPENN	YKTPPVVLDS	DGSFFLYSKL	420
TVDKSRWQQG	NVFSQSVMHE	ALHNYHTQKS	LSLSPGKGGG	GSGGGGSNPQ	IAAHVISEAS	480
SKTTSVLQWA	NVFSQSVMHE	LVTLENGKQL	TVKRQGLYYI	YAQVTFCSNR	EASSQAPFIA	540
SLCLKSPGRF	ERILLRAANT	HSSAKPCGQQ	SIHLGGVFEL	QPGASVFVNV	TDPSQVSHGT	600
GFTSFGLLKL						610

SEQ ID NO: 69 moltype = AA length = 916
 FEATURE Location/Qualifiers
 source 1..916
 mol_type = protein
 note = chain 1
 organism = synthetic construct

SEQUENCE: 69

EVQLLESGGG	LVQPGGSLRL	SCAASGFTFS	SYMSWVRQA	PGKLEWVAD	ISVSGSYTNY	60
ADSVKGRFTI	SRDNSKNTLY	LQMNSLRAED	TAVYYCARIG	SYRFFFTDD	GDSLQYWGQG	120
TLVTVSSAST	KGPSVFPLAP	SSKSTSGGTA	ALGCLVKDYF	PEPVTVSWNS	GALTSGVHTF	180
PAVLQSSGLY	SLSSVVTGPS	SSLGTQTYIC	NVNHKPSNTK	VDKRVEPKSC	DKTHTCPPCP	240
APEFEGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVEHED	PEVKFNWYVD	GVEVHNAKTK	300
PREEQYNSTY	RVVSVLTVLH	QDWLNKEYK	CKVSNKALPA	SIEKTISKAK	GQPREPQVYT	360
LPPCRKKLT	NQVSLTCLVK	GFYPSPDIAE	WESNGQPENN	YKTPPVVLKS	DGSFFLYSKL	420
TVDKSRWQQG	NVFSQSVMHE	ALHNYHTQKS	LSLSPGKGGG	GSGGGGSNPQ	IAAHVISEAS	480
SKTTSVLQWA	EKGYTMSNN	LVTLENGKQL	TVKRQGLYYI	YAQVTFCSNR	EASSQAPFIA	540
SLCLKSPGRF	ERILLRAANT	HSSAKPCGQQ	SIHLGGVFEL	QPGASVFVNV	TDPSQVSHGT	600
GFTSFGLLKL						660
KQLTVKRQGL	YYIYAQVTF	SNREASSQAP	FIASLCLKSP	GRFERILLRA	ANTHSSAKPC	720
GQGSIHGGV	FELQPGASVF	VNVTDPQSV	HGTGFTSFL	LKLGGGGSGG	GGSNPQIAAH	780
VISEASSKTT	SVLQWAEKGY	YTMNNLVTL	ENGKQLTVKR	QGLYYIYAQV	TFCSNREASS	840
QAPFIASLCL	KSPGRFERIL	LRAANTHSSA	KPCGQSIHL	GGVFELQPGA	SVFVNVTDP	900
QVSHGTGFTS	FGLLKL					916

SEQ ID NO: 70 moltype = AA length = 725
 FEATURE Location/Qualifiers
 source 1..725
 mol_type = protein
 note = chain 1
 organism = synthetic construct

US 2025/0051444 A1

Feb. 13, 2025

32

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SEQUENCE: 70
EVQLLESQGG LVQPGGSLRL SCAASGFTFS SYMSWVRQA PGKGLEWVAD ISVSGSYTNY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIG SYRSFFSTDD GDSLDYWGQG 120
TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF 180
PAVLQSSGLY SLSSVVTVPSS SLGTQTYIC NVNHKPSNTK VDKRVEPKSC DKHTTCCPPCP 240
APEFEGGPSV FLFPKPKDT LMISRTPEVT CVVDVEHED PEVKFNWYVD GVEVHNAKTK 300
PREEQYNSTY RVVSVLTVLH DWLNGKEYK CKVSNKALPA SIEKTSKAK QPREPQVYT 360
LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPEMN YKTTTPVLDS DGSFFLYSKL 420
TVDKSRWQGG NVFSCSMHE ALHNHYTQKS LSLSPGKGGG GSGGGSGGGG GSQVQLVQSG 480
AEVKKPGASV KVSCKASGYT FTGYMHVVR QAPQGQLEWM GWINPDSGGT NYAQKFGQGRV 540
TMTRDTSIST AYMELNRLRS DDTAVYYCAR DQPLGYCTNG VCSYFDYWGQ GTLVTVSSGG 600
GSGGGSGGGG GSGGGSGGDI QMTQSPSSVS ASVGDRTIT CRASQGIYSW LAWYQKPGK 660
APNLLIYTAS TLQSGVPSRF SSGSGGTDFE LTISSLQPED FATYYCQQAN IFPLTFGGGT 720
KVEIK 725

