

arthromeningitis and is mostly accompanied by organ and tissue damages of different degrees. Its main manifestations are fever, rash, and arthralgia, and it has poor prognosis such as low long-term remission rate, high dysfunction rate and disability rate, and high mortality rate. (Woerner A *et al. Expert Rev Clin Immunol.* 11(5):575-88. (2015)). It is generally accepted that the sJIA is an autoinflammatory disease instead of an autoimmune disease (Sun Juan *et al. Progress in Modern Biomedicine.* 8:1584-1588 (2016)). Clinical studies have demonstrated that the anti-human IL-1 β monoclonal antibody canakinumab can effectively treat active sJIA accompanied by fever and reduce steroid doses, and significantly reduces the recurrence of sJIA (Orrock JE *et al. Expert Rev Clin Pharmacol.* 9:1015-24. (2016)). Therefore the anti-IL-1 β monoclonal antibody canakinumab is approved for treating systemic juvenile idiopathic arthritis by FDA and the like.

There is still a need to develop new anti-IL-1 β antibodies.

SUMMARY OF THE INVENTION

In one aspect, the present disclosure refers to an anti-IL-1 β antibody or an antigen-binding fragment thereof, wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively; and the antibody or the antigen-binding fragment thereof comprises a light chain variable region comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22, respectively.

In another aspect, the present disclosure refers to a bispecific antibody comprising a first protein functional region and a second protein functional region, wherein the first protein functional region targets IL-1 β , and the second protein functional region targets a target other than IL-1 β , wherein the first protein functional region is the antibody or the antigen-binding fragment thereof as disclosed herein; preferably, the bispecific antibody is in an IgG-scFv form; preferably, the first protein functional region is the antibody or the antigen-binding fragment thereof as disclosed herein, and the second protein functional region is a single chain antibody; or preferably, the first protein functional region is the single chain antibody as disclosed herein, and the second protein functional region is an antibody or an antigen-binding fragment thereof.

In another aspect, the present disclosure refers to an isolated nucleic acid molecule comprising a nucleotide sequence encoding an antibody heavy chain variable region and a nucleotide sequence encoding an antibody light chain variable region, wherein the antibody heavy chain variable region comprises HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19, respectively, and the antibody light chain variable region comprises LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22, respectively; preferably, the amino acid sequence of the antibody heavy chain variable region is selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, and SEQ ID NO: 14, and the amino acid sequence of the antibody light chain variable region is selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, and SEQ ID NO: 16; more preferably, the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 2, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 4; the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 6, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 8; the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 10, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 12; or the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 14, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 16; and even more preferably, the nucleic acid molecule comprises: nucleotide sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 3, nucleotide sequences set forth in SEQ ID NO: 5 and SEQ ID NO: 7, nucleotide sequences set forth in SEQ ID NO: 9 and SEQ ID NO: 11, or nucleotide sequences set forth in SEQ ID NO: 13 and SEQ ID NO: 15.

After intensive study and creative effort, the inventors used mammalian cell expression systems to express recombinant IL-1 β -His as an antigen to immunize mice, and obtained hybridoma cells by fusion of mouse spleen cells and myeloma cells. The inventors acquired the following hybridoma cell line by screening a large number of the samples:

the hybridoma cell line LT010 deposited at China Center for Type Culture Collection (CCTCC) on June 21, 2018 with an accession number of CCTCC NO: C2018133.

The inventors surprisingly found:

the hybridoma cell line LT010 may secrete and produce a specific monoclonal antibody (named as 3H6) that specifically binds to human IL-1 β , and the monoclonal antibody can block the binding of IL-1 β to IL-1R1 very effectively;

Furthermore, the inventors creatively prepared anti-human IL-1 β humanized antibodies (respectively named as 3H6H1L1, 3H6H2L2, 3H6H3L3, and 3H6H4L1), all of which may bind to the human IL-1 β effectively, block the binding of IL-1 β to a receptor thereof (IL-1R1), and inhibit the activation of a downstream signaling pathway of IL-1 β . And these anti-human IL-1 β humanized antibodies have the potential of being used in preparing medicaments for reducing, preventing or treating diseases such as rheumatoid arthritis, gout, multiple sclerosis, cardiovascular events and/or cardiovascular diseases, tumors, cryopyrin-associated periodic syndromes in children and adults, periodic fever syndromes, and systemic juvenile idiopathic arthritis.

The present ~~invention~~disclosure is detailed below.

One ~~aspect~~example of the present ~~invention~~disclosure relates to an anti-IL-1 β antibody or an antigen-binding fragment thereof, wherein the antibody comprises a heavy chain variable region comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NO: 17-SEQ ID NO: 19, respectively; and the antibody comprises a light chain variable region comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NO: 20-SEQ ID NO: 22, respectively.

