

SPECIFICATION

ANTI-INTERLEUKIN-17A ANTIBODY, PHARMACEUTICAL COMPOSITION THEREOF AND USE THEREOF

5

TECHNICAL FIELD

The present invention belongs to the field of molecular immunology, and relates to an anti-interleukin-17A antibody, a pharmaceutical composition thereof and use thereof. In particular, the present invention
10 relates to an anti-interleukin-17A monoclonal antibody.

TECHNICAL BACKGROUND

Interleukin-17A (abbreviated as IL-17A or IL 17A) is a member of IL-17 cytokine family which has 6 members, i.e. IL-17A (the IL-17A is
15 discovered first and also called IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also named as IL-25) and IL-17F (Shi Peiqing et al., Chinese Journal of Cell Biology 33: 345-357 (2011)). IL-17F shares about 50% homology with IL-17A, and their coding genes are located in the same segment of chromosome 6p12 (Gaffen et al., Nat Rev Immunol 9:556-67 (2009)).
20 IL-17A and IL-17F can exist in the form of homodimers such as IL-17A/IL-17A and IL-17F/IL-17F, as well as the heterodimer IL-17A/IL-17F. IL-17A and IL-17F exhibit biological effects by binding to receptors (Wright et al., J Immunol 181: 2799-805 (2008)).

The IL-17 receptor (IL-17R) family consists of 5 members, i.e. IL-17RA,
25 IL-17RB, IL-17RC, IL-17RD, and IL-17RE. Members of the IL-17 receptor family can form different receptor complexes, in which IL-17RA, the largest molecule discovered to date in this family, is a

common subunit that transmits signals for at least four ligands, and exhibits major biological effects (Gaffen et al., Nat Rev Immunol 9: 556-67 (2009)). IL-17RA and IL-17RC complex mediates cell responses to IL-17A and IL-17F (Toy et al., J Immunol 177: 36-9 (2006)).

- 5 IL-17A is more critical than IL-17F in the autoimmune inflammatory response, the key reason is that IL17RA has a hundred times greater affinity for IL-17A than that for IL-17F (Ely et al., Nat Immunol 10 1245-51 (2009)), the response of a cell to IL-17A is 10 times stronger than that to IL-17F (Dubin et al., Immunity 30: 9-11 (2009)). An
10 anti-IL-17A antibody or an anti-IL-17-receptor antibody can be used to block the binding of the IL-17A to its receptor thereof, thereby blocking the biological activity of IL-17A.

- IL-17A plays an important role in several autoimmune diseases (e.g. psoriasis, psoriatic arthritis, rheumatoid arthritis, ankylosing
15 spondylitis, systemic lupus erythematosus, etc.). The anti-IL-17A monoclonal antibody Secukinumab is approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of moderate to severe plaque psoriasis, psoriatic arthritis, and ankylosing spondylitis.

- 20 Psoriasis, also known as psora, is a chronic autoimmune skin disease. The skin histological characteristics thereof are epidermal keratinocyte hyperproliferations, angiogenesis, as well as dendritic cell, macrophage, neutrophil, and T cell infiltrations. (Nestle et al., N Engl J Med, 361: 496-509 (2009)). Psoriasis has various manifestations, among which
25 plaque psoriasis is the most common type, accounting for more than 90% of all patients with psoriasis. Psoriatic arthritis (PsA) is a special type of psoriasis, which causes psoriasis rash as well as pain, swelling, tenderness, stiffness, and dyskinesia in the joints and surrounding soft

tissues. Some patients may have sacroiliitis and (or) spondylitis with prolonged course, easy relapse and late-stage joint stiffness, leading to disability. The existence of psoriasis is an important difference between psoriatic arthritis and other inflammatory joint diseases, and the severity of skin lesions is not directly related to the degree of joint inflammations (Tan Zhen et al., Chinese Journal of Rheumatology 20:354-357 (2016)).

The IL-17A expression is significantly increased in psoriatic pathogenic skin tissues, and this increase is closely related to psoriasis disease activity (Johansen et al., Brit J Dermatol, 160:319-24 (2009); Lowes et al. J Invest Dermatol 128:1207-11 (2008)). Among patients with psoriasis, the anti-IL-17A monoclonal antibody Secukinumab has shown excellent efficacy, which can significantly alleviate the disease activity of patients with psoriasis and reduce the area of psoriasis plaques (Langley et al., N Engl J Med, 371:326-38 (2014)); Secukinumab can also significantly reduce arthritis symptoms and significantly improve joint functions of patients with psoriatic arthritis (Gottlieb et al., J Drugs Dermatol, 14:821-33 (2015)). Secukinumab is approved by the FDA for the treatment of moderate to severe plaque psoriasis and psoriatic arthritis.

Rheumatoid arthritis (RA) is mainly characterized by inflammatory joint synovial fibroblast proliferation, joint and cartilage damage, infiltrations of CD4⁺ helper T cells and plasma cells producing autoantibodies. IL-17A can cause both inflammation and bone damage in rheumatoid arthritis. IL-17A is highly expressed in rheumatoid synovial monocytes of patients with rheumatoid arthritis relative to healthy people or patients with osteoarthritis (Sarkar et al., Clin Exp Immunol. 177: 652-61 (2014)), cytological studies suggest that IL-17A can stimulate bone resorption and collagen destruction (Kitami et al.,

Biochimie. 92: 398-404 (2010)). IL-17A can induce cartilage, synovial cells, macrophages and osteoblasts to secrete proinflammatory cytokines such as TNF α , IL-1b and IL-6, and exert biological effects. These proinflammatory cytokines cause sudden onset of rheumatoid arthritis and can maintain the number of TH17 cells through IL-17A-induced IL-6, thereby forming a positive feedback and acting synergistically to amplify their inflammatory effects, and establishing a chronic inflammatory state (Ogura et al., Immunity 29: 628-36 (2008)).

Antagonizing IL-17A can effectively alleviate rheumatoid arthritis symptoms. In a mouse model with collagen-induced arthritis, neutralizing IL-17A or its receptor thereof can resolve the symptoms of rheumatoid arthritis (Chao et al., Autoimmunity. 2011 May; 44 (3):243-52); IL-17 deficiency can protect a host mouse from collagen-induced arthritis (Nakae et al., J Immunol, 171: 6173-7 (2003)) while IL-17A overexpression can aggravate such conditions (Lubberts et al., Inflamm Res 51: 102-4 (2002)).

Ankylosing spondylitis (AS) is a chronic autoimmune disease. The early pathological features of AS are acute or chronic inflammations at the bone attachment points of sacroiliac joints, tendons, and ligaments, which could develop to discitis and facet arthritis at a later stage; and there is a phenomenon of decreased bone density in all patients with AS. Studies have shown that both the number of Th17 cells secreting IL-17A in peripheral blood and the concentration of the IL-17A of patients with AS are significantly elevated than those of healthy people (Gracey et al., Arthritis Rheumatol. 68: 679-89. (2016)). IL-17A can activate a variety of cells such as macrophages, dendritic cells, endothelial cells, fibroblasts, chondrocytes, and osteoblasts, which can produce a large number of inflammatory destructive factors (Ogura et al., Immunity 29:

628-36 (2008)). In bone tissues, IL-17A induces osteoblasts to express receptor activator of nuclear factor- κ B ligand (RANKL), activates osteoclasts, thus inducing bone resorption, cumulatively exacerbating bone loss, and causing bone destruction directly or indirectly (Gaffen, Curr Rheumatol Rep 11:365-370 (2009)). Among patients with AS, the anti-IL-17A monoclonal antibody Secukinumab has shown excellent efficacy, which can significantly reduce the symptoms and signs of ankylosing spondylitis (Baeten et al., N Engl J Med, 373:2534-48 (2015)). Based on these results, Secukinumab is approved by the FDA for the treatment of ankylosing spondylitis.

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects multiple systems. Specifically, antibodies against autoantigens appear in patients' bodies, which attack various tissues or organs directly or indirectly, and the most commonly affected areas include skin, joints and kidneys. Studies have shown that IL-17A plays a role in SLE. The ratio of cells producing IL-17A in peripheral blood of patients with SLE is increased, and the level of IL-17A in serum of patients is abnormally high (Chen et al., J Clin Immunol, 30:221-5 (2010)).

Peripheral blood mononuclear cells of patients with SLE accompanied by renal damage can produce more total IgG, anti-dsDNA IgG and IL-6 when cultured with IL-17, indicating that IL-17 can participate in B cells activation (Dong Et al., Chin Med J (Engl), 116:543-8 (2003)). It has also been recently found that IL-17A can cooperate with BAFF (B-cell activating factor) to protect B cells from apoptosis, thereby increasing the number of cells producing autoantibodies (Onishi et al., Immunology 129: 311-21 (2010)).

SUMMARY OF THE INVENTION

After intensive study and creative effort, the inventors used mammalian cell expression systems to express recombinant IL-17A (24-155) as an antigen to immunize mice, and obtained a large number of hybridoma cell samples by fusion of mouse spleen cells and myeloma cells. The inventors obtained the following two hybridoma cell lines separately by screening a large number of the samples:

hybridoma cell line LT006 (IL-17A-13E9), which was deposited at China Center for Type Culture Collection (CCTCC) on September 12, 2017 with an accession number of CCTCC NO: C2017102;

and hybridoma cell line LT007 (IL-17A-2G2), which was deposited at China Center for Type Culture Collection (CCTCC) on September 12, 2017 with an accession number of CCTCC NO: C2017165.

The inventors surprisingly found:

the hybridoma cell line LT006 may secrete and produce a specific monoclonal antibody (named as 13E9) that specifically binds to IL-17A, and the monoclonal antibody can block the binding of IL-17A to IL-17RA very effectively;

the hybridoma cell line LT007 may secrete and produce a specific monoclonal antibody (named as 2G2) that specifically binds to IL-17A, and the monoclonal antibody can block the binding of IL-17A to IL-17RA very effectively;

furthermore, the inventors creatively prepared anti-IL-17A humanized antibodies (named as 13E9 H1L1, 13E9 H2L2, 13E9 H3L2; and 2G2 H1L1, 2G2 H2L2, 2G2 H3L3, respectively), all of which may bind to human IL-17A effectively, block the binding of IL-17A to IL-17A

receptors, and inhibit the activation of downstream signaling pathways of the IL-17A receptors;

the antibody of the present invention has the potential to produce drugs for preventing and/or treating autoimmune diseases such as psoriasis,
5 rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and systemic lupus erythematosus.

The following invention is thus provided:

one aspect of the present invention relates to a monoclonal antibody or
10 an antigen binding fragment thereof, wherein,

the heavy chain variable region (VH) of the monoclonal antibody comprises: HCDR1-HCDR3 with the amino acid sequences shown in SEQ ID NOs: 31-33 respectively, or HCDR1-HCDR3 with the amino acid sequences shown in SEQ ID NOs: 37-39 respectively; and

15 the light chain variable region (VL) of the monoclonal antibody comprises: LCDR1-LCDR3 with the amino acid sequences shown in SEQ ID NOs: 34-36 respectively, or LCDR1-LCDR3 with the amino acid sequences shown in SEQ ID NOs: 40-42 respectively.

The variable regions of the light chain and the heavy chain determine
20 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
25 of Immunological Interest, Fifth Edition (1991), Volumes 1-3, NIH Publication 91-3242, Bethesda Md).