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SEQ ID NO: 71      moltype = AA length = 725
FEATURE           Location/Qualifiers
source            1..725
                  mol_type = protein
                  note = chain 1
                  organism = synthetic construct

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SEQUENCE: 71
QVQLVQSGAE VKKPGASVKV SCKASGYTFT GYMHVVRQA PGQGLEWMGW INPDSSGTNY 60
AQKFGQGRVT TRDTSISTAY MELNRLRSDD TAVYYCARDQ PLGYCTNGVC SYFDYWGQGT 120
LVTVSSASTK GPSVFPLAPS SKSTSGGTA ALGCLVKDYF EPVTVSWNSG ALTSGVHTFP 180
AVLQSSGLYS LSSVVTVPSS SLGTQTYIC NVNHKPSNTKV DKRVEPKSCD KHTTCCPPCPA 240
PEFEGGPSVF FLFPKPKDTL MISRTPEVTC VVDVEHEDP EVKFNWYVDG VEVHNAKTKP 300
REEQYNSTYR VVSVLTVLHQ DWLNGKEYK CKVSNKALPA IEKTSKAKG QPREPQVYTL 360
PPSRDELTKN QVSLTCLVKG FYPSPDIAVEW ESNGQPENNY KTTTPVLDSG GSFFLYSKLT 420
VDKSRWQGGN VFSVSMHEA LHNHYTQKSL SLSPGKGGGG GSGGGSGGGG SEVQLLESQGG 480
GLVQPGGSLR LSCAASGFTF SSYMSWVRQ APKGLEWVA DISVSGSYTN YADSVKGRFT 540
ISRDNSKNTL YLQMNSLRAE DTAVYYCARI GSYSFFSTD DGSLDYWGQ GTLVTVSSGG 600
GSGGGSGGGG GSGGGSGGDI QMTQSPSTLS ASVGDRTIT CRASQSISSW LAWYQKPGK 660
APKLLIYDAS SLESGVPSRF SSGSGGTFT LTISSLQPD FATYYCQQYN SYSLTFGGGT 720
KVEIK 725

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SEQ ID NO: 72      moltype = AA length = 713
FEATURE           Location/Qualifiers
source            1..713
                  mol_type = protein
                  note = chain 1
                  organism = synthetic construct

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SEQUENCE: 72
EVQLVESGGG LVQPGGSLRL SCAASGYSFT GYIHVVRQA PGKGLEWVAR VIPNAGGTSY 60
NQKFKGRFTL SVDNSKNTAY LQMNSLRAED TAVYYCAREG IYWWGQGTLV TVSSASTKGP 120
SVFPLAPSSK STSGTAALG CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSLS 180
SVVTVPSSSL GTQTYICNVN HKPSNTKVDK RVEPKSCDKT HTCPPCPAPE FEGGPSVFLF 240
PPKPKDTLMI SRTEPEVTCV VDVHEHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV 300
SVLTVLHQDW LNKKEYKCKV SNKALPASIE KTISKAKGQP REPQVYTLPP SRDELTKNQV 360
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGGS FFLYSKLTVD KSRWQGGNVP 420
SCSVMEALH NHYTQKLSL SPKGKGGGSG GSGGGGGGSE VQLLESQGGG VQPGGSLRLS 480
CAASGFTFSS YMSWVRQAP GKCLEWVADI SVSGSYTNYA DSVKGRFTIS RDNSKNTLYL 540
QMNSLRAEDT AVYYCARIGS YRSFFSTDDG DSLDYWGQGT LVTVSSGGGG SGGGGSGGGG 600
SGGGGSDIQM TQSPSTLSAS VGDRTITCR ASQSISSWLA WYQKPGKAP KLLIYDASSL 660
ESGVPSRFSG SSGGTFTLT ISSLQPDFA TYCQQYNSY SLTFGCGTKV EIK 713