Preferably, the IL-1 β is human IL-1 β .

The variable regions of the light chain and the heavy chain determine the binding of the antigen; the variable region of each chain contains three hypervariable regions, namely complementarity determining regions (CDRs) (the CDRs of the heavy chain (H) include HCDR1, HCDR2, HCDR3, and the CDRs of the light chain (L) include LCDR1, LCDR2, LCDR3; defined by Kabat *et al.*, see Sequences of Proteins of Immunological Interest, Fifth Edition (1991), Volumes 1-3, NIH Publication 91-3242, Bethesda Md).

The antibodies 3H6, 3H6H1L1, 3H6H2L2, 3H6H3L3, and 3H6H4L1 disclosed herein have the same HCDR1-3 and LCDR1-3, according to analysis by technical means well known to those skilled in the art, for example by a VBASE2 database.

The amino acid sequences of the three HCDR regions of the heavy chain variable region are as follows:

HCDR 1: GFSLSTSGMG (SEQ ID NO: 17),

HCDR 2: IYWDDDK (SEQ ID NO: 18),

HCDR 3: ARSAYYSFAY (SEQ ID NO: 19);

and the amino acid sequences of the three CDR regions of the light chain variable region are as follows:

LCDR 1: QDVDTD (SEQ ID NO: 20),

LCDR 2: WAS (SEQ ID NO: 21),

LCDR 3: QQYSSYPT (SEQ ID NO: 22).

In one or more ~~embodiment~~examples of the present ~~invention~~disclosure, the antibody or the antigen-binding fragment thereof is provided, wherein

the heavy chain variable region of the antibody comprises an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, and SEQ ID NO: 14; and

the light chain variable region of the antibody comprises an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, and SEQ ID NO: 16.

In some ~~embodiment~~examples of the present ~~invention~~disclosure, the antibody or the antigen-binding fragment thereof is provided, wherein the antibody is selected from:

(1) VH set forth in SEQ ID NO: 2 and VL set forth in SEQ ID NO: 4;

(2) VH set forth in SEQ ID NO: 6 and VL set forth in SEQ ID NO: 8;

(3) VH set forth in SEQ ID NO: 10 and VL set forth in SEQ ID NO: 12; and

(4) VH set forth in SEQ ID NO: 14 and VL set forth in SEQ ID NO: 16.

In one or more ~~embodiment~~examples of the present ~~invention~~disclosure, the antibody or the antigen-binding fragment thereof is provided, wherein the antibody or the antigen-binding fragment thereof is selected from Fab, Fab', F(ab')₂, Fd, Fv, dAb, a complementarity determining region fragment, a single chain antibody (*e.g.*, scFv), a humanized antibody, a chimeric antibody, and a diabody.

In one or more ~~embodiment~~examples of the present ~~invention~~disclosure, the antibody or the antigen-binding fragment thereof is provided, wherein the antibody binds to IL-1 β protein with a K_D less than 10⁻⁵ M, such as less than 10⁻⁶ M, less than 10⁻⁷ M, less than 10⁻⁸ M, less than 10⁻⁹ M, or less than 10⁻¹⁰ M or less; preferably, the K_D is measured by a Biacore molecular interaction instrument.

In some ~~embodiment~~examples of the present ~~invention~~disclosure, the antibody or the antigen-binding fragment thereof is provided, wherein the antibody binds to IL-1 β protein with an EC₅₀ less than about 100 nM, such as less than about 10 nM, less than about 1 nM, less than about 0.9 nM, less than about 0.8 nM, less than about 0.7 nM, less than about 0.6 nM, less than about 0.5 nM, less than about 0.4 nM, less than about 0.3 nM, less than about 0.2 nM, less than about 0.1 nM or less. Specifically, the EC₅₀ is measured by an indirect ELISA method.

In one or more ~~embodiment~~examples of the present ~~invention~~disclosure, the antibody or the antigen-binding fragment thereof is provided, wherein the antibody comprises a non-CDR region derived from a species other than murine, such as from a human antibody.

In some ~~embodiment~~examples of the present ~~invention~~disclosure, the constant regions of the antibodies are humanized, *e.g.*, the heavy chain constant regions are Ig gamma-1 chain C region such as ACCESSION: P01857 or Ig gamma-4 chain C region such as ACCESSION: P01861.1; and the light chain

constant regions are Ig kappa chain C region such as **ACCESSION: P01834**.