The amino acid sequences of the CDR regions of the monoclonal antibody in (1) to (2) above are analyzed by technical means well known to those skilled in the art, for example, by a VBASE2 database:

the antibodies 13E9, 13E9 H1L1, 13E9 H2L2, and 13E9 H3L2 of the
5 present invention have the same CDRs:

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

HCDR1: SYSFTSDYA (SEQ ID NO: 31),

HCDR2: ITYSGVT (SEQ ID NO: 32),

10 HCDR3: ARADYDSYYTMDY (SEQ ID NO: 33); and

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

LCDR1: QSLVHSNGNTY (SEQ ID NO: 34),

LCDR2: KVS (SEQ ID NO: 35),

15 LCDR3: SQSTHFWT (SEQ ID NO: 36).

The antibodies 2G2, 2G2 H1L1, 2G2 H2L2, and 2G2 H3L3 of the present invention have the same CDRs:

the amino acid sequences of the three CDR regions of the heavy chain
20 variable region are as follows:

HCDR1: SEVFPIAD (SEQ ID NO: 37),

HCDR2: ILPSFGRT (SEQ ID NO: 38),

HCDR3: ARGNYGFAY (SEQ ID NO: 39); and

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

LCDR1: QSLNSDGKTY (SEQ ID NO: 40),

LCDR2: LVS (SEQ ID NO: 41),
LCDR3: WQGSHPQT (SEQ ID NO: 42).

In one or more embodiments of the present invention, the monoclonal
antibody or the antigen binding fragment thereof, wherein,
the heavy chain variable region (VH) of the monoclonal antibody
comprises HCDR1-HCDR3 with the amino acid sequences shown in
SEQ ID NOs: 31-33 respectively, and
the light chain variable region (VL) of the monoclonal antibody
comprises LCDR1-LCDR3 with the amino acid sequences shown in SEQ
ID NOs: 34-36 respectively.

In one or more embodiments of the present invention, the monoclonal
antibody or the antigen binding fragment thereof, wherein,
the heavy chain variable region (VH) of the monoclonal antibody
comprises HCDR1-HCDR3 with the amino acid sequences shown in
SEQ ID NOs: 37-39 respectively, and
the light chain variable region (VL) of the monoclonal antibody
comprises LCDR1-LCDR3 with the amino acid sequences shown in SEQ
ID NOs: 40-42 respectively.

In one or more embodiments of the present invention, the monoclonal
antibody or the antigen binding fragment thereof, wherein,
the amino acid sequence of the heavy chain variable region is selected
from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14,
SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 24, and SEQ ID NO: 28;

and

the amino acid sequence of the light chain variable region is selected from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 26, and SEQ ID NO: 30.

- 5 **In one or more embodiments of the present invention, the monoclonal antibody or the antigen binding fragment thereof, wherein,**
the amino acid sequence of the 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 light chain variable region is
10 **selected from SEQ ID NO: 4, SEQ ID NO: 8, and SEQ ID NO: 12;**

or

- the amino acid sequence of the heavy chain variable region is selected from SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 24, and SEQ ID NO: 28, and the amino acid sequence of the light chain variable region is**
15 **selected from SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 26, and SEQ ID NO: 30.**

- In one or more embodiments of the present invention, the monoclonal antibody or the antigen binding fragment thereof, wherein the heavy chain variable region and light chain variable region are selected from**
20 **any one of the following (1) to (8):**

- (1) a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO: 2, and**
a light chain variable region comprising the amino acid sequence shown in SEQ ID NO: 4;
25 **(2) a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO: 6, and**

a light chain variable region comprising the amino acid sequence shown in SEQ ID NO: 8;

(3) a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO: 10, and

5 a light chain variable region comprising the amino acid sequence shown in SEQ ID NO: 12;

(4) a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO: 14, and

10 a light chain variable region comprising the amino acid sequence shown in SEQ ID NO: 12;

(5) a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO: 16, and

a light chain variable region comprising the amino acid sequence shown in SEQ ID NO: 18;

15 (6) a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO: 20, and

a light chain variable region comprising the amino acid sequence shown in SEQ ID NO: 22;

20 (7) a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO: 24, and

a light chain variable region comprising the amino acid sequence shown in SEQ ID NO: 26; and

(8) a heavy chain variable region comprising the amino acid sequence shown in SEQ ID NO: 28, and

25 a light chain variable region comprising the amino acid sequence shown in SEQ ID NO: 30.

In one or more embodiments of the present invention, the monoclonal antibody or the antigen binding fragment thereof, wherein the monoclonal antibody or the antigen binding fragment thereof is selected from a Fab, a Fab', an F(ab')₂, an Fd, an Fv, a dAb, a complementarity determining region fragment, a single chain antibody, a humanized antibody, a chimeric antibody and a diabody.

In one or more embodiments of the present invention, the monoclonal antibody or the antigen binding fragment thereof, wherein, the monoclonal antibody binds to IL-17A protein with an EC₅₀ of less than about 100 nM, such as less than about 10 nM, 5 nM, 4 nM, 3 nM, 2.5 nM, 2 nM, or less; preferably, the EC₅₀ is measured by a competitive ELISA method.

In some embodiments of the present invention, the monoclonal antibody or the antigen binding fragment thereof, wherein the monoclonal antibody binds to IL-17A protein with a K_D of less than about 10⁻⁵ M, such as less than about 10⁻⁶ M, 10⁻⁷ M, 10⁻⁸ M, 10⁻⁹ M, 10⁻¹⁰ M, or less; preferably, the K_D is measured by a Fortebio molecular interaction instrument.

In some embodiments of the present invention, the monoclonal antibody or the antigen binding fragment thereof, wherein the monoclonal antibody binds to IL-17A protein with an EC₅₀ of less than about 100 nM, such as less than about 10 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, or less; in particular, the EC₅₀ is measured by an indirect ELISA method.

In one or more embodiments of the present invention, the monoclonal antibody comprises non-CDR regions, and the non-CDR regions are from species other than murine, such as from a human antibody.

In some embodiments of the present invention, the constant region of the immunoglobulin is humanized, for example, the heavy chain constant regions use Ig gamma-1 chain C region, ACCESSION: P01857; and the light chain constant regions use Ig kappa chain C region,
5 **ACCESSION: P01834.**

In one or more embodiments of the present invention, the monoclonal antibody or the antigen binding fragment thereof, wherein:
the monoclonal antibody is produced by the hybridoma cell line LT006,
10 **which was deposited at China Center for Type Culture Collection (CCTCC) with an accession number of CCTCC NO: C2017102; or**
the monoclonal antibody is produced by the hybridoma cell line LT007,
which was deposited at China Center for Type Culture Collection (CCTCC) with an accession number of CCTCC NO: C2017165.

In one or more embodiments of the present invention, the monoclonal
15 **antibody or the antigen binding fragment thereof is used to prevent and/or treat tumors or autoimmune diseases, or to diagnose autoimmune diseases; preferably, the autoimmune disease is selected from psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and systemic lupus erythematosus; preferably, the psoriasis**
20 **is moderate to severe plaque psoriasis.**

In one or more embodiments of the present invention, the monoclonal antibody or an antigen binding fragment thereof is used for:
blocking the binding of IL-17A to IL-17RA,
regulating (e.g., down-regulating) IL-17A activity or level, or
25 **inhibiting IL-6 expression in cells.**

Another aspect of the present invention relates to an isolated nucleic acid molecule comprising nucleotide sequences encoding the heavy chain variable region and light chain variable region of any one of the monoclonal antibodies described in the present invention.

5 In one or more embodiments of the present invention, the isolated nucleic acid molecule comprises nucleotide sequences selected from any of the following (1) to (8):

(1) SEQ ID NO: 1, SEQ ID NO: 3;

(2) SEQ ID NO: 5, SEQ ID NO: 7;

10 (3) SEQ ID NO: 9, SEQ ID NO: 11;

(4) SEQ ID NO: 13, SEQ ID NO: 11;

(5) SEQ ID NO: 15, SEQ ID NO: 17;

(6) SEQ ID NO: 19, SEQ ID NO: 21;

(7) SEQ ID NO: 23, SEQ ID NO: 25; and

15 (8) SEQ ID NO: 27, SEQ ID NO: 29.

Another aspect of the present invention relates to a recombinant vector comprising the isolated nucleic acid molecule of the present invention.

20 Preferably, the recombinant vector is a recombinant expression vector, such as a recombinant prokaryotic expression vector or a recombinant eukaryotic expression vector.

Another aspect of the present invention relates to a host cell comprising the recombinant vector of the present invention.

25 Another aspect of the present invention relates to a method for preparing any one of the monoclonal antibodies or the antigen binding

fragments thereof described in the present invention, comprising the steps of culturing the host cell in the present invention under appropriate conditions and isolating the monoclonal antibody or the antigen binding fragment thereof from the cell cultures.

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Another aspect of the present invention relates to a hybridoma cell line selected from:

the hybridoma cell line LT006, which was deposited at China Center for Type Culture Collection (CCTCC) with an accession number of

10 **CCTCC NO: C2017102; and**

the hybridoma cell line LT007, which was deposited at China Center for Type Culture Collection (CCTCC) with an accession number of CCTCC NO: C2017165.

15 **Another aspect of the present invention relates to a conjugate comprising a monoclonal antibody or an antigen binding fragment thereof and a conjugated portion, wherein the monoclonal antibody is any one of the monoclonal antibodies or the antigen binding fragments thereof described in the present invention, the conjugated portion is a**
20 **detectable label; preferably, the detectable label is a radioactive isotope, a luminescent substance such as a fluorescent substance, a colored substance, or an enzyme.**

Another aspect of the present invention relates to a kit comprising any
25 **one of the monoclonal antibodies or the antigen binding fragments thereof described in the present invention or comprising the conjugate of the present invention;**

preferably, the kit further comprises a second antibody which specifically recognizes the monoclonal antibody or the antigen binding fragment thereof; optionally, the second antibody further comprises a detectable label; preferably, the detectable label is a radioactive isotope,
5 a luminescent substance such as a fluorescent substance, a colored substance, or an enzyme.

Another aspect of the present invention relates to use of any one of the monoclonal antibodies or the antigen binding fragments thereof
10 described in the present invention or the conjugate of the present invention in the preparation of a kit for qualitative or quantitative detection for IL-17A. The qualitative detection refers to detecting the presence of IL-17A in the sample to be tested, and the quantitative detection refers to detecting the concentration or content of IL-17A in
15 the sample to be tested.

Another aspect of the present invention relates to a pharmaceutical composition comprising any one of the monoclonal antibodies or the antigen binding fragments thereof described in the present invention or
20 the conjugate of the present invention; optionally, the pharmaceutical composition further comprises pharmaceutically acceptable carriers or excipients.

Another aspect of the present invention relates to use of any one of the
25 monoclonal antibodies or the antigen binding fragments thereof described in the present invention or the conjugate of the present invention in the preparation of a drug for preventing and/or treating

tumors or autoimmune diseases, or use in the preparation of a drug for diagnosing autoimmune diseases; preferably, the autoimmune disease is selected from psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and systemic lupus erythematosus; preferably, the psoriasis is moderate to severe plaque psoriasis.

In particular, the inventors found from animal experiments (Example 11) that 13E9H3L2 can effectively inhibit the increase in the epidermal thickness of a C57BL/6 mouse model with psoriasis, which is shown as the antibody drug 13E9H3L2 can significantly inhibit the increase of epidermal thickness of the mouse caused by IL-17A and subcutaneous injection, having an efficacy equivalent to the marketed monoclonal antibody drug Secukinumab for the same target.