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SEQ ID NO: 73      moltype = AA length = 713
FEATURE           Location/Qualifiers
source            1..713
                  mol_type = protein
                  note = chain 1
                  organism = synthetic construct

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SEQUENCE: 73
EVQLVESGGG LVQPGGSLRL SCAASGYSFT GYIHVVRQA PGKGLEWVAR VIPNAGGTSY 60
NQKFKGRFTL SVDNSKNTAY LQMNSLRAED TAVYYCAREG IYWWGQGTLV TVSSASTKGP 120
SVFPLAPSSK STSGTAALG CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSLS 180
SVVTVPSSSL GTQTYICNVN HKPSNTKVDK RVEPKSCDKT HTCPPCPAPE FEGGPSVFLF 240
PPKPKDTLMI SRTEPEVTCV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV 300
SVLTVLHQDW LNKKEYKCKV SNKALPASIE KTISKAKGQP REPQVYTLPP SRDELTKNQV 360
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGGS FFLYSKLTVD KSRWQGGNVP 420
SCSVMEALH NHYTQKLSL SPKGKGGGSG GSGGGGGGSE VQLLESQGGG VQPGGSLRLS 480
CAASGFTFSS YMSWVRQAP GKCLEWVADI SVSGSYTNYA DSVKGRFTIS RDNSKNTLYL 540
QMNSLRAEDT AVYYCARIGS YRSFFSTDDG DSLDYWGQGT LVTVSSGGGG SGGGGSGGGG 600
SGGGGSDIQM TQSPSTLSAS VGDRTITCR ASQSISSWLA WYQKPGKAP KLLIYDASSL 660
ESGVPSRFSG SSGGTFTLT ISSLQPDFA TYCQQYNSY SLTFGCGTKV EIK 713

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US 2025/0051444 A1

Feb. 13, 2025

33

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SEQ ID NO: 74      moltype = AA  length = 651
FEATURE           Location/Qualifiers
source            1..651
                  mol_type = protein
                  note = chain 1
                  organism = synthetic construct

SEQUENCE: 74
EVQLLESQGGG LVQPGGSLRL SCAASGFTFS SYMSWVRQA PGKGLEWVAD ISVSGSYTNY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIG SYRSFFSTDD GDSLDYWGQG 120
TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF 180
PAVLQSSGLY SLSSVVTVP SSGLTQTYIC NVNHKPSNTK VDKRVEPKSC DKHTHTCPPCP 240
APEFEGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVEHED PEVKFNWYVD GVEVHNAKTK 300
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA SIEKTISKAK GQPREPQVYT 360
LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPPVLD SDFSFLYSKL 420
TVDKSRWQGG NVFSCSVME ALHNHYTQKS LSLSPGKGGG GSGGGGSGREG PELSPDDPAG 480
LDDLQGMFA QLVQAQNVLLI DGPLSWYSDP GLAGVSLTGG LSYKEDTKEL VVAKAGVYV 540
FFQLELRVYV AGEKSGSVSL ALHLQPLRSA AGAALALTV DLPPASSEAR NSAFGFQGR 600
LHLSAQQLRG VHLHTEARAR HAWQLTQGAT VLGLFRVTPE IPAGLPSPRS E 651

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SEQ ID NO: 75      moltype = AA  length = 1039
FEATURE           Location/Qualifiers
source            1..1039
                  mol_type = protein
                  note = chain 1
                  organism = synthetic construct