In one or more **embodiment examples** of the present **invention disclosure**, the antibody or the antigen-binding fragment thereof is provided, wherein the antibody is a monoclonal antibody produced by a hybridoma cell line LT010 deposited at China Center for Type Culture Collection (CCTCC) with an accession number of CCTCC NO: C2018133.

In one or more **embodiment examples** of the present **invention disclosure**, the antibody is a monoclonal antibody.

Another aspect of the present **invention disclosure** relates to an antibody-drug conjugate (ADC) comprising an antibody or an antigen-binding fragment thereof and a small molecule drug, wherein the antibody or the antigen-binding fragment thereof is any one of the antibodies or the antigen-binding fragments thereof disclosed herein; preferably, the small molecule drug is a small molecule cytotoxic drug; and more preferably, the small molecule drug is a chemotherapeutic drug.

The chemotherapeutic drug may be a conventional tumor chemotherapeutic drug, such as an alkylating agent, an antimetabolite, an anti-tumor antibiotic, a plant-based anticancer agent, a hormone, and an immunological agent.

In one or more **embodiment examples** of the present **invention disclosure**, the antibody-drug conjugate is provided, wherein the antibody or the antigen-binding fragment thereof is linked to a small molecule drug via a linker; the linker may be one known to those skilled in the art, for example, a hydrazone bond, a disulfide bond, or a peptide bond.

In one or more **embodiment examples** of the present **invention disclosure**, the antibody-drug conjugate is provided, wherein the molar ratio of the antibody or the antigen-binding fragment thereof to the small molecule drug is 1:1-1:4, for example, 1:1, 1:2, 1:3, or 1:4.

Yet another ~~aspect~~example of the present ~~invention~~disclosure relates to a bispecific antibody (also known as bifunctional antibody) comprising a first protein functional region and a second protein functional region, wherein:

the first protein functional region targets IL-1 β ,

the second protein functional region targets a target other than IL-1 β ,
e.g., IL-17A;

wherein the first protein functional region is any one of the antibodies or the antigen-binding fragments thereof disclosed herein;

preferably, the bispecific antibody is in an IgG-scFv form;

preferably,

(1) the first protein functional region is any one of the antibodies or the antigen-binding fragments thereof disclosed herein, and the second protein functional region is a single chain antibody;

or,

(2) the first protein functional region is a single chain antibody, the heavy chain variable region thereof comprises HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NO: 17-SEQ ID NO: 19, and the light chain variable region thereof comprises LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NO: 20-SEQ ID NO: 22, and the second protein functional region is an antibody (*e.g.*, a monoclonal antibody).

In some ~~embodiment~~examples of the present ~~invention~~disclosure, the bispecific antibody is provided, wherein the first protein functional region and the second protein functional region are linked directly or via a linker fragment;

preferably, the linker fragment is (GGGGS)*m*, *m* being a positive integer such as 1, 2, 3, 4, 5, or 6; or

preferably, the linker fragment is SS(GGGGS)*n*, *n* being a positive integer such as 1, 2, 3, 4, 5, or 6.

In some ~~embodiment~~examples of the present ~~invention~~disclosure, the bispecific antibody is provided, wherein in item (2),

the heavy chain variable region of the single chain antibody comprises an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, and SEQ ID NO: 14; and

the light chain variable region of the single chain antibody comprises an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, and SEQ ID NO: 16.

In some ~~embodiment~~examples of the present ~~invention~~disclosure, the bispecific antibody is provided, wherein in item (2),

the heavy chain variable region of the single chain antibody comprises an amino acid sequence set forth in SEQ ID NO: 2, and the light chain variable region of the single chain antibody comprises an amino acid sequence set forth in SEQ ID NO: 4;

the heavy chain variable region of the single chain antibody comprises an amino acid sequence set forth in SEQ ID NO: 6, and the light chain variable region of the single chain antibody comprises an amino acid sequence set forth in SEQ ID NO: 8;

the heavy chain variable region of the single chain antibody comprises an amino acid sequence set forth in SEQ ID NO: 10, and the light chain variable region of the single chain antibody comprises an amino acid sequence set forth in SEQ ID NO: 12; or

the heavy chain variable region of the single chain antibody comprises an amino acid sequence set forth in SEQ ID NO: 14, and the light chain variable region of the single chain antibody comprises an amino acid sequence set forth in SEQ ID NO: 16.

In some ~~embodiment~~examples of the present ~~invention~~disclosure, the bispecific antibody is provided, wherein the numbers of the first protein functional region and the second protein functional region are each independently 1, 2 or more.

In some ~~embodiment~~examples of the present ~~invention~~disclosure, the bispecific antibody is provided, wherein in item (2), the constant region of the monoclonal antibody is selected from constant regions of human IgG1, IgG2, IgG3, and IgG4.