In some embodiments of the present invention, the autoimmune diseases (e.g., psoriasis such as moderate to severe plaque psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis or systemic lupus erythematosus) are caused by an elevated IL-17A expression level. The elevated expression level described here refers to the expression level higher than that of healthy people and has reached a pathogenic level.

Another aspect of the present invention relates to use of any one of the monoclonal antibodies or the antigen binding fragments thereof described in the present invention or the conjugate of the present invention in the preparation of the following drugs:

drugs blocking the binding of IL-17A to IL-17RA,
drugs regulating (e.g., down-regulating) IL-17A activity or level, or
drugs inhibiting IL-6 expression in cells.

Another aspect of the present invention relates to an in vivo or in vitro method comprising the step of administering to a cell or a subject in need an effective amount of any one of the monoclonal antibodies or the antigen binding fragments thereof described in the present invention or the conjugate of the present invention, and the method is selected from the following:

- methods for blocking the binding of IL-17A to IL-17RA,
- methods for regulating (e.g., down-regulating) IL-17A activity or level, or
- methods for inhibiting IL-6 expression in fibroblasts.

In a specific embodiment of the present invention, the in vitro method is non-therapeutic and/or non-diagnostic.

Interleukin 6 (abbreviated as IL-6 or IL 6) can be produced by fibroblasts, monocytes/macrophages, T lymphocytes, B lymphocytes, epithelial cells, keratinocytes, and various tumor cells. Interleukin 6 is an important factor regulating immune response, and IL-6 and IL-1 can synergistically promote T cell proliferation, stimulate B cell differentiation, and participate in the body's inflammatory responses (Schoenborn et al. Advances in Immunology 96: 41-101 (2007)). The in vitro experiment (Example 10) of the present invention shows that the anti-IL-17A antibody can significantly reduce the secretion of IL-6 and inhibit IL-6-mediated immune response.

Another aspect of the present invention relates to a method for treating and/or preventing tumors or autoimmune diseases, and the method comprises the step of administering to a subject in need an effective amount of any one of the monoclonal antibodies or the antigen binding fragments thereof described in the present invention or the conjugate

of the present invention; preferably, the autoimmune disease is selected from psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and systemic lupus erythematosus; preferably, the psoriasis is moderate to severe plaque psoriasis.

- 5 Another aspect of the present invention relates to a method for diagnosing autoimmune diseases, and the method comprises the step of applying a sample to be tested (such as a tissue sample, a cell sample, or a blood sample) or administering to a subject in need an effective amount of any one of the monoclonal antibodies or the antigen binding
10 fragments thereof described in the present invention or the conjugate of the present invention; preferably, the autoimmune disease is selected from psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and systemic lupus erythematosus; preferably, the psoriasis is moderate to severe plaque psoriasis.
- 15 In some embodiments of the present invention, considering that autoimmune diseases (e.g., psoriasis, such as moderate to severe plaque psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis or systemic lupus erythematosus) are caused by an elevated IL-17A expression level, thus the diagnosis can be performed by
20 detecting the IL-17A expression level using any one of the monoclonal antibodies or the antigen binding fragments thereof described in the present invention or the conjugate of the present invention. If the IL-17A expression level is higher than that of healthy people and has reached the pathogenic level, the diagnosis is a positive result; otherwise,
25 the diagnosis is a negative result.

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 herein are the routine operations widely used in the corresponding fields. Meanwhile, in order to better understand the present invention,
5 the definitions and explanations of the relevant terms are provided below.

As used herein, when the amino acid sequence of the IL-17A (interleukin-17A) protein is mentioned, it includes the full length of the IL-17A protein; also a fusion protein of IL-17A, such as a fragment
10 fused to a mouse or human IgG Fc protein fragment (mFc or hFc). However, those skilled in the art will understand that in the amino acid sequence of the IL-17A protein, mutations or variations (including but not limited to, substitutions, deletions and/or additions) can be naturally generated or artificially introduced without affecting biological
15 functions thereof. Therefore, in the present invention, the term "IL-17A protein" should include all such sequences, including their natural or artificial variants. In addition, when the sequence fragment of the IL-17A protein is described, the IL-17A protein also includes the corresponding sequence fragments in natural or artificial variants
20 thereof.

The full-length sequence (155aa) of IL-17A is as follows, wherein the signal peptide sequence (23aa) is underlined.

MTPGKTSLVSLLLLLLSLEAIVKAGITIPRNPGCPNSEDKNFPRTVM
VNLNIHNRNTNTNPKRSSDYYNRSTSPWNLHRNEDPERYPSVIWE
25 AKCRHLGCINADGNVDYHMNSVPIQQEILVLRREPPHCPNSFRLE
KILVSVGCTCVTPIVHHVA (SEQ ID NO: 43)

As used herein, unless otherwise defined, the IL-17R is IL-17RA; specific protein sequence thereof is a sequence known in the prior art,

and reference may be made to the sequence disclosed in the existing literature or GenBank. For example, IL-17RA (CD217, NCBI Gene ID: NP_055154.3).

As used herein, the term EC₅₀ refers to the concentration for 50% of maximal effect, i.e. the concentration that can cause 50% of the maximal effect.

As used herein, the term "antibody" refers to an immunoglobulin molecule that generally consists of two pairs of polypeptide chains (each pair with one "light" (L) chain and one "heavy" (H) chain). In a general sense, the heavy chain can be interpreted as a polypeptide chain with a larger molecular weight in an antibody, and the light chain refers to a polypeptide chain with a smaller molecular weight in an antibody. Light chains are classified as κ and λ light chains. Heavy chains are generally classified as μ , δ , γ , α , or ϵ , and isotypes of antibodies are defined as IgM, IgD, IgG, IgA, and IgE, respectively. In light chains and heavy chains, the variable region and constant region are linked by a "J" region of about 12 or more amino acids, and the heavy chain also comprises a "D" region of about 3 or more amino acids. Each heavy chain consists of a heavy chain variable region (V_H) and a heavy chain constant region (C_H). The heavy chain constant region consists of 3 domains (C_{H1}, C_{H2} and C_{H3}). Each light chain consists of a light chain variable region (V_L) and a light chain constant region (C_L). The light chain constant region consists of one domain C_L. The constant region of the antibody can mediate the binding of immunoglobulins to host tissues or factors, including the binding of various cells of the immune system (e.g., effector cells) to the first component (C1q) of classical complement system. The V_H and V_L regions can be further subdivided into highly variable regions (called complementarity determining regions (CDRs)),

and between which conservative regions called framework regions (FRs) are distributed. Each V_H and V_L consists of 3 CDRs and 4 FRs arranged from amino terminus to carboxyl terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions (V_H and V_L) of each heavy chain/light chain pair form an antibody binding site, respectively. The assignment of amino acids to each region or domain follows the definition of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk (1987) J. Mol. Biol. 196:901-917; Chothia et al. (1989) Nature 342:878-883. In particular, the heavy chain may also comprise more than 3 CDRs, such as 6, 9, or 12. For example, in the bispecific antibody of the present invention, the heavy chain may be a ScFv with the C-terminus of the heavy chain of IgG antibody linked to another antibody, and in this case, the heavy chain comprises 9 CDRs. The term "antibody" is not restricted by any specific method for producing antibody. For example, the antibody includes, in particular, a recombinant antibody, a monoclonal antibody or a polyclonal antibody. Antibodies can be different isotypes, such as antibodies IgG (e.g., subtypes IgG1, IgG2, IgG3 or IgG4), IgA1, IgA2, IgD, IgE or IgM. As used herein, the term "antigen binding fragment" refers to the polypeptide comprising the fragment of a full-length antibody, which maintains the ability to specifically bind to the same antigen to which the full-length antibody binds, and/or competing with the full-length antibody for the specific binding to antigen, which is also known as the "antigen binding portion". See generally, Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd edition, Raven Press, N.Y. (1989)), which is incorporated herein by reference in its entirety for all purposes. Antigen binding fragment of the antibody can be produced by recombinant DNA

technology or by enzymatic or chemical cleavage of intact antibodies. In some cases, the antigen binding fragment includes a Fab, a Fab', an F (ab')₂, an Fd, an Fv, a dAb, a complementarity determining region (CDR) fragment, a single chain antibody fragment (e.g., scFv), a
5 chimeric antibody, a diabody and such polypeptide, which comprises at least a portion of the antibody sufficient to impart specific antigen binding ability to a polypeptide.

As used herein, the term "Fd fragment" refers to an antibody fragment consisting of V_H and C_H1 domains; the term "Fv fragment" refers to an
10 antibody fragment consisting of the V_L and V_H domains of a single arm of an antibody; the term "dAb fragment" refers to an antibody fragment consisting of a V_H domain (Ward et al., Nature 341:544-546 (1989)); the term "Fab fragment" refers to an antibody fragment consisting of V_L, V_H, C_L, and C_H1 domains; and the term "F (ab')₂
15 fragment" refers to an antibody fragment comprising two Fab fragments linked by the disulfide bridge on a hinge region.

In some cases, the antigen binding fragment of the antibody is a single chain antibody (e.g., scFv) in which the V_L and V_H domains are paired to form a monovalent molecule via a linker that enables them to
20 produce a single polypeptide chain (see, e.g., Bird et al., Science 242:423-426 (1988) and Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988)). Such scFv molecules may have a general structure: NH₂-V_L-linker-V_H-COOH or NH₂-V_H-linker-V_L-COOH. An appropriate prior art linker consists of a repeating GGGGS amino acid sequence or
25 a variant thereof. For example, a linker having the amino acid sequence (GGGGS)₄ can be used, but variants thereof can also be used (Holliger et al. (1993), Proc. Natl. Acad. Sci. USA 90: 6444-6448). Other linkers that can be used in the present invention are described by Alfthan et al.

(1995), Protein Eng. 8:725-731, Choi et al. (2001), Eur. J. Immunol. 31: 94-106, Hu et al. (1996), Cancer Res. 56:3055-3061, Kipriyanov et al. (1999), J. Mol. Biol. 293:41-56 and Roovers et al. (2001), Cancer Immunol.

5 In some cases, the antigen binding fragment of the antibody is a diabody, that is, a bivalent antibody, in which the V_H and V_L domains are expressed on a single polypeptide chain. However, the linker used is too short to allow the pairing of the two domains on the same chain, thereby the domains are forced to pair with the complementary domains on the
10 other chain and two antigen binding sites are generated (see, e.g., Holliger P. et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993), and Poljak RJ et al., Structure 2:1121-1123 (1994)).

Antigen binding fragments (e.g., the above mentioned antibody fragments) of antibodies can be obtained from given antibodies by using
15 conventional techniques known to those skilled in the art (e.g., recombinant DNA technology or enzymatic or chemical cleavage), and the antigen binding fragments of the antibodies are screened for specificity in the same way as for intact antibodies.

As used herein, unless otherwise clearly defined in the context, when
20 referring to the term "antibody", it includes not only intact antibodies but also antigen binding fragments of antibodies.

As used herein, the terms "mAb" and "monoclonal antibody" refer to an antibody or a fragment of an antibody that is derived from a group of highly homologous antibodies, i.e. from a group of identical antibody
25 molecules, except for natural mutations that may occur spontaneously. The monoclonal antibody has a high specificity for a single epitope on an antigen. The Polyclonal antibody, relative to the monoclonal antibody, generally comprises at least two or more different antibodies

which generally recognize different epitopes on an antigen. Monoclonal antibodies can generally be obtained by hybridoma technology first reported by Kohler et al. (Nature, 256:495, 1975), but can also be obtained by recombinant DNA technology (for example, see U.S.P
5 4,816,567).