SEQUENCE: 75
EVQLLESQGGG LVQPGGSLRL SCAASGFTFS SYMSWVRQA PGKGLEWVAD ISVSGSYTNY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIG SYRSFFSTDD GDSLDYWGQG 120
TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF 180
PAVLQSSGLY SLSSVVTVP SSGLTQTYIC NVNHKPSNTK VDKRVEPKSC DKHTHTCPPCP 240
APEFEGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVEHED PEVKFNWYVD GVEVHNAKTK 300
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA SIEKTISKAK GQPREPQVYT 360
LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPPVLD SDFSFLYSKL 420
TVDKSRWQGG NVFSCSVME ALHNHYTQKS LSLSPGKGGG GSGGGGSGREG PELSPDDPAG 480
LDDLQGMFA QLVQAQNVLLI DGPLSWYSDP GLAGVSLTGG LSYKEDTKEL VVAKAGVYV 540
FFQLELRVYV AGEKSGSVSL ALHLQPLRSA AGAALALTV DLPPASSEAR NSAFGFQGR 600
LHLSAQQLRG VHLHTEARAR HAWQLTQGAT VLGLFRVTPE IPAGLPSPRS EGGGGSGGGG 660
SREGPELSPD DPAGLLDLRQ GMFAQLVAQN VLLIDGPLSW YSDPGLAGVS LTGGLSYKED 720
TKELVVAKAG VYVFFQLEL RRVVAGEGSG SVSLALHLQP LRSAGAGAAAL ALTVDLPPAS 780
SEARNNSAFG QGRLLHLSAG QRLGVHLHTE ARARHAWQLT QGATVVLGLF VTEIPAGLP 840
SPRSEGGGGS GGGGSGREGPE LSPDDPAGLL DLRQGMFAQL VAQNVLLIDG PLSWYSDPGL 900
AGVSLTGGLS YKEDTKELV AKAGVYVFF QLELRVYVAG EGSGSVSLAL HLQPLRSAG 960
AAALALTVDL PPASSEARN AFGFQGRLLH LSAQQLGVH LHTEARARHA WQLTQGATVL 1020
GLFRVTPEIP AGLPSRSE 1039

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SEQ ID NO: 76      moltype = AA  length = 722
FEATURE           Location/Qualifiers
source            1..722
                  mol_type = protein
                  note = chain 1
                  organism = synthetic construct

SEQUENCE: 76
EVQLLESQGGG LVQPGGSLRL SCAASGFTFS SYMSWVRQA PGKGLEWVAD ISVSGSYTNY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIG SYRSFFSTDD GDSLDYWGQG 120
TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF 180
PAVLQSSGLY SLSSVVTVP SSGLTQTYIC NVNHKPSNTK VDKRVEPKSC DKHTHTCPPCP 240
APEFEGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK 300
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA SIEKTISKAK GQPREPQVYT 360
LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPPVLD SDFSFLYSKL 420
TVDKSRWQGG NVFSCSVME ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSQVQLQQWG 480
AGLLKPSSETL SLTCAVYGG FSGYYWSWIR QSPEKGLEWI GEINHGQVYT YNPSLESRTV 540
ISVDTSKNQF SLKLSSVTAA DTAVYYCARD YGPGNYDWYF DLWGRGTLVT VSSGGGGSGG 600
GGSGGGSGGG GGSEIVLTQS PATLSLSPGE RATLSCRASQ SVSSYLAWYQ QKPGQAPRL 660
IYDASNRATG IPARFSGSGS GTDFTLTISS LEPEDFAVY CQQRSNWPPA LTPGGGTVKE 720
IK 722

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SEQ ID NO: 77      moltype = AA  length = 716
FEATURE           Location/Qualifiers
source            1..716
                  mol_type = protein
                  note = chain 1
                  organism = synthetic construct

SEQUENCE: 77
EVQLLESQGGG LVQPGGSLRL SCAASGFTFS SYMSWVRQA PGKGLEWVAD ISVSGSYTNY 60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIG SYRSFFSTDD GDSLDYWGQG 120
TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF 180

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US 2025/0051444 A1

Feb. 13, 2025

34

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PAVLQSSGLY	SLSSVVTVP	SSLGTQTYIC	NVNHKPSNTK	VDRKVEPKSC	DKTHTCPPCP	240
APEFEGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVEHED	PEVKFNWYVD	GVEVHNAKTK	300
PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	SIEKTISKAK	GQPREPQVYT	360
LPSPRDELTK	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTPPVLDSD	DGSFFLYSKL	420
TVDKSRWQQG	NVFSQSVME	ALHNHYTQKS	LSLSPGKGGG	GSGGGSGGGG	GSEVQLVQSG	480
AEVKKPGESL	RISCKGSGYS	FSTYIWSVVR	QMPGKGLEWM	GKIYPGDSYT	NYSFSPQGGV	540
TISADKSIST	AYLQWSSLKA	SDTAMYYCAR	GYGIFDYWGQ	GTLVTVSSGG	GSGGGSGGGG	600
GSGGGGSSSY	ELTQPPPSVS	SPGQTASITC	SGDNIGDQYA	HWYQQKPGQS	PVLVIYQDKN	660
RPSGIPERFS	GSNSGNTATL	TISGTQAMDE	ADYYCATYTG	FGSLAVFGGG	TKLTVL	716