In some ~~embodiment~~examples of the present ~~invention~~disclosure, the bispecific antibody is provided, wherein the single chain antibody is linked to the C-terminus of the heavy chain of the antibody or the monoclonal antibody.

Yet another ~~aspect~~example of the present ~~invention~~disclosure relates to an isolated nucleic acid molecule comprising a nucleotide sequence encoding an antibody heavy chain variable region and a nucleotide sequence encoding an antibody light chain variable region, wherein the antibody heavy chain variable region comprises HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NO: 17-SEQ ID NO: 19, respectively, and the antibody light chain variable region comprises LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NO: 20-SEQ ID NO: 22, respectively;

preferably, the amino acid sequence of the antibody heavy chain variable region is selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, and SEQ ID NO: 14, and the amino acid sequence of the antibody light chain variable region is selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, and SEQ ID NO: 16;

more preferably, the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 2, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 4; the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 6, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 8; the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 10, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 12; or the amino

acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 14, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 16; and

even more preferably, the isolated nucleic acid molecule comprises:

nucleotide sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 3,

nucleotide sequences set forth in SEQ ID NO: 5 and SEQ ID NO: 7,

nucleotide sequences set forth in SEQ ID NO: 9 and SEQ ID NO: 11, or

nucleotide sequences set forth in SEQ ID NO: 13 and SEQ ID NO: 15.

The isolated nucleic acid molecule may be a single nucleic acid molecule or multiple nucleic acid molecules, such as two nucleic acid molecules. In the case of a single nucleic acid molecule, the heavy chain variable region and the light chain variable region of the antibody may be expressed by the same nucleic acid molecule, *e.g.*, by the same or different expression cassettes located on the same nucleic acid molecule. In the case of multiple nucleic acid molecules, for example, two nucleic acid molecules, the heavy chain variable region and the light chain variable region of the antibody may be expressed by different nucleic acid molecules.

Yet another aspect of the present ~~invention~~disclosure relates to a recombinant vector comprising the isolated nucleic acid molecule disclosed herein. The number of the recombinant vector may be one or more. In the case of multiple (*e.g.*, two) nucleic acid molecules, the multiple (*e.g.*, two) nucleic acid molecules may be expressed by the same recombinant vector or by different recombinant vectors.

Yet another aspect of the present ~~invention~~disclosure relates to a host cell comprising the isolated nucleic acid molecule or the recombinant vector disclosed herein.

Yet another aspect of the present ~~invention~~disclosure relates to a method for preparing any one of the antibodies or the antigen-binding fragments thereof disclosed herein, comprising cultivating the host cell disclosed

herein in a suitable condition, and isolating the antibody or the antigen-binding fragment thereof from the cell cultures.

Yet another aspect of the present ~~invention~~disclosure relates to a hybridoma cell line LT010 deposited at China Center for Type Culture Collection (CCTCC) with an accession number of CCTCC NO: C2018133.

Yet another aspect of the present ~~invention~~disclosure relates to a pharmaceutical composition comprising any one of the antibodies or the antigen-binding fragments thereof disclosed herein, the antibody-drug conjugate disclosed herein, or the bispecific antibody disclosed herein; optionally, the pharmaceutical composition further comprises pharmaceutically acceptable carriers and/or excipients.

Yet another aspect of the present ~~invention~~disclosure relates to use of any one of the antibodies or the antigen-binding fragments thereof disclosed herein, the antibody-drug conjugate disclosed herein, or the bispecific antibody disclosed herein in preparing a medicament for the treatment and/or prevention of autoimmune diseases, cardiovascular and cerebrovascular diseases, tumors, cryopyrin-associated periodic syndromes in children and adults, systemic juvenile idiopathic arthritis, or gouty arthritis;

preferably, the autoimmune disease is selected from rheumatoid arthritis, multiple sclerosis, and periodic fever syndromes;

preferably, the periodic fever syndrome is selected from TNF receptor-associated periodic syndrome (TRAPS), hyper-IgD syndrome (HIDS)/mevalonate kinase deficiency (MKD), and familial mediterranean fever (FMF);

preferably, the cryopyrin-associated periodic syndrome in children and adults is selected from familial cold auto-inflammatory syndrome, Muckle-Wells syndrome, neonatal-onset multisystem inflammatory

disease, chronic infantile neurological cutaneous and articular syndrome, and familial cold urticaria;

preferably, the cardiovascular and cerebrovascular disease is selected from myocardial infarction, atherosclerosis, arterial thrombosis, and cerebralvascular accident;

preferably, the tumor is selected from lung cancer, hepatocellular carcinoma, and acute myeloid leukemia; and

preferably, the gouty arthritis is acute gouty arthritis or chronic gouty arthritis.