As used herein, the term "chimeric antibody" refers to an antibody of which a part of the light or/and heavy chains is derived from an antibody (which may be derived from a specific species or belong to a specific antibody class or subclass), and the other part of the light
10 or/and heavy chains are derived from another antibody (which may be derived from the same or different species or belong to the same or different antibody class or subclass). But in any case, it retains the binding activity to the target antigen (USP 4,816,567 to Cabilly et al.; Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851 6855 (1984)).

15 As used herein, the term "humanized antibody" refers to an antibody or antibody fragment obtained when all or a part of CDR regions of a human immunoglobulin (receptor antibody) are replaced by the CDR regions of a non-human antibody (donor antibody), wherein the donor antibody may be a non-human (e.g., mouse, rat or rabbit) antibody
20 having expected specificity, affinity or reactivity. In addition, some amino acid residues in the framework regions (FRs) of the receptor antibody can also be replaced by the amino acid residues of corresponding non-human antibodies or by the amino acid residues of other antibodies to further improve or optimize the performance of the
25 antibody. For more details on humanized antibodies, see, for example, Jones et al., Nature, 321:522 525 (1986); Reichmann et al., Nature, 332:323 329 (1988); Presta, Curr. Op Struct. Biol., 2:593 596 (1992); and Clark, Immunol. Today 21: 397 402 (2000).

As used herein, the term "epitope" refers to a site on the antigen that an immunoglobulin or antibody specifically binds to. "Epitope" is also called in the art as an "antigenic determinant". The epitope or antigenic determinant generally consists of chemically active surface groups of a molecule such as amino acids or carbohydrates or sugar side chains, and usually has specific three-dimensional structural characteristics and specific charge characteristics. For example, the epitope generally includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive or non-consecutive amino acids in a unique spatial conformation, which can be "linear" or "conformational". See, for example, Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, G. E. Morris, Ed. (1996). In a linear epitope, all interacting points between a protein and an interacting molecule (e.g., an antibody) exist linearly along the primary amino acid sequence of the protein. In a conformational epitope, the interacting points exist across the protein amino acid residues that are separated from each other.

As used herein, the term "isolated" refers to obtained by artificial means from natural state. If a certain "isolated" substance or component appears in nature, it may be due to the change in its natural environment, or it is isolated from the natural environment, or both. For example, a certain non-isolated polynucleotide or polypeptide naturally exists in a certain living animal, and the same polynucleotide or polypeptide with a high purity isolated from such a natural state is called isolated polynucleotide or polypeptide. The term "isolated" does not exclude the existence of artificial or synthetic substances or other impurities that do not affect the activity of the substance.

As used herein, the term "E. coli expression system" refers to an expression system consisting of E. coli (strain) and a vector, wherein the

E. coli (strain) is derived from a commercially available strain, such as but not limited to GI698, ER2566, BL21 (DE3), B834 (DE3), and BLR (DE3).

As used herein, the term "vector" refers to a nucleic acid vehicle into which a polynucleotide can be inserted. When the vector allows for the expression of the protein encoded by the inserted polynucleotide, the vector is called an expression vector. A vector can be introduced into a host cell by transformation, transduction, or transfection so that the genetic substance elements carried by the vector can be expressed in the host cell. Vectors are well known to those skilled in the art, including, but not limited to: plasmids; phagemids; cosmids; artificial chromosomes, such as yeast artificial chromosomes (YAC), bacterial artificial chromosomes (BAC) or P1-derived artificial chromosomes (PAC); phages such as lambda phages or M13 phages, and animal viruses, etc. Animal viruses that can be used as vectors include, but are not limited to, retroviruses (including lentiviruses), adenoviruses, adeno-associated viruses, herpes viruses (such as herpes simplex virus), poxviruses, baculoviruses, papillomaviruses, and papovaviruses (such as SV40). A vector can contain a variety of elements that control expression, including, but not limited to, promoter sequences, transcription initiation sequences, enhancer sequences, selection elements, and reporter genes. In addition, the vector may further contain a replication initiation site.

As used herein, the term "host cell" refers to cells that can be used to introduce vectors, including, but not limited to, prokaryotic cells such as E. coli or bacillus subtilis, fungal cells such as yeast cells or aspergillus, insect cells such as S2 drosophila cells or Sf9, or animal cells such as

fibroblast, CHO cells, COS cells, NSO cells, HeLa cells, BHK cells, HEK 293 cells, or human cells.

As used herein, the term " K_D " refers to a dissociation equilibrium constant for a specific antibody-antigen interaction, which is used to describe the binding affinity between the antibody and the antigen. The smaller the equilibrium dissociation constant, the tighter the antibody-antigen binding, and the higher the affinity between the antibody and the antigen. Generally, antibodies bind to antigens with a dissociation equilibrium constant (K_D) of less than about 10^{-5} M, such as less than about 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, or less, and the dissociation equilibrium constant can be measured by, for example, using a Fortebio molecular interaction instrument.

As used herein, the terms "monoclonal antibody" and "mAb" have the same meaning and can be used interchangeably; the terms "polyclonal antibody" and "PcAb" have the same meaning and can be used interchangeably; the terms "polypeptide" and "protein" have the same meaning and can be used interchangeably. And in the present invention, amino acids are generally represented by single-letter and three-letter abbreviations known in the art. For example, alanine can be represented by A or Ala.

As used herein, the terms "hybridoma" and "hybridoma cell line" can be used interchangeably, and when referring to the terms "hybridoma" and "hybridoma cell line", they also include subclones and progeny cells of the hybridoma. For example, when referring to hybridoma cell line LT006 or LT007, it also refers to subclones and progeny cells of the hybridoma cell line LT006 or LT007.

As used herein, the term "pharmaceutically acceptable carrier and/or excipient" refers to a carrier and/or excipient that is pharmacologically

and/or physiologically compatible with the subject and the active ingredient, which is well known in the art (see, e.g., Remington's Pharmaceutical Sciences. Edited by Gennaro AR, 19th ed. Pennsylvania: Mack Publishing Company, 1995), and includes but is not limited to pH regulators, surfactants, adjuvants, and ionic strength enhancers. For example, the pH regulators include, but are not limited to, phosphate buffer; the surfactants include, but are not limited to, cationic, anionic, or non-ionic surfactants, such as Tween-80; and the ionic strength enhancers include, but are not limited to, sodium chloride.

As used herein, the term "adjuvant" refers to a non-specific immune enhancer, which can enhance the immune response of the body to antigens or change the type of immune response when delivered into the body together with the antigens or delivered into the body in advance. There are various adjuvants, including, but not limited to, aluminum adjuvant (e.g., aluminum hydroxide), Freund's adjuvant (e.g., complete Freund's adjuvant and incomplete Freund's adjuvant), corynebacterium parvum, lipopolysaccharide, cytokine, etc. The Freund's adjuvant is the most commonly used adjuvant in animal experiments. The aluminum hydroxide adjuvant is used more in clinical trials.

As used herein, the term "effective amount" refers to an amount sufficient to obtain or at least partially obtain desired effect. For example, the effective amount for preventing diseases (e.g., diseases related to IL-17A binding to IL-17A receptor or excessive IL-17A activity, such as autoimmune diseases) is an amount sufficient to prevent, stop, or delay the onset of diseases (e.g., diseases related to IL-17A binding to IL-17A receptor or excessive IL-17A activity, such as autoimmune diseases); a therapeutically effective amount is an amount

sufficient to cure or at least partially stop a disease and its complications in patients who have already had the disease. It is well within the ability of those skilled in the art to determine such an effective amount. For example, the amount effective for therapeutic use will depend on the severity of the disease to be treated, the overall state of the patient's own immune system, the general condition of the patient such as age, weight and gender, the manner of drug administration, and other treatments administered concurrently, etc.

10 The Beneficial Effects of The Invention

The present invention achieves at least one of the following technical effects:

- (1) the monoclonal antibodies of the present invention such as 13E9 H1L1, 13E9 H2L2, 13E9 H3L2, and 2G2 H1L1, 2G2 H2L2, 2G2 H3L3 all can specifically bind to IL-17A very well, and can effectively block the binding of IL-17A to the IL-17A ligand and specifically reduce secretion of IL-6 in the IL-17A-mediated fibroblasts;
- (2) the monoclonal antibodies of the present invention, especially 13E9 H2L2, have the same effect as the marketed drug Secukinumab for the same target in inhibiting the secretion of IL-6;
- (3) the monoclonal antibodies of the present invention have the potential to be applied in the treatment and/or prevention of anti-autoimmune diseases such as psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis or systemic lupus erythematosus.

25

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: SDS-PAGE detection results of the monoclonal murine antibody 13E9. The samples of the four lanes from left to right and their respective loading amounts are: antibody in non-reducing protein electrophoresis loading buffer, 1 µg; antibody in reducing protein electrophoresis loading buffer, 1 µg; Marker, 5 µL; BSA, 1 µg.

Figure 2: SDS-PAGE detection results of the monoclonal murine antibody 2G2. The samples of the four lanes from left to right and their respective loading amounts are: antibody in non-reducing protein electrophoresis loading buffer, 1 µg; antibody in reducing protein electrophoresis loading buffer, 1 µg; Marker, 5 µL; BSA, 1 µg.

Figure 3: SDS-PAGE detection results of monoclonal humanized antibody 13E9 H3L2. The samples of the three lanes from left to right and their respective loadings are: BSA, 1 µg; Marker, 5 µL; antibody in reducing protein electrophoresis loading buffer, 1 µg; antibody in non-reducing protein electrophoresis loading buffer, 1 µg.

Figure 4: detection results of the kinetic characteristic parameters of antibody 13E9 H1L1. In the figure, ordinate is signal value with nm as unit; abscissa is time with sec as unit.

Figure 5: detection results of the kinetic characteristic parameters of antibody 13E9 H2L2. In the figure, ordinate is signal value with nm as unit; abscissa is time with sec as unit.

Figure 6: detection results of the kinetic characteristic parameters of antibody 13E9 H3L2. In the figure, ordinate is signal value with nm as unit; abscissa is time with sec as unit.

Figure 7: detection results of the kinetic characteristic parameters of antibody Secukinumab. In the figure, ordinate is signal value with nm as unit; abscissa is time with sec as unit.

Figure 8: detection results of the kinetic characteristic parameters of antibody 2G2 H1L1. In the figure, ordinate is signal value with nm as unit; abscissa is time with sec as unit.

5 **Figure 9: detection results of the kinetic characteristic parameters of antibody 2G2 H2L2. In the figure, ordinate is signal value with nm as unit; abscissa is time with sec as unit.**

Figure 10: detection results of the kinetic characteristic parameters of antibody 2G2 H3L3. In the figure, ordinate is signal value with nm as unit; abscissa is time with sec as unit.

10 **Figure 11: detection results of the kinetic characteristic parameters of antibody Secukinumab. In the figure, ordinate is signal value with nm as unit; abscissa is time with sec as unit.**

Figure 12: detection of the binding activity of antibodies 13E9 H1L1 and 13E9 H2L2 to antigen IL17A-His with an indirect ELISA method. The
15 **marketed drug Secukinumab is used as a positive control.**

Figure 13: detection of the binding activity of antibody 13E9 H3L2 to antigen IL17A-His by indirect ELISA method. The marketed drug Secukinumab is used as a positive control.