SEQ ID NO: 78 moltype = AA length = 720
 FEATURE Location/Qualifiers
 source 1..720
 mol_type = protein
 note = chain 1
 organism = synthetic construct

SEQUENCE: 78

QVQLQQWGGAG	LLKPSSETLSL	TCAVYGGGFS	GYWSWIRQS	PEKGLEWIGE	INHGQVVTYN	60
PSLESRVITIS	VDTSKNQFSL	KLSSVTAADT	AVYYCARDYG	PGNYDWYFDL	WGRGTLVTVS	120
SASTKGPSVF	PLAPSSKSTS	GGTAALGCLV	KDYFPEPVTV	SWNSGALTSG	VHTFPAVLQS	180
SGLYSLSSVV	TVPSSSLGTQ	TYICNVNHKP	SNTKVDKRVK	PKSCDKTHTC	PPCPAPEFEG	240
GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN	AKTKPREEQY	300
NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	ALPASIEKTI	SKAKGQPREP	QVYTLPPSRD	360
ELTKNQVSLT	VMKGFYPSD	IAVEWESNGQ	PENNYKTPPP	VLDSDGSEFFL	YSKLTVDKSR	420
WQQGNVVFSC	VMHEALHNHY	TQKSLSLSPG	KGGGGSGGGG	GSGGGSEVQL	LESQGLVQVP	480
GGSLRLSCAA	SGFTFSSYYM	SWVRQAPGKG	LEWVADISVS	GSYTNYADSV	KGRFTISRDN	540
SKNTLYLQMN	SLRAEDTAVY	YCARIGSYRS	FFSTDGDSL	DYWGQGTLLV	VSSGGGGSGG	600
GSGGGGGSGG	GGSDIQMTQS	PSTLSASVGD	RVTITCRASQ	SISSWLAWYQ	QKPKKAPKLL	660
IYDASSLESG	VPSRFSGSGS	GTEFTLTISS	LQPDDEFATYY	CQQYNSYSLT	FGGGTKVEIK	720

SEQ ID NO: 79 moltype = AA length = 715
 FEATURE Location/Qualifiers
 source 1..715
 mol_type = protein
 note = chain 1
 organism = synthetic construct

SEQUENCE: 79

EVQLVQSGAE	VKKPGESLRI	SCKGSGYSFS	TYWISWVRQM	PGKGLEWMGK	IYPGDSYNTY	60
SPSPQQQVTI	SADKSISTAY	LQWSSSLKASD	TAMYYCARGY	GIPDYWGQGT	LVTVSSASTK	120
GPSVFPLAPS	SKSTSGGTAA	LGCLVKDYFP	EPVTVSWNSG	ALTSQVHTFP	AVLQSSGLYS	180
LSSVTVTPSS	SLGTQTYICN	VNHKPSNTKV	DKRVEPKSCD	KTHTCPPCPA	PEFEGGPSVF	240
LFPPKPKDTL	MISRTPEVTC	VVVDVEHEDP	EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	300
VVSVLTVLHQ	DWLNGKEYKC	KVSNKALPAS	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	360
QVSLTCLVKG	FYPSPDIAVEW	ESNGQPENNY	KTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN	420
VFSCSVMEHA	LHNHYTQKSL	SLSPGKGGGG	GSGGGSGGGG	SEVQLLESQG	GLVQPGGSLR	480
LSCAASGFTF	SSYYMSWVRQ	APGKGLEWVA	DISVSGSYTN	YADSVKGRFT	ISRDNKNTL	540
YLQMNSLRAE	DTAVYYCARI	GSYRSFFSTD	DGDSLQDWYQ	GTLVTVSSGG	GSGGGGGSGG	600
GSGGGGGSDI	QMTQSPSTLS	ASVGDRVTIT	CRASQSISSW	LAWYQQKPGK	APKLLIYDAS	660
SLSGVPSRPF	SGSGSGTEFT	LTISSLQPD	FATYYCQYN	SYSLTFGGGT	KVEIK	715