Yet another aspect of the present ~~invention~~disclosure relates to use of any one of the antibodies or the antigen-binding fragments thereof disclosed herein, the antibody-drug conjugate disclosed herein, or the bispecific antibody disclosed herein in preparing:

a medicament for blocking the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2,

a medicament for down-regulating the activity or level of human IL-1 β , or

a medicament for inhibiting the activation of downstream signaling pathways mediated by the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2.

In one ~~embodiment~~example of the present ~~invention~~disclosure, the human IL-1R1 and/or human IL-1R2 is human IL-1R1 and/or human IL-1R2 on the cell surface.

In one ~~embodiment~~example of the present ~~invention~~disclosure, the use is non-therapeutic and/or non-diagnostic.

In one or more ~~embodiment~~examples of the present ~~invention~~disclosure, the antibody or the antigen-binding fragment thereof disclosed herein, the antibody-drug conjugate disclosed herein, or the bispecific antibody disclosed herein is for use in the treatment and/or prevention of autoimmune diseases,

cardiovascular and cerebrovascular diseases, tumors, cryopyrin-associated periodic syndromes in children and adults, systemic-onset juvenile idiopathic arthritis, or gouty arthritis;

preferably, the autoimmune disease is selected from rheumatoid arthritis, multiple sclerosis, and periodic fever syndromes;

preferably, the periodic fever syndrome is selected from TNF receptor-associated periodic syndrome (TRAPS), hyper-IgD syndrome (HIDS)/mevalonate kinase deficiency (MKD), and familial mediterranean fever (FMF);

preferably, the cryopyrin-associated periodic syndrome in children and adults is selected from familial cold auto-inflammatory syndrome, Muckle-Wells syndrome, neonatal-onset multisystem inflammatory disease, chronic infantile neurological cutaneous and articular syndrome, and familial cold urticaria;

preferably, the cardiovascular and cerebrovascular disease is selected from myocardial infarction, atherosclerosis, arterial thrombosis, and cerebralvascular accident;

preferably, the tumor is selected from lung cancer, hepatocellular carcinoma, and acute myeloid leukemia; and

preferably, the gouty arthritis is acute gouty arthritis or chronic gouty arthritis.

In one or more ~~embodiment~~examples of the present ~~invention~~disclosure, the antibody or the antigen-binding fragment disclosed herein, the antibody-drug conjugate disclosed herein, or the bispecific antibody disclosed herein is for use in:

blocking the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2,

down-regulating the activity or level of human IL-1 β , or

inhibiting the activation of downstream signaling pathways mediated by the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2.

In one ~~embodiment~~example of the present ~~invention~~disclosure, the human IL-1R1 and/or human IL-1R2 is human IL-1R1 and/or human IL-1R2 on the cell surface.

Yet another aspect of the present ~~invention~~disclosure relates to an *in vivo* or *in vitro* method comprising: administering to a cell an effective amount of any one of the antibodies or the antigen-binding fragments thereof disclosed herein, the antibody-drug conjugate disclosed herein, or the bispecific antibody disclosed herein, wherein the method is selected from:

a method for blocking the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2,

a method for down-regulating the activity or level of human IL-1 β , and

a method for inhibiting the activation of downstream signaling pathways mediated by the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2.

In one ~~embodiment~~example of the present ~~invention~~disclosure, the human IL-1R1 and/or human IL-1R2 is human IL-1R1 and/or human IL-1R2 on the cell surface.

In one ~~embodiment~~example of the present ~~invention~~disclosure, the *in vitro* method is non-therapeutic and/or non-diagnostic.

Yet another aspect of the present ~~invention~~disclosure relates to a method for treating and/or preventing autoimmune diseases, cardiovascular and cerebrovascular diseases, tumors, cryopyrin-associated periodic syndromes in children and adults, systemic juvenile idiopathic arthritis, or gouty arthritis, comprising: administering to a subject in need an effective amount of any one of the antibodies or the antigen-binding fragments thereof disclosed herein, the antibody-drug conjugate disclosed herein, or the bispecific antibody disclosed herein;

preferably, the autoimmune disease is selected from rheumatoid arthritis, multiple sclerosis, and periodic fever syndromes;

preferably, the periodic fever syndrome is selected from TNF receptor-associated periodic syndrome (TRAPS), hyper-IgD syndrome (HIDS)/mevalonate kinase deficiency (MKD), and familial mediterranean fever (FMF);

preferably, the cryopyrin-associated periodic syndrome in children and adults is selected from familial cold auto-inflammatory syndrome, Muckle-Wells syndrome, neonatal-onset multisystem inflammatory disease, chronic infantile neurological cutaneous and articular syndrome, and familial cold urticaria;

preferably, the cardiovascular and cerebrovascular disease is selected from myocardial infarction, atherosclerosis, arterial thrombosis, and cerebralvascular accident;

preferably, the tumor is selected from lung cancer, hepatocellular carcinoma, and acute myeloid leukemia; and

preferably, the gouty arthritis is acute gouty arthritis or chronic gouty arthritis.