Figure 14: detection of the activity of antibodies 13E9 H1L1 and 13E9
20 **H2L2 competing with receptor IL17RA-His (biotin) for binding by competitive ELISA method. The marketed drug Secukinumab is used as a positive control.**

Figure 15: detection of the activity of antibody 13E9 H3L2 competing with receptor IL17RA-His (biotin) for binding by competitive ELISA
25 **method. The marketed drug Secukinumab is used as a positive control.**

Figure 16: detection of the binding activity of antibodies 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3 to antigen IL17A-His by indirect ELISA method. The marketed drug Secukinumab is used as a positive control.

Figure 17: detection of the activity of antibodies 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3 competing with the receptor IL17A-His (biotin) for binding to antigen IL17A-His by competitive ELISA method. The marketed drug Secukinumab is used as a positive control.

Figure 18: effect of antibodies 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2 as well as 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3 on the secretion of cytokine IL-6 by mixed lymphocytes.

Figure 19: effect of the antibody drug 13E9 H3L2 on epidermal thickness of the C57BL/6 mouse model with psoriasis.

Notes on the preservation of biological materials:

hybridoma cell line LT006 (IL17A-13E9), which was deposited at China Center for Type Culture Collection (CCTCC) on September 12, 2017 with an accession number of CCTCC NO: C2017102, at Wuhan University, Wuhan, China, 430072.

hybridoma cell line LT007 (IL17A-2G2), which was deposited at China Center for Type Culture Collection (CCTCC) on September 12, 2017 with an accession number of CCTCC NO: C2017165, at Wuhan University, Wuhan, China, 430072.

DETAILED DESCRIPTION

The embodiments of the present invention will be described in detail below with reference to the examples. Those skilled in the art will understand that the following examples are only used to illustrate the

present invention, and should not be regarded as limiting the scope of the present invention. An example is performed according to the technologies or conditions described in the literature in the art (e.g., see, *Guide to Molecular Cloning Experiments*, authored by J. Sambrook et al.,
5 and translated by Huang Peitang et al., third edition, Science Press) or according to the product manual if specific technologies or conditions are not specified therein. Reagents or instruments used are all commercially available conventional products if the manufacturers thereof are not specified.

10 In the following examples, the BALB/C mice used were purchased from Guangdong Medical Laboratory Animal Center; the C57BL/6 mice used were from Nanjing Galaxy Biopharma Co., Ltd.; the MRC5 cells used were from Shanghai Fudan IBS Cell Center; the monoclonal antibody
15 Secukinumab (Cosentyx®) used was purchased from Novartis Corporation.

Example 1: Preparation of anti-IL-17A antibodies 13E9 and 2G2

1. Preparation of the hybridoma cell lines LT006 and LT007

20 Antigen IL17A (24-155)-his used to generate the anti-IL-17A antibody is the fusion protein of human IL-17A (GenBank ID: Q16552) mature peptide and the His tag. Spleen cells from the immunized BALB/C mice (purchased from Guangdong Medical Laboratory Animal Center) and mouse myeloma cells were fused into hybridoma cells, and established
25 methods (e.g., “Monoclonal Antibody Production”, in *Basic Methods in Antibody Production and Characterization*, Eds. G.C. Howard and D.R.

Bethell, Boca Raton: CRC Press, 2000) were referred to for specific operations.

The fusion protein IL17A-His was enzyme-digested with TEV protease and purified by column to obtain IL-17A (24-155) protein. Indirect
5 ELISA screening was performed in coated microplates with the IL-17A (24-155) protein as the antigen to obtain hybridoma cells that secreted new antibodies specifically bound to IL-17A (24-155).

Hybridoma cells obtained by indirect ELISA screening were screened by competitive ELISA to obtain hybridoma cell lines capable of
10 secreting a monoclonal antibody that competed with the receptor IL-17RA (CD217, NCBI Gene ID: NP_055154.3) for binding to IL-17A, and two stable hybridoma cell lines were obtained by limited dilution.

Hybridoma cell line LT006 (IL17A-13E9) was deposited at China Center for Type Culture Collection (CCTCC) on Saturday, September
15 12, 2015 with an accession number of CCTCC NO: C2017102, at Wuhan University, Wuhan, China, 430072. The monoclonal antibody secreted by hybridoma cell line LT006 was named as 13E9.

Hybridoma cell line LT007 (IL17A-2G2) was deposited at China Center for Type Culture Collection (CCTCC) on September 12, 2017 with an
20 accession number of CCTCC NO: C2017165, at Wuhan University, Wuhan, China, 430072. The monoclonal antibody secreted by hybridoma cell line LT007 was named as 2G2.

2. Preparation of anti-IL-17A antibody 13E9

The LT006 cell line prepared above was cultured (1×10^5 cells per well)
25 with hybridoma serum-free medium (containing 1% Penicillin-Streptomycin and 4% Glutamax, cultured in a cell incubator at 37°C with 5% CO₂), and the cell culture supernatant was collected

when the survival rate was around 20% after 7 days of culturing, which was then subjected to high-speed centrifugation, vacuum filtration through a microporous membrane, and purification through a HiTrap protein A HP column to obtain antibody 13E9. The purified 13E9 samples were detected by SDS-PAGE electrophoresis, and the results are shown in Figure 1.

3. Preparation of anti-IL-17A antibody 2G2

The LT007 cell line prepared above was cultured (1×10^5 cells per well) with hybridoma serum-free medium (containing 1%

Penicillin-Streptomycin and 4% Glutamax, cultured in a cell incubator at 37°C with 5% CO₂), and the cell culture supernatant was collected when the survival rate was around 20% after 7 days of culturing, which was then subjected to high-speed centrifugation, vacuum filtration through a microporous membrane, and purification through a HiTrap protein A HP column to obtain antibody 2G2. The purified 2G2 samples were detected by SDS-PAGE electrophoresis, and the results are shown in Figure 2.

Example 2: Sequence analysis of antibody 13E9

LT006 cells were cultured according to the method in step 2 of Example 1. Using the cell/bacterial total RNA extraction kit (Tiangen, article number DP430), mRNA was extracted from the cultured LT006 cells according to the method in the kit manual.

cDNA was synthesized according to the kit manual of the Invitrogen SuperScript® III First-Strand Synthesis System for RT-PCR, and amplified by PCR.

The PCR-amplified products were directly TA cloned, and the kit manual of the pEASY-T1 Cloning Kit (Transgen CT101) was referred to for specific operations.

The TA-cloned products were directly sequenced, and the sequencing results are as follows:

nucleotide sequence of the heavy chain variable region: (360 bp)

GAAGTAAAGCTGCAGGAGTCGGGACCTGGCCTGGTGAAACCT
TCTCAGTCTCTGTCCCTCACCTGCACTGTCACTAGCTACTCAT
TCACCAGTGATTATGCCTGGAGCTGGATCCGGCAGTTTCCAG
10 GAATCAAACCTGGAGTGGATGGGCTACATAACCTACAGTGGTG
TCACTAGCTACAACCCCTCTCTCAAAAGTCGAATCTCTATCAC
TCGAGACACATCCAAGAACCAGTTCTTCCTACAGTTGAATTCT
GTGACTACTGAGGACACGGCCACATATTACTGTGCAAGGGCA
GACTATGATAGCTACTATACTATGGACTACTGGGGTCAAGGAA
15 CCTCAGTCACCGTCTCCTCA (SEQ ID NO: 1)

its encoded amino acid sequence: (120 aa)

EVKLQESGPGLVKPSQSLSLTCTVTSYSFTSDYAWSWIRQFPGIK
LEWMGYITYSGVTSYNPSLKSRISITRDTSKNQFFLQLNSVTTEDT
ATYYCARADYDSYYTMDYWGQGTSVTVSS (SEQ ID NO: 2)

nucleotide sequence of the light chain variable region: (333 bp)

GACATCCAGCTGACTCAGTCTCCACTCTCCCTGCCTGTCAGTC
TTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCCT
TGTACACAGTAATGGAAACACCTATTTACATTGGTACCTGCAG
AAGCCAGGCCAGTCTCCAAGGCTCCTGATCTACAAAGTTTCCA
25 ACCGATTTTCTGGGGTCCCAGACAGGTTTCAGTGGCAGTGGATC
AGGGACAGATTTCACTCAAGATCAGCAGAGTGGAGGCTGA

**GGATCTGGGAGTTTATTTCTGCTCTCAAAGTACACATTTTGG
ACGTTTCGGTGGAGGCACCAAGCTGGAAATAAAA (SEQ ID NO: 3)**

its encoded amino acid sequence: (111 aa)

**DIQLTQSPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKP
5 GQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVY
FCSQSTHFWTFGGG**TKLEIK** (SEQ ID NO: 4)**

The underlined regions are the CDR regions.

Example 3: Design, preparation and detection of anti-IL-17A

10 humanized antibodies 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2

1. Design of the light chain and heavy chain sequences of anti-IL-17A humanized antibodies 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2

**Based on the three-dimensional crystal structure of the IL-17A protein
(EMBO J. (2001) 20 p: 5332-41) and the sequence of the antibody 13E9
15 obtained in Example 2, the antibody model was simulated by computer,
and mutations were designed according to the model to obtain the
variable region sequences of antibodies 13E9 H1L1, 13E9 H2L2 and
13E9 H3L2 (antibody constant region sequences are from the NCBI
database, in which the heavy chain constant region is Ig gamma-1 chain
20 C region, ACCESSION: P01857, and the light chain constant region is
Ig kappa chain C region, ACCESSION: P01834).**

The designed variable region sequences are as follows:

**(1) heavy chain and light chain sequences of humanized monoclonal
antibody 13E9 H1L1**

25 nucleotide sequence of the heavy chain variable region: (360 bp)

**GATGTGCAGCTGCAGGAAAGCGGACCAGGACTGGTGAAGCCT
AGCCAGACCCTGAGCCTGACTTGCACCGTGTCCAGCTACAGC**

5 TTCACCAGCGACTACGCTTGGTCTTGGATCAGACAGTTCCCAG
GAATTGGCCTCGAGTGGATGGGCTACATCACCTACAGCGGCG
TGACCAGCTACAACCCCAGCCTGAAGAGCAGGATCACCATCA
GCCGGGACACCAGCAAGAACCAGTTCTTCCTGCAGCTGAACA
GCGTGACAGCAGCCGATACCGCAGTGTACTATTGCGCCAGGG
CCGACTACGACAGCTACTACACCATGGACTATTGGGGCCAGG
GAACCAGCGTGACAGTGTCTAGC (SEQ ID NO: 5)

its encoded amino acid sequence: (120 aa)

10 DVQLQESGPGLVKPSQTL~~SLTCTVSSYSFTSDYA~~WSWIRQFPGIG
LEWMGYITYSGVTSYNPSLKSRITISRDTSKNQFFLQLNSVTAADT
AVYYC~~ARADYDSYYTMDY~~WGQGTSVTVSS (SEQ ID NO: 6)

The underlined regions are the CDR regions.