SEQ ID NO: 80 moltype = AA length = 457
 FEATURE Location/Qualifiers
 source 1..457
 mol_type = protein
 note = chain 2
 organism = synthetic construct

SEQUENCE: 80

EVQLLESQGG	LVQPGGSLRL	SCAASGFTFS	SYMSWVRQA	PGKGLEWVAD	ISVSGSYNTY	60
ADSVKGRFTI	SRDNSKNTLY	LQMNSLRAED	TAVYYCARIG	SYRSFFSTD	GDSDLQWYQG	120
TLVTVSSAST	KGPSVFPLAP	SSKSTSGGTA	ALGCLVKDYF	PEPVTVSWNS	GALTSGVHTF	180
PAVLQSSGLY	SLSSVVTVP	SSLGTQTYIC	NVNHKPSNTK	VDRKVEPKSC	DKTHTCPPCP	240
APEFEGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK	300
PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	SIEKTISKAK	GQPREPQVCT	360
LPSPRDELTK	NQVSLTCLVE	GFYPSDIAVE	WESNGQPENN	YKTPPVLDSD	DGSFFLYSDL	420
TVDKSRWQQG	NVFSQSVME	ALHNHYTQES	LSLSPGK			457

SEQ ID NO: 81 moltype = AA length = 457
 FEATURE Location/Qualifiers
 source 1..457
 mol_type = protein
 note = chain 2
 organism = synthetic construct

SEQUENCE: 81

QVQLVESGGG	LVKPGGSLRL	SCAASGFTFS	DYMSWIRQA	PGKGLEWVSD	ISVSSSYNTY	60
ADSVKGRFTI	SRDNAKNSLY	LQMNSLRAED	TAVYYCARLG	GYRSFFSTD	GDSDLQWYQG	120
TLVTVSSAST	KGPSVFPLAP	SSKSTSGGTA	ALGCLVKDYF	PEPVTVSWNS	GALTSGVHTF	180

US 2025/0051444 A1

Feb. 13, 2025

35

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PAVLQSSGLY	SLSSVVTVP	SSLGTQTYIC	NVNHKPSNTK	VDKRVEPKSC	DKTHTCPPCP	240
APEFEGGSPV	FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK	300
PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	SIEKTISKAK	GQPREPQVCT	360
LPPSRDELTK	NQVSLTCLVE	GFYPSDIAVE	WESNGQPENN	YKTTTPVLDS	DGSFFLYSDL	420
TVDKSRWQQG	NVFSCSVMHE	ALHNHYTQES	LSLSPGK			457

SEQ ID NO: 82 moltype = AA length = 457
 FEATURE Location/Qualifiers
 source 1..457
 mol_type = protein
 note = chain 2
 organism = synthetic construct

SEQUENCE: 82

EVQLLES	GGG	LVQPGG	SLRL	SCAASG	FTFS	SYMSWVR	QA	PGKGLE	WVAD	ISVSGS	YNTY	60
ADSVKGR	FTI	SRDNSK	NLTLY	LQMNSL	RAED	TAVYYC	ARIG	SYRSFF	STDD	GDSL	DYWG	120
TLVTVSS	AST	KGPSVF	PLAP	SSKSTG	GGTA	ALGCLV	KDYF	PEPVTV	SWNS	GALTSG	VHTF	180
PAVLQSS	GLY	SLSSVVT	VP	SSLGTQ	TYIC	NVNHKPS	NTK	VDKRVE	PKSC	DKTHTC	PPCP	240
APEFEGG	SPV	FLFPPK	PKDT	LMISRT	PEVT	CVVVDV	EHED	PEVKFN	WYVD	GVEVHN	AKTK	300
PREEQYN	STY	RVVSVL	TVLH	QDWLNG	KEYK	CKVSNKA	LPA	SIEKTIS	KAK	GQPREP	QVCT	360
LPPSRDE	LTK	NQVSLT	CLVE	GFYPSD	IAVE	WESNGQP	ENN	YKTTTP	PVLD	DGSFFL	YSDL	420
TVDKSRW	QQG	NVFSCS	VMHE	ALHNHY	TQES	LSLSPG	K					457