The inventors found from animal experiments that the antibodies disclosed herein, particularly 3H6H4L1, can effectively alleviate pathological changes in a rheumatoid arthritis model induced by NIH/3T3 cells transfected with human IL-1 β in BALB/c mice, characterized in that the administration of the antibody 3H6H4L1 can effectively improve the pathological behaviors and reduce the swelling area of affected limbs of the rheumatoid mice.

DEFINITIONS

In the present invention, unless otherwise defined, the scientific and technical terms used herein have the meanings generally understood by those skilled in the art. In addition, the laboratory operations of cell culture, molecular genetics, nucleic acid chemistry and immunology used in the present invention are the routine operations widely used in the

CLAIMS

1. An anti-IL-1 β antibody or an antigen-binding fragment thereof, wherein

the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NO: 17, SEQ ID NO: 18, and -SEQ ID NO: 19, respectively; and

the antibody or the antigen-binding fragment thereof comprises a light chain variable region comprising LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NO: 20, SEQ ID NO: 21, and -SEQ ID NO: 22, respectively.

2. The antibody or the antigen-binding fragment thereof according to claim 1, wherein

the heavy chain variable region of the antibody comprises an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, and SEQ ID NO: 14; and

the light chain variable region of the antibody comprises an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, and SEQ ID NO: 16.

3. The antibody or the antigen-binding fragment thereof according to claim 1, wherein

the heavy chain variable region of the antibody comprises an amino acid sequence set forth in SEQ ID NO: 2, and the light chain variable region

~~of the antibody~~ comprises an amino acid sequence set forth in SEQ ID NO: 4;

the heavy chain variable region ~~of the antibody~~ comprises an amino acid sequence set forth in SEQ ID NO: 6, and the light chain variable region ~~of the antibody~~ comprises an amino acid sequence set forth in SEQ ID NO: 8;

the heavy chain variable region ~~of the antibody~~ comprises an amino acid sequence set forth in SEQ ID NO: 10, and the light chain variable region ~~of the antibody~~ comprises an amino acid sequence set forth in SEQ ID NO: 12; or

the heavy chain variable region ~~of the antibody~~ comprises an amino acid sequence set forth in SEQ ID NO: 14, and the light chain variable region ~~of the antibody~~ comprises an amino acid sequence set forth in SEQ ID NO: 16.

4. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 3, wherein the antibody or the antigen-binding fragment thereof is selected from Fab, Fab', F(ab')₂, ~~FvFd, Fv, dAb, a-complementarity-determining-region-fragment~~, a single chain antibody, a humanized antibody, a chimeric antibody, and a diabody.

5. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 3, wherein the antibody or the antigen-binding fragment thereof binds to IL-1 β protein with a K_D less than 10⁻⁵ M, such as less than 10⁻⁶ M, less than 10⁻⁷ M, less than 10⁻⁸ M, less than 10⁻⁹ M, or less than 10⁻¹⁰ M ~~or less~~; preferably, the K_D is measured by a Biacore molecular interaction instrument.

6. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 3, wherein

the antibody or the antigen-binding fragment thereof comprises a non-CDR region derived from a species other than murine, such as from a human antibody.

7. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 3, wherein

the antibody or antigen-binding fragment thereof comprises a constant region ~~derived from a human antibody;~~

~~preferably, the constant region of the antibody is~~ selected from constant regions of human IgG1, IgG2, IgG3, and IgG4~~IgG-4~~.

8. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 3, wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain constant region and a light chain constant region, wherein

the heavy chain constant region ~~of the antibody~~ is Ig gamma-1 chain C region or Ig gamma-4 chain C region, and the light chain constant region is Ig kappa chain C region.

9. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 3, wherein the antibody is a monoclonal antibody produced by a hybridoma cell line LT010 deposited at China Center for Type Culture Collection (CCTCC) with an accession number of CCTCC NO: C2018133.

10. An antibody-drug conjugate comprising an antibody or an antigen-binding fragment thereof and a small molecule drug, wherein the antibody or the antigen-binding fragment thereof is the antibody or the

antigen-binding fragment thereof according to any one of claims 1 to 9; preferably, the small molecule drug is a small molecule cytotoxic drug; and more preferably, the small molecule drug is a tumor chemotherapeutic drug.