15 nucleotide sequence of the light chain variable region: (333 bp)
GATGTCGTGATGACCCAGACCCCTCTGTCTCTGCCAGTGACAC
TGGGACAGCAGGCTAGCATCTCTTGCAGAAGCAGCCAGAGCC
TGGTGCACAGCAACGGCAACACCTACCTGCATTGGTACCTGC
AGAAGCCAGGCCAGTCTCCTAGACTGCTGATCTACAAGGTGT
20 CCAACCGGTTCAGCGGCGTGCCAGATAGATTCAGCGGAAGCG
GAAGCGGCACCGACTTCACCCTGAAGATCAGCAGAGTGGAGG
CCGAGGATCTGGGAGTGTACTTCTGCAGCCAGAGCACCCACT
TTTGGACCTTCGGCGGAGGCACCAAGCTGGAGATCAAG (SEQ
ID NO: 7)

25 its encoded amino acid sequence: (111 aa)

DVVMTQTPLSLPVTLGQQASISCRSSQSLVHSNGNTYLHWYLQK
PGQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGV
YFCSQSTHFWTFGGGTKLEIK (SEQ ID NO: 8)

The underlined regions are the CDR regions.

5

(2) heavy chain and light chain sequences of humanized monoclonal
antibody 13E9 H2L2

nucleotide sequence of the heavy chain variable region: (360 bp)

GATGTGCAGCTGCAGGAAAGCGGACCAGGACTGGTGAAGCCT
10 AGCCAGACCCTGAGCCTGACTTGCACCGTGTCCAGCTACAGC
TTCACCAGCGACTACGCTTGGTCTTGGATCAGACAGCCACCA
GGAAAGGGACTCGAGTGGATCGGCTACATCACCTACAGCGGC
GTGACCAGCTACAACCCAGCCTGAAGAGCAGGATCACCATC
AGCCGGGACACCAGCAAGAACCAGTTCTTCCTGCAGCTGTCT
15 AGCGTGACAGCAGCCGATACCGCAGTGTACTATTGCGCCAGG
GCCGACTACGACAGCTACTACACCATGGACTATTGGGGCCAG
GGAACCAGCGTGACAGTGTCTAGC (SEQ ID NO: 9)

its encoded amino acid sequence: (120 aa)

DVQLQESGPGLVKPSQTLSTCTVSSYSFTSDYAWSWIRQPPGKG
20 LEWIGYITYSGVTSYNPSLKSRLTISRDTSKNQFFLQLSSVTAADTA
VYYCARADYDSYYTMDYWGQGTSTVTVSS (SEQ ID NO: 10)

The underlined regions are the CDR regions.

nucleotide sequence of the light chain variable region: (333 bp)

25 GATGTCGTGATGACCCAGACCCCTCTGTCTCTGCCAGTGACAC
TGGGACAGCCAGCTAGCATCTCTTGCAGAAGCAGCCAGAGCC
TGGTGCACAGCAACGGCAACACCTACCTGCATTGGTACCTGC

AGAAGCCAGGCCAGTCTCCTAGACTGCTGATCTACAAGGTGT
CCAACCGGTTTCAGCGGCGTGCCAGATAGATTCAGCGGAAGCG
GAAGCGGCACCGACTTCACCCTGAAGATCAGCAGAGTGGAGG
CCGAGGATCTGGGAGTGTACTACTGCAGCCAGAGCACCCACT
5 TTTGGACCTTCGGCGGAGGCACCAAGCTGGAGATCAAG (SEQ
ID NO: 11)

its encoded amino acid sequence: (111 aa)

DVVMTQTPLSLPVTLGQPASISCRSSQSLVHSNGNTYLHWYLQKP
GQSPRLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLG
10 YCSQSTHFWTFGGGGTKLEIK (SEQ ID NO: 12)

The underlined regions are the CDR regions.

(3) heavy chain and light chain sequences of humanized monoclonal
antibody 13E9 H3L2

15 nucleotide sequence of the heavy chain variable region: (360 bp)
GATGTGCAGCTGCAGGAAAGCGGACCAGGACTGGTGAAGCCT
AGCCAGACCCTGAGCCTGACTTGCACCGTGTCCAGCTACAGC
TTCACCAGCGACTACGCTTGGTCTTGGATCAGACAGCCACCA
GGAAAGGGACTCGAGTGGATCGGCTACATCACCTACAGCGGC
20 GTGACCAGCTACAACCCTAGCCTGAAGAGCCGCGTGACCATT
AGCGTGGACACCAGCAAGAACCAGTTCTCCCTGAAGCTGAGC
AGCGTGACAGCCGCCGATACAGCAGTGTACTATTGCGCCCGG
GCCGATTACGACAGCTACTACACCATGGACTATTGGGGGCCAG
GGAACCAGCGTGACAGTGTCTAGC (SEQ ID NO: 13)

25 its encoded amino acid sequence: (120 aa)

**DVQLQESGPGLVKPSQTLSTCTVSSYSFTSDYAWSWIRQPPGKG
LEWIGYITYSGVTSYNPSLKSRTISVDTSKNQFSLKLSSVTAADT
AVYYCARADYSYYTMDYWGQGTSVTVSS (SEQ ID NO: 14)**

The underlined regions are the CDR regions.

5

The nucleotide sequence of the light chain variable region is the same as the nucleotide sequence of the light chain variable region of 13E9 H2L2, as shown in SEQ ID NO: 11.

10 **Its encoded amino acid sequence is also the same as the amino acid sequence of the light chain variable region of 13E9 H2L2, as shown in SEQ ID NO: 12.**

2. Preparation of humanized antibodies 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2

15 **Heavy chain constant regions all use Ig gamma-1 chain C region, ACCESSION: P01857; the light chain constant regions use Ig kappa chain C region, ACCESSION: P01834.**

20 **Heavy chain cDNA and light chain cDNA of 13E9 H1L1, heavy chain cDNA and light chain cDNA of 13E9 H2L2, and heavy chain cDNA and light chain cDNA of 13E9 H3L2 were cloned into pUC57simple (provided by Genscript) vectors, respectively, to obtain pUC57simple-13E9H1, pUC57simple-13E9L1, pUC57simple-13E9H2, pUC57simple-13E9L2 and pUC57simple-13E9H3, respectively, and fragments containing corresponding heavy chains and fragments containing corresponding light chains were subcloned into pcDNA3.1**
25 **vectors, respectively, to obtain recombinant plasmids pcDNA3.1-13E9H1, pcDNA3.1-13E9L1, pcDNA3.1-13E9H2, pcDNA3.1-13E9L2,**

pcDNA3.1-13E9H3 and pcDNA3.1-13E9L2. Then, the corresponding light chain recombinant plasmids and heavy chain recombinant plasmids (pcDNA3.1-13E9H1 and pcDNA3.1-13E9L1; pcDNA3.1-13E9H2 and pcDNA3.1-13E9L2; pcDNA3.1-13E9H3 and pcDNA3.1-13E9L2) were co-transfected into 293F cells, the cell culture was collected and purified to obtain humanized antibodies 13E9 H1L1, 13E9 H2L2, and 13E9 H3L2 respectively. The purified 13E9 H3L2 sample was detected by SDS-PAGE electrophoresis, and the results are shown in Figure 3.

Example 4: Sequence analysis of antibody 2G2

LT007 cells were cultured according to the method in step 3 of Example 1.

Using the cell/bacterial total RNA extraction kit (Tiangen, article number DP430), mRNA was extracted from the cultured LT007 cells according to the method in the kit manual.

cDNA was synthesized according to the kit manual of the Invitrogen SuperScript® III First-Strand Synthesis System for RT-PCR, and amplified by PCR.

The PCR-amplified products were directly TA cloned, and the kit manual of the pEASY-T1 Cloning Kit (Transgen CT101) was referred to for specific operations.

The TA-cloned products were directly sequenced, and the sequencing results are as follows:

nucleotide sequence of the heavy chain variable region: (351 bp)

GAGGTTTCAGCTGGAGCAGTCTGGTTCTGAACTGAGGAGTCCT
GGATCTTCAGTAAAGCTTTCATGCAAGGATTTTGATTCAGAAG

TCTTCCCTATTGCTGATATGAGTTGGGTTAGGCAGAAGCCTGG
GCATGGATTTGAATGGATTGGAGACATACTCCCAAGTTTTGGT
AGAACAATCTATGGAGAGAAGTTTGAGGACAAAGCCAAAGTG
GATGCAGACACAGTGTCCAACACAGCCTACTTGGA ACTCAAC
5 AGTCTGACATCTGAGGACTCTGCTATCTACTACTGTGCAAGGG
GTA ACTACGGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCA
CTGTCTCTGCA (SEQ ID NO: 15)

its encoded amino acid sequence: (117 aa)

EVQLEQSGSEL RSPGSSVKLSCKDFD SEVFPIADMSWVRQKPGHG
10 FEWIGDIL PSFGRTIYGEKFEDKAKVDADTVSNTAYLELNSLTSED
SAIYYC ARGNYGFAYWGQGTLVTVSA (SEQ ID NO: 16)

The underlined regions are the CDR regions.

nucleotide sequence of the light chain variable region: (336 bp)

15 GATGTTTTGATGACCCAAACTCCACTCACTTTGTCGGTTATCA
TTGGACAACCAGCCTCCATCTCTTGCAAGCCAAGTCAGAGCCT
CTTAAATAGTGATGGAAAGACATATTTGAATTGGTTGTTGCAG
AGGCCAGGCCAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTA
AACTGGACTCTGGAGTCCCTGACAGGTTCACTGGCAGTGGAT
20 CAGGGACAGATTTCACTGAAAATCAGCAGAGTGGAGGCTG
AGGATTTGGGAGTTTATTATTGCTGGCAAGGTTACATTTTCC
TCAGACGTTTCGGTGGAGGCACAAAGTTGGAAATAAAA (SEQ ID
NO: 17)

its encoded amino acid sequence: (112 aa)

25 DVLMTQTPLTLSVIIGQPASISCKPS QSLLNSDGKTYLNWLLQRP
GQSPKRLIY LVSKLDSGVPDRFTGSGSGTDFTLKISRVEAEDLG
YYC WQGSHPQTFGGGTKLEIK (SEQ ID NO: 18)

The underlined regions are the CDR regions.