SEQ ID NO: 83 moltype = AA length = 214
 FEATURE Location/Qualifiers
 source 1..214
 mol_type = protein
 note = chain 3
 organism = synthetic construct

SEQUENCE: 83

DIQMTQS	PST	LSASVG	DRV	ITCRASQ	SIS	SWLAWY	QQKP	GKAPKL	LIYD	ASSLES	GVPS	60
RFSGSGS	GTE	FTLTIS	LQP	DDFATY	YCQ	YNSYSL	TFGG	GTKVEI	KRTV	AAPSVF	IFPP	120
SDEQLKS	GT	SVVCLL	NNFY	PREAKV	QWKV	DNALQS	GN	ESVTEQ	DSKD	STYSL	SSTLT	180
LSKADYE	KHK	VYACEV	THQG	LSSPVT	KSFN	RGEC						214

SEQ ID NO: 84 moltype = AA length = 214
 FEATURE Location/Qualifiers
 source 1..214
 mol_type = protein
 note = chain 3
 organism = synthetic construct

SEQUENCE: 84

DIQMTQS	PST	LSASVG	DRV	ITCRASQ	SIS	SWLAWY	QQKP	GKAPKL	LIYD	AFSLES	GVPS	60
RFSGSGS	GTE	FTLTIS	LQP	DDFATY	YCQ	YNSYSI	TFGG	GTKVEI	KRTV	AAPSVF	IFPP	120
SDEQLKS	GT	SVVCLL	NNFY	PREAKV	QWKV	DNALQS	GN	ESVTEQ	DSKD	STYSL	SSTLT	180
LSKADYE	KHK	VYACEV	THQG	LSSPVT	KSFN	RGEC						214

SEQ ID NO: 85 moltype = AA length = 214
 FEATURE Location/Qualifiers
 source 1..214
 mol_type = protein
 note = chain 3
 organism = synthetic construct

SEQUENCE: 85

DIQMTQS	PSS	VSASVG	DRV	ITCRASQ	GIY	SWLAWY	QQKP	GKAPNL	LIYT	ASTLQS	GVPS	60
RFSGSGS	GT	FTLTIS	LQP	EDFATY	YCQ	ANIFPL	TFGG	GTKVEI	KRTV	AAPSVF	IFPP	120
SDEQLKS	GT	SVVCLL	NNFY	PREAKV	QWKV	DNALQS	GN	ESVTEQ	DSKD	STYSL	SSTLT	180
LSKADYE	KHK	VYACEV	THQG	LSSPVT	KSFN	RGEC						214

SEQ ID NO: 86 moltype = AA length = 219
 FEATURE Location/Qualifiers
 source 1..219
 mol_type = protein
 note = chain 3
 organism = synthetic construct

SEQUENCE: 86

DIQMTQS	PSS	LSASVG	DRV	ITCRSSQ	SLV	HSNGNT	FLHW	YQKPKG	KAPK	LLIYTV	SNRF	60
SGVPSR	FSGS	GS	GTDF	TLTI	SSLQPE	DFAT	YFCS	QT	THVP	WTFGQ	GKVE	120
FIFPPS	DEQL	KSGTAS	VVCL	LNNFY	P	REAK	VQWK	VDNAL	Q	SGNSQ	ESVTE	180
SSTLTLS	KAD	YEKHKV	YACE	VTHQGL	SSPV	TKSFN	RGEC					219

SEQ ID NO: 87 moltype = AA length = 216
 FEATURE Location/Qualifiers
 source 1..216
 mol_type = protein
 note = chain 3
 organism = synthetic construct

US 2025/0051444 A1

Feb. 13, 2025

36

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SEQUENCE: 87
EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA 60
RFGSGSGSTD FTLTISLSEP EDFAVYQCQQ RSNWPPALTF GGGTKVEIKR TVAAPSVFIF 120
PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYLSLST 180
LTLISKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC 216

SEQ ID NO: 88      moltype = AA length = 214
FEATURE           Location/Qualifiers
source            1..214
                  mol_type = protein
                  note = chain 3
                  organism = synthetic construct