11. The antibody-drug conjugate according to claim 10, wherein the antibody or the antigen-binding fragment thereof is linked to the small molecule drug via a linker, wherein; for example, the linker is a hydrazone bond, a disulfide bond or a peptide bond.

12. The antibody-drug conjugate according to claim 10 or 11, wherein the molar ratio of the antibody or the antigen-binding fragment thereof to the small molecule drug is 1:1–1:4.

13. A bispecific antibody comprising a first protein functional region and a second protein functional region, wherein

the first protein functional region targets IL-1 β , and

the second protein functional region targets a target other than IL-1 β , — (e.g., IL-17A);

wherein the first protein functional region is the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 9;

preferably, the bispecific antibody is in an IgG-scFv form;

preferably, the first protein functional region is the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 9, and the second protein functional region is a single chain antibody; or

preferably, the first protein functional region is the single chain antibody according to claim 4, and the second protein functional region is an antibody or an antigen-binding fragment thereof.

14. The bispecific antibody according to claim 13, wherein the first and second protein functional regions are linked directly or via a linker fragment;

preferably, the linker fragment is (GGGGS)_m, m being ~~a positive integer such as~~ 1, 2, 3, 4, 5, or 6; or

preferably, the linker fragment is SS(GGGGS)_n, n being ~~a positive integer such as~~ 1, 2, 3, 4, 5, or 6.

15. The bispecific antibody according to claim 13, wherein the numbers of the first and second protein functional regions are each independently 1, 2 or more.

16. The bispecific antibody according to ~~claim any one of claims 13 to 15,~~ wherein the single chain antibody is linked to the C-terminus of the heavy chain of the antibody or the antigen-binding fragment thereof.

17. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an antibody heavy chain variable region and a nucleotide sequence encoding an antibody light chain variable region, wherein the antibody heavy chain variable region comprises HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NO: 17, SEQ ID NO: 18, and -SEQ ID NO: 19, respectively, and the antibody light chain variable region comprises LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NO: 20, SEQ ID NO: 21, and -SEQ ID NO: 22, respectively;

preferably, the amino acid sequence of the antibody heavy chain variable region is selected from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, and SEQ ID NO: 14, and the amino acid sequence of the antibody light

chain variable region is selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, and SEQ ID NO: 16;

more preferably, the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 2, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 4; the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 6, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 8; the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 10, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 12; or the amino acid sequence of the antibody heavy chain variable region is set forth in SEQ ID NO: 14, and the amino acid sequence of the antibody light chain variable region is set forth in SEQ ID NO: 16; and

even more preferably, the nucleic acid molecule comprises:

nucleotide sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 3, nucleotide sequences set forth in SEQ ID NO: 5 and SEQ ID NO: 7, nucleotide sequences set forth in SEQ ID NO: 9 and SEQ ID NO: 11, or nucleotide sequences set forth in SEQ ID NO: 13 and SEQ ID NO: 15.

18. A recombinant vector comprising the isolated nucleic acid molecule according to claim 17.

19. A host cell comprising the isolated nucleic acid molecule according to claim 17 or the recombinant vector according to claim 18.

20. A method for preparing the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 9, comprising: cultivating the host cell according to claim 19 in a suitable condition, and isolating the antibody or the antigen-binding fragment thereof from the cell cultures.

21. A hybridoma cell line LT010 deposited at China Center for Type Culture Collection (CCTCC) with an accession number of CCTCC NO: C2018133.

22. A pharmaceutical composition comprising the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 9, the antibody-drug conjugate according to any one of claims 10 to 12, or the bispecific antibody according to any one of claims 13 to 16; and optionally, a pharmaceutically acceptable carrier and/or excipient.

23. Use of the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 9, the antibody-drug conjugate according to any one of claims 10 to 12, or the bispecific antibody according to any one of claims 13 to 16 in preparing a medicament for the treatment and/or prevention of autoimmune diseases, cardiovascular and cerebrovascular diseases, tumors, cryopyrin-associated periodic syndromes in children and adults, systemic juvenile idiopathic arthritis, or gouty arthritis;

preferably, the autoimmune disease is selected from rheumatoid arthritis, multiple sclerosis, and periodic fever syndromes;

preferably, the periodic fever syndrome is selected from TNF receptor-associated periodic syndrome (TRAPS), hyper-IgD syndrome (HIDS)/mevalonate kinase deficiency (MKD), and familial mediterranean fever (FMF);

preferably, the cryopyrin-associated periodic syndrome in children and adults is selected from familial cold auto-inflammatory syndrome, Muckle-Wells syndrome, neonatal-onset multisystem inflammatory disease, chronic infantile neurological cutaneous and articular syndrome, and familial cold urticaria;

preferably, the cardiovascular and cerebrovascular disease is selected from myocardial infarction, atherosclerosis, arterial thrombosis, and cerebral stroke;

preferably, the tumor is selected from lung cancer, hepatocellular carcinoma, and acute myeloid leukemia; and

preferably, the gouty arthritis is acute gouty arthritis or chronic gouty arthritis.