Example 5: Design, preparation and detection of anti-IL-17A
humanized antibodies 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3

5 (1) heavy chain and light chain sequences of humanized monoclonal antibody 2G2 H1L1

nucleotide sequence of the heavy chain variable region: (348 bp)

GTGCAGCTGGTGCAGAGCGGAAGCGAACTGAGAAAGCCAGGC
TCCAGCGTGAAGCTGTCTTGCAAGGACTTCGACAGCGAGGTG
10 TTCCCCATCGCCGATATGTCTTGGGTCCGACAGGCTCCAGGC
CAGGGATTCGAGTGGATCGGTGACATTCTGCCCAGCTTCGGA
AGAACCAACTACGCCCAGAAGTTCGAGGGCAAGGCCAAGGTG
GACGCAGACAAGAGCACCAACACCGCCTACCTGGAGCTGAAC
AGCCTGAGAAGCGAGGACACCGCCATCTACTATTGCGCCAGG
15 GGCAACTACGGATTCGCCTATTGGGGCCAGGGAACACTGGTG
ACAGTGTCCGCC (SEQ ID NO: 19)

its encoded amino acid sequence: (116 aa)

VQLVQSGSELRKPGSSVKLSCKDFDSEVFPIADMSWVRQAPGQG
FEWIGDILPSFGRTNYAQKFEGKAKVDADKSTNTAYLELNSLRSE
20 DTAIYYCARGNYGFAYWGQGTLVTVSA (SEQ ID NO: 20)

The underlined regions are the CDR regions.

nucleotide sequence of the light chain variable region: (336 bp)

GATGTCGTGATGACCCAGACCCCTCTGTCTCTGAGCGTGACA
25 CTGGGACAGCCAGCTAGCATCAGCTGCAGAAGCAGCCAGAGC
CTGCTGAACAGCGACGGCAAGACCTACCTGAATTGGCTGCTG
CAGAGACCAGGCCAGTCTCCTAGAAGGCTGATCTACCTGGTG

TCCAAGCTGGACAGCGGCGTGCCAGATAGATTTCAGCGGAAGC
GGAAGCGGCACCGACTTCACCCTGAAGATCAGCAGAGTGGAG
GCCGAGGATCTGGGAGTGTACTACTGTTGGCAGGGCAGCCAC
TTCCCTCAGACATTCGGCGGCGGCACAAAGCTGGAGATCAAG

5 (SEQ ID NO: 21)

its encoded amino acid sequence: (112 aa)

DVVMQTPLSLSVTLGQPASISCRSSQSLNSDGKTYLNWLLQRP
GQSPRRLIYLYSKLDSGVPDRFSGSGSGTDFTLKISRVEAEDLGVY
YCWQGSHFPQTFGGGTKLEIK (SEQ ID NO: 22)

10 The underlined regions are the CDR regions.

(2) heavy chain and light chain sequences of humanized monoclonal
antibody 2G2 H2L2

nucleotide sequence of the heavy chain variable region: (348 bp)

15 GTGCAGCTGGTGCAGAGCGGAGCAGAAGTGAAGAAGCCAGG
CTCCAGCGTGAAGCTGTCTTGCAAGGACTTCGACAGCGAGGT
GTTCCCCATCGCCGATATGTCTTGGGTCCGACAGGCTCCAGG
CCAGGGATTCGAGTGGATCGGTGACATTCTGCCCAGCTTCGG
GAGAACCAATTACGCCCAGAAGTTCCAGGGCAGAGTGACCGT
20 GACCGCAGACAAGAGCACCAACACCGCCTACCTGGAGCTGAA
CAGCCTGAGGAGCGAGGATACCGCCGTGTACTATTGCGCCAG
GGGCAACTACGGCTTCGCCTATTGGGGACAGGGAACACTGGT
GACAGTGTCCGCC (SEQ ID NO: 23)

its encoded amino acid sequence: (116 aa)

25 VQLVQSGAEVKKPGSSVKLSCKDFDSEVFPIADMSWVRQAPGQG
FEWIGDILPSEGRTNYAQKFQGRVTVTADKSTNTAYLELNSLRSE
DTAVYYCARGNYGFAYWGQGLVTVSA (SEQ ID NO: 24)

The underlined regions are the CDR regions.

nucleotide sequence of the light chain variable region: (336 bp)

GATGTCGTGATGACCCAGACCCCTCTGTCTCTGAGCGTGACA
5 **CTGGGACAGCCAGCTAGCATCAGCTGCAGAAGCAGCCAGAGC**
CTGCTGAACAGCGACGGCAAGACCTACCTGAATTGGCTGCTG
CAGAGACCAGGCCAGTCTCCTAGAAGGCTGATCTACCTGGTG
TCCAACCTGGACAGCGGCGTGCCAGATAGATTCAGCGGAAGC
GGAAGCGGCACCGACTTCACCCTGAAGATCAGCAGAGTGGAA
10 **GCCGAGGACGTGGGAGTGTACTACTGTTGGCAGGGCAGCCAC**
TTCCCTCAGACATTCGGCGGGCGGCACAAAGCTGGAGATCAAG
(SEQ ID NO: 25)

its encoded amino acid sequence: (112 aa)

DVVMQTPLSLSVTLGQPASISCRSSQSLLNSDGKTYLNWLLQRP
15 **GQSPRRLIYLVSNLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVY**
YCWQGSHFPQTFGGGTKLEIK (SEQ ID NO: 26)

The underlined regions are the CDR regions.

(3) heavy chain and light chain sequences of humanized monoclonal
20 **antibody 2G2 H3L3**

nucleotide sequence of the heavy chain variable region: (348 bp)

GTGCAGCTGGTGCAGAGCGGAGCAGAAGTGAAGAAGCCAGG
CAGCAGCGTGAAGGTGTCTTGCAAGGACTTCAGCAGCGAGGT
GTTCCCCATCGCCGATATGTCTTGGGTCCGACAGGCTCCAGG
25 **CCAGGGACTGGAGTGGATCGGTGACATTCTGCCCAGCTTCGG**
GAGAACCAATTACGCCCAGAAGTTCCAGGGCAGAGTGACCGT
GACCGCAGACAAGAGCACCAACACCGCCTACCTGGAGCTGTC

**TAGCCTGAGAAGCGAGGACACCGCCGTGTACTATTGCGCCAG
GGGCAACTACGGCTTCGCCTATTGGGGACAGGGAACACTGGT
GACAGTGTCCGCC (SEQ ID NO: 27)**

its encoded amino acid sequence: (116 aa)

**5 VQLVQSGAEVKKPGSSVKVSCKDFSESEVFPIADMSWVRQAPGQG
LEWIGDILPSFGRNTNYAQKFQGRVTVTADKSTNTAYLELSSLRSE
DTAVYYCARGNYGFAYWGQGLVTVSA (SEQ ID NO: 28)**

The underlined regions are the CDR regions.

10 nucleotide sequence of the light chain variable region: (336 bp)

**GATGTCGTGATGACCCAGACCCCTCTGTCTCTGAGCGTGACA
CTGGGACAGCCAGCTAGCATCAGCTGCAGAAGCAGCCAGAGC
CTGCTGAACAGCGACGGCAAGACCTACCTGAATTGGTTCCTG
CAGAGACCAGGCCAGTCTCCTAGAAGGCTGATCTACCTGGTG
15 TCCAACCTGGACAGCGGCGTGCCAGATAGATTCAGCGGAAGC
GGAAGCGGCACCGACTTCACCCTGAAGATCAGCAGAGTGGA
GCCGAGGACGTGGGAGTGTACTACTGTTGGCAGGGCAGCCAC
TTCCCTCAGACATTCGGCGGCGGCACAAAGCTGGAGATCAAG
(SEQ ID NO: 29)**

20 its encoded amino acid sequence: (112 aa)

**DVVMQTPLSLSVTLGQPASISCRSSQSLLNSDGKTYLNWFLQRP
GQSPRRLIYLVSNLDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVY
YCWQGSHFPQTFGGGTKLEIK (SEQ ID NO: 30)**

The underlined regions are the CDR regions.

25

**2. Preparation and SDS-PAGE electrophoresis detection of humanized
antibodies 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3**

Heavy chain constant regions used Ig gamma-1 chain C region, ACCESSION: P01857; the light chain constant regions used Ig kappa chain C region, ACCESSION: P01834.

Heavy chain cDNA and light chain cDNA of 2G2 H1L1, heavy chain
5 cDNA and light chain cDNA of 2G2 H2L2, and heavy chain cDNA and
light chain cDNA of 2G2 H3L3 were cloned into pUC57simple (provided
by Genscript) vectors, respectively, to obtain pUC57simple-2G2H1,
pUC57simple-2G2L1; pUC57simple-2G2H2, pUC57simple-2G2L2; and
pUC57simple-2G2H3, pUC57simple-2G2L3, respectively. Nucleotide
10 fragments containing corresponding heavy chains and nucleotide
fragments containing corresponding light chains were then subcloned
into pcDNA3.1 vectors, respectively, to obtain recombinant plasmids
pcDNA3.1-2G2H1, pcDNA3.1-2G2L1, pcDNA3.1-2G2H2,
pcDNA3.1-2G2L2, pcDNA3.1-2G2H3 and pcDNA3.1-2G2L3. Then, the
15 corresponding light chain recombinant plasmids and heavy chain
recombinant plasmids (pcDNA3.1-2G2H1 and pcDNA3.1-2G2L1;
pcDNA3.1-2G2H2 and pcDNA3.1-2G2L2; pcDNA3.1-2G2H3 and
pcDNA3.1-2G2L3) were co-transfected into 293F cells, the cell culture
was collected and purified to obtain humanized antibodies 2G2 H1L1,
20 2G2 H2L2 and 2G2 H3L3 respectively. The purified samples were
detected by SDS-PAGE electrophoresis.

**Example 6: Measurement of kinetic parameters for the binding of 13E9
H1L1, 13E9 H2L2 and 13E9 H3L2 to the antigen IL-17A (24-155)
25 protein**

The kinetic parameters for the binding of humanized antibodies 13E9
H1L1, 13E9 H2L2 and 13E9 H3L2 to the antigen IL-17A (24-155) were
measured using a Fortebio molecular interaction instrument.

The AR2G sensor was activated by EDC/NHS, and the antibody was fixed to the activated AR2G sensor by amine coupling, and the sensor was blocked with 1 M ethanolamine (pH 8.5). After the sensor was equilibrated in PBST for 300 s, the antibody fixed on the sensor binded to the antigen IL-17A (24-155) protein (same as the antigen used in Example 1), in which the antigen concentration was 6.25-400 nM (double gradient dilution) and the binding time was 420 s, and the antigen and the antibody were dissociated in PBST for 600 s.

The kinetic parameters of antibodies 13E9 H1L1, 13E9 H2L2, 13E9 H3L2 and Secukinumab are shown in Table 1, and detection results of the kinetic characteristic parameters are shown in Figure 4, Figure 5, Figure 6 and Figure 7, respectively.

Table 1: the kinetic parameters of 13E9 humanized antibody

Antibody	K_D (M)	K_{on} (1/Ms)	K_{on} error	K_{dis} (1/s)	K_{dis} error	R_{max} (nm)
13E9 H1L1	9.72E-10	1.34E+05	2.60E+03	1.31E-04	9.06E-06	0.0858-0.3326
13E9 H2L2	1.03E-09	9.86E+04	1.51E+03	1.01E-04	7.57E-06	0.0546-0.3049
13E9 H3L2	5.08E-10	2.90E+05	8.22E+03	1.47E-04	1.02E-05	0.1457-0.3546
Secukinumab	6.74E-10	9.28E+04	3.03E+03	6.26E-05	1.57E-05	0.0379-0.2064

K_D is affinity constant; K_{on} is binding rate of antigen and antibody; K_{dis} is dissociation rate of antigen and antibody; $K_D = K_{dis}/K_{on}$.

The results show that 13E9 H1L1, 13E9 H2L2, and 13E9 H3L2 all have good affinity to the antigen IL-17A (24-155), and the affinity is equivalent to that of the control antibody Secukinumab.

Example 7: Measurement of kinetic parameters for the binding of 2G2 H1L1, 2G2 H2L2, and 2G2 H3L3 to the antigen IL-17A (24-155)

The kinetic parameters for the binding of humanized antibodies 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3 to the antigen IL-17A (24-155) were measured using a Fortebio molecular interaction instrument.

The AR2G sensor was activated by EDC/NHS, and the antibody was fixed to the activated AR2G sensor by amine coupling, and the sensor was blocked with 1 M ethanolamine (pH 8.5). After the sensor is equilibrated in PBST for 300 s, the antibody fixed on the sensor bound to the antigen IL-17A (24-155), in which the antigen concentration was 6.25-400 nM (double gradient dilution) and the binding time was 420 s, and the antigen and the antibody were dissociated in PBST for 600 s.

The kinetic parameters of antibodies 2G2 H1L1, 2G2 H2L2, 2G2 H3L3, and Secukinumab are shown in Table 2, and detection results of the kinetic characteristic parameters are shown in Figure 8, Figure 9, Figure 10, and Figure 11, respectively.