SEQUENCE: 88
SYELTQPPSV SVSPGQTASI TCSGDNIGDQ YAHWYQQKPG QSPVLVIYQD KNRPSGIPER 60
FSGSNSGNTA TLTISGTQAM DEADYYCATY TGFGLAVFG GGTKLTVLGQ PKAAPSVTLF 120
PPSSEELQAN KATLVCLISD FYPGAVTVAW KADSSPVKAG VETTPSKQS NNKYAASSYL 180
SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP TECS 214

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1. A multi-specific T cell engager, which comprises:
a first targeting domain comprising one or more LRRC15 antigen binding domains;
a second targeting domain, wherein the second targeting domain binds to a target protein selected from the group consisting of: CD3, CD28, CD40, and CD137; and/or
an optional third targeting domain, wherein the third targeting domain binds to a target protein selected from the group consisting of: CD3, CD28, CD40, and CD137.

2. The multi-specific T cell engager of claim 1, wherein the targeting domain is in the form of a single-domain antibody (sdAb), a fragment variable (Fv) heterodimer, a single-chain Fv (scFv), a Fab fragment, a TriFab, or a combination thereof.

3. The multi-specific T cell engager of claim 1, wherein the LRRC15 antigen binding domain comprises a heavy chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 1, 2, 3, 4, or 53, and a light chain variable region having at least 80% sequence identity to the amino acid sequence shown in SEQ ID NO: 5, 6, 7, 8, or 54.

4. The multi-specific T cell engager of claim 3, wherein the LRRC15 antigen binding domain comprises a heavy chain variable region as shown in SEQ ID NO: 1, and a light chain variable region as shown in SEQ ID NO: 5.

5. The multi-specific T cell engager of claim 3, wherein the LRRC15 antigen binding domain comprises a heavy chain variable region as shown in SEQ ID NO: 53, and a light chain variable region as shown in SEQ ID NO: 54.

6. The multi-specific T cell engager of claim 1, wherein the second targeting domain is selected from the group consisting of: a CD3 antigen binding domain, a CD28 antigen binding domain, a CD40 antigen binding domain, a CD137 antigen binding domain, a CD40L sequence, and a CD137L sequence.

7. The multi-specific T cell engager of claim 1, wherein the third targeting domain is selected from the group consisting of: a CD3 antigen binding domain, a CD28 antigen binding domain, a CD40 antigen binding domain, a CD137 antigen binding domain, a CD40L sequence, and a CD137L sequence.

8. An anti-LRRC15 antibody or antigen-binding fragment thereof, which comprises the following three heavy chain variable region CDRs:
HCDR1 having an amino acid sequence as shown in SEQ ID NO: 32, 35, 38, or 41;
HCDR2 having an amino acid sequence as shown in SEQ ID NO: 33, 36, 39, or 42; and
HCDR3 having an amino acid sequence as shown in SEQ ID NO: 34, 37, 40, or 43;
and the following three light chain variable region CDRs:
LCDR1 having an amino acid sequence as shown in SEQ ID NO: 44 or 49;
LCDR2 having an amino acid sequence as shown in SEQ ID NO: 45, 47, or 50; and
LCDR3 having an amino acid sequence as shown in SEQ ID NO: 46, 48, 51, or 52.

9-10. (canceled)

11. An immunoconjugate comprising:
(i) the multi-specific T cell engager of claim 1; and
(ii) a coupling moiety selected from the group consisting of: a detectable label, a drug, a toxin, a cytokine, a radioisotope, and an enzyme.

12. A pharmaceutical composition comprising: (a) the multi-specific T cell engager of claim 1; and (b) a pharmaceutically acceptable carrier.

13. An immunoconjugate comprising:
(i) the antibody or antigen binding fragment thereof of claim 8; and
(ii) a coupling moiety selected from the group consisting of: a detectable label, a drug, a toxin, a cytokine, a radioisotope, and an enzyme.

14. A pharmaceutical composition comprising: (a) the antibody or antigen binding fragment thereof of claim 8; and (b) a pharmaceutically acceptable carrier.

15. A method for treating a cancer or tumor, which comprises a step of administering the multi-specific T cell engager of claim 1 to a subject in need thereof.

16. The method of claim 15, wherein the cancer or tumor is a cancer or tumor with high LRRC15 expression.

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