24. Use of the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 9, the antibody-drug conjugate according to any one of claims 10 to 12, or the bispecific antibody according to any one of claims 13 to 16 in preparing:

a medicament for blocking the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2,

**a medicament for down-regulating the activity or level of human IL-1 β ,
or**

a medicament for inhibiting the activation of downstream signaling pathways mediated by the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2.

~~**25. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 9, the antibody-drug conjugate according to any**~~

~~one of claims 10 to 12, or the bispecific antibody according to any one of claims 13 to 16, for use in the treatment and/or prevention of autoimmune diseases, cardiovascular and cerebrovascular diseases, tumors, cryopyrin-associated periodic syndromes in children and adults, systemic juvenile idiopathic arthritis, or gouty arthritis;~~

~~preferably, the autoimmune disease is selected from rheumatoid arthritis, multiple sclerosis, and periodic fever syndromes;~~

~~preferably, the periodic fever syndrome is selected from TNF receptor-associated periodic syndrome (TRAPS), hyper-IgD syndrome (HIDS)/mevalonate kinase deficiency (MKD), and familial mediterranean fever (FMF);~~

~~preferably, the cryopyrin-associated periodic syndrome in children and adults is selected from familial cold auto-inflammatory syndrome, Muckle-Wells syndrome, neonatal-onset multisystem inflammatory disease, chronic infantile neurological cutaneous and articular syndrome, and familial cold urticaria;~~

~~preferably, the cardiovascular and cerebrovascular disease is selected from myocardial infarction, atherosclerosis, arterial thrombosis, and cerebral stroke;~~

~~preferably, the tumor is selected from lung cancer, hepatocellular carcinoma, and acute myeloid leukemia; and~~

~~preferably, the gouty arthritis is acute gouty arthritis or chronic gouty arthritis.~~

~~26. The antibody or the antigen-binding fragment thereof according to any one of claims 1 to 9, the antibody-drug conjugate according to any one of claims 10 to 12, or the bispecific antibody according to any one of claims 13 to 16, for use in:~~

~~blocking the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2,~~

~~down-regulating the activity or level of human IL-1 β , or~~

~~inhibiting the activation of downstream signaling pathways mediated by the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2.~~

~~27. A method for treating and/or preventing autoimmune diseases, cardiovascular and cerebrovascular diseases, tumors, cryopyrin-associated periodic syndromes in children and adults, systemic juvenile idiopathic arthritis, or gouty arthritis, comprising: administering to a subject in need an effective amount of the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 9, the antibody-drug conjugate according to any one of claims 10 to 12, or the bispecific antibody according to any one of claims 13 to 16;~~

~~preferably, the autoimmune disease is selected from rheumatoid arthritis, multiple sclerosis, and periodic fever syndromes;~~

~~preferably, the periodic fever syndrome is selected from TNF receptor-associated periodic syndrome (TRAPS), hyper-IgD syndrome (HIDS)/mevalonate kinase deficiency (MKD), and familial mediterranean fever (FMF);~~

~~preferably, the cryopyrin-associated periodic syndrome in children and adults is selected from familial cold auto-inflammatory syndrome, Muckle-Wells syndrome, neonatal-onset multisystem inflammatory disease, chronic infantile neurological cutaneous and articular syndrome, and familial cold urticaria;~~

~~preferably, the cardiovascular and cerebrovascular disease is selected from myocardial infarction, atherosclerosis, arterial thrombosis, and cerebral stroke;~~

~~preferably, the tumor is selected from lung cancer, hepatocellular carcinoma, and acute myeloid leukemia; and~~

~~preferably, the gouty arthritis is acute gouty arthritis or chronic gouty arthritis.~~

~~28. An *in vivo* or *in vitro* method comprising: administering to a cell an effective amount of the antibody or the antigen-binding fragment thereof according to any one of claims 1 to 9, the antibody-drug conjugate according to any one of claims 10 to 12, or the bispecific antibody according to any one of claims 13 to 16, wherein the method is selected from:~~

~~a method for blocking the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2,~~

~~a method for down-regulating the activity or level of human IL-1 β , and~~

~~a method for inhibiting the activation of downstream signaling pathways mediated by the binding of human IL-1 β to human IL-1R1 and/or human IL-1R2.~~