Table 2: the kinetic parameters of 2G2 H1L1, 2G2 H2L2, 2G2 H3L3 and Secukinumab

Antibody	K_D (M)	K_{on} (1/Ms)	Kon error	K_{dis} (1/s)	Kdis error	Rmax(nm)
2G2 H1L1	1.53E-09	1.46E+05	3.15E+03	2.24E-04	9.67E-06	0.0035-0.2192
2G2 H2L2	8.43E-10	1.42E+05	2.18E+03	1.20E-04	6.73E-06	0.0444-0.2732
2G2 H3L3	1.54E-09	1.98E+05	5.30E+03	3.06E-04	1.08E-05	0.011-0.2109
Secukinumab	1.08E-09	1.33E+05	2.38E+03	1.43E-04	8.09E-06	0.0118-0.2584

K_D is affinity constant; K_{on} is binding rate of antigen and antibody; K_{dis} is dissociation rate of antigen and antibody; $K_D = K_{dis}/K_{on}$.

The results show that compared with the control antibody Secukinumab, 2G2 H2L2 has a higher affinity to the antigen IL-17A (24-155); the affinity of 2G2 H1L1 and 2G2 H3L3 is equivalent to that of the control antibody Secukinumab.

5

Example 8: Detection of the binding activity of the antibodies 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2 to the antigen with an ELISA method

1. The binding activity of the antibodies 13E9 H1L1, 13E9 H2L2 and
10 13E9 H3L2 to the antigen IL17A-His was detected with an indirect ELISA and compared with the marketed drug Secukinumab for the same target.

IL17A-His can be prepared by referring to the published sequences and conventional technical means in the art, or referring to the following
15 steps:

Preparation of IL17A-His: the full-length protein sequence of human IL-17A was found in the NCBI protein database, and fused with His*6 purification tag. Genscript in Nanjing was entrusted to synthesize the nucleic acid encoding the fusion protein, and by referring to the
20 standard technologies introduced in the *Guide to Molecular Cloning Experiments (Second Edition)* and using standard molecular cloning technologies such as PCR, enzyme digestion, gel recovery, ligation transformation, colony PCR or enzyme digestion identification, the target gene was subcloned into mammalian cell expression vectors, and
25 the target gene with the recombinant expression vectors was further sequenced and analyzed. After the sequence was verified to be correct, a medium and large amount of endotoxin-free expression plasmids were

prepared, and transiently transfected HEK293 cells for protein expression. After 7 days of culture, the cell culture fluid was collected and affinity purified using a Ni Sepharose column (GE), and the quality of the resulting protein samples was determined using SDS-PAGE and SEC-HPLC standard analysis techniques to be up to standard.

ELISA: IL17A-His was added to the microplate and incubated at 4 °C overnight; after blocking with 1% BSA in PBS at 37 °C for 2 h, antibodies were added respectively, and incubated at 37 °C for 30 min; and Goat Anti Human IgG (H + L)-HRP (Jackson, 109-035-088) was added and incubated at 37 °C for 30 min; and then the color reaction was performed with TMB (Neogen, 308177) for 5 min, and the absorbance at 450 nm was detected in a microplate reader. The obtained experimental data were analyzed and processed with SoftMax Pro 6.2.1 software, and the 4-parameter fitted curve was plotted for analysis with the antibody concentration as the abscissa and the absorbance value as the ordinate.

The experimental results are shown in Figure 12, Figure 13, and Table 3 and Table 4 below.

Table 3: detection results of the binding activity of 13E9 H1L1 and 13E9 H2L2 to the antigen IL17A-His

Antibody concentration/ gradient	Coating antigen: IL17A-His, 1 µg/mL					
	13E9 H1L1		13E9 H2L2		Secukinumab	
1 µg/mL	2.726	2.731	2.804	2.737	2.184	2.227
1:3	2.875	2.852	2.832	2.873	2.595	2.505
1:9	2.858	2.815	2.717	2.712	2.297	2.364
1:27	2.564	2.494	2.479	2.481	2.049	2.064
1:81	1.934	1.925	1.891	1.834	1.372	1.314

1:243	1.159	1.116	1.097	1.062	0.672	0.697
1:729	0.522	0.537	0.514	0.511	0.313	0.309
0	0.047	0.048	0.048	0.048	0.046	0.046
EC ₅₀ (nM)	0.044		0.048		0.082	

Table 4: detection results of the binding activity of 13E9 H3L2 to the antigen IL17A-His

Antibody concentration/ gradient	Coating antigen: IL17A-His: 1 µg/mL			
	13E9 H3L2		Secukinumab	
0.5 µg/mL	3.074	3.068	2.929	2.929
1:3	3.175	3.026	2.924	2.885
1:9	2.944	2.895	2.660	2.666
1:27	2.472	2.393	1.947	1.862
1:81	1.610	1.617	1.140	1.092
1:243	0.786	0.783	0.521	0.522
1:729	0.343	0.348	0.252	0.253
0	0.091	0.084	0.087	0.087
EC ₅₀ (nM)	0.043		0.078	

The experimental results show that the antibodies 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2 all can effectively bind to the antigen IL17A-His, and their binding efficiency is dose-dependent. Under the same experimental conditions, the binding EC₅₀ of 13E9 H1L1 is 0.044 nM, the binding EC₅₀ of 13E9 H2L2 is 0.048 nM, and the EC₅₀ of the marketed drug Secukinumab for the same target is 0.082 nM (Figure 12, Table 3); under the same experimental conditions, the binding EC₅₀ of 13E9 H3L2 is 0.043 nM, and the EC₅₀ of the marketed drug Secukinumab for the same target is 0.078 nM (Figure 13, Table 4).

The above experimental results show that under the same experimental conditions, the EC₅₀ values of 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2 are all smaller than those of the positive control drug Secukinumab for

the same target, indicating that the binding activity of 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2 to IL17A-His is better than that of the marketed control drug Secukinumab for the same target.

- 5 **2. The activity of antibodies 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2 blocking the binding of IL17RA-His (biotin) to the antigen IL17A-His is detected with a competitive ELISA.**

IL17RA-His (biotin) can be prepared by referring to the published sequences and conventional technical means in the art, or referring to
10 **the following steps:**

Preparation of IL17RA-His (biotin): the extracellular domain sequence of human IL-17RA was found in the NCBI protein database, and fused with His*6 purification tag. Genscript in Nanjing was entrusted to synthesize the nucleic acid encoding the fusion protein, and by referring
15 **to the standard technologies introduced in the *Guide to Molecular Cloning Experiments (Second Edition)* and using standard molecular cloning technologies such as PCR, enzyme digestion, gel recovery, ligation transformation, colony PCR or enzyme digestion identification, the target gene was subcloned into mammalian cell expression vectors,**
20 **and the target gene with the recombinant expression vectors was further sequenced and analyzed. After the sequence was verified to be correct, a medium and large amount of endotoxin-free expression plasmids were prepared, and transiently transfected HEK293 cells for protein expression. After 7 days of culture, the cell culture was collected and**
25 **affinity purified using a Ni Sepharose column (GE), and the quality of the resulting protein samples was determined using SDS-PAGE and SEC-HPLC standard analysis techniques to be up to standard. After the quality determination was complete, the biotinylated human**

IL-17RA-His protein samples were labeled and obtained with the commercial kit EZ-Link®Sulfo-NHS-LC-Biotinylation of Thermo scientific, and the specific preparation method was performed according to the manual of the kit.

5 **ELISA: IL17A-His** was added to the microplate and incubated at 4°C overnight; after blocking with 1% BSA in PBST at 37°C for 2 h, antibodies are added respectively, and the antigen and the antibody reacted at room temperature for 10 min; then the receptor IL17RA-His (biotin) was added which was mixed well with the antibody at a volume
10 **ratio of 1:1 and incubated at 37 °C for 30 min; and SA-HRP (KPL, 14-30-00) was added and incubated at 37 °C for 30 min; and then the color reaction was performed with TMB (Neogen, 308177) for 5 min, and the absorbance at 450 nm was detected in a microplate reader. The obtained experimental data were analyzed and processed with SoftMax**
15 **Pro 6.2.1 software, and the 4-parameter fitted curve was plotted for analysis with the antibody concentration as the abscissa and the absorbance value as the ordinate. The experimental results are shown in Figure 14, Figure 15, and Table 5 and Table 6 below.**

20 **Table 5: detection results of the activity of 13E9 H1L1and 13E9 H2L2 competing with the receptor IL17RA-His (biotin) for binding to the antigen IL17A-His**

Antibody concentration/ gradient	Antigen coating: IL17A-His (20150213) 0.5 µg/mL					
	13E9 H1L1		13E9 H2L2		Secukinumab	
10 µg/mL	0.273	0.249	0.274	0.290	0.402	0.392
1:3	0.299	0.220	0.313	0.339	0.469	0.502
1:9	0.418	0.379	0.431	0.379	0.642	0.708

1:27	0.673	0.610	0.646	0.636	0.842	0.855
1:81	0.986	1.008	1.011	1.069	1.169	1.201
1:243	1.342	1.362	1.428	1.331	1.418	1.468
1:729	1.363	1.375	1.447	1.519	1.467	1.657
0	1.495	1.406	1.429	1.561	1.610	1.495
Receptor	IL17RA-His(bio) 0.1 µg/mL					
EC ₅₀ (nM)	1.437		1.281		1.807	

Table 6: detection results of the activity of 13E9 H3L2 competing with the receptor IL17RA-His (biotin) for binding to the antigen IL17A-His

Antibody concentration/ gradient	Antigen coating: IL17A-His 0.5 µg/mL			
	13E9 H3L2		Secukinumab	
10µg/mL	0.193	0.182	0.263	0.282
1:3	0.231	0.206	0.318	0.327
1:9	0.265	0.267	0.419	0.462
1:27	0.424	0.415	0.630	0.663
1:81	0.725	0.627	0.813	0.859
1:243	1.069	1.018	1.120	1.182
1:729	1.218	1.095	1.133	1.250
0	1.184	1.334	1.182	1.232
Receptor	IL17RA-His(bio) 0.1 µg/mL			
EC ₅₀ (nM)	0.805		1.580	

- 5 The experimental results show that the antibodies 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2 all can effectively block the binding of the receptor IL17RA-His (biotin) to the antigen IL17A-His, and the blocking efficiency is dose-dependent. Under the same experimental conditions, the EC₅₀ of 13E9 H1L1 competing with IL17AR-His (biotin) for binding to IL17A-His is 1.437 nM, the EC₅₀ of 13E9 H2L2 competing
- 10

with IL17AR-His (biotin) for binding to IL17A-His is 1.281 nM, and the EC₅₀ of the positive control drug Secukinumab for the same target competing with IL17AR-His (biotin) for binding to IL17A-His is 1.807 nM (Table 5, Figure 14); the EC₅₀ of 13E9 H3L2 competing with
5 IL17AR-His (biotin) for binding to IL17A-His is 0.805 nM, and the EC₅₀ of the marketed drug Secukinumab for the same target competing with IL17AR-His (biotin) for binding to IL17A-His is 1.580 nM (Table 6, Figure 15).

The above experimental results show that under the same experimental
10 conditions, the EC₅₀ values of 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2 competing with IL17AR-His (biotin) for binding to IL17A-His are all smaller than those of the marketed control drug Secukinumab for the same target, indicating the activity of 13E9 H1L1, 13E9 H2L2 and 13E9 H3L2 competing with IL17AR-His (biotin) for binding to IL17A-His is
15 better than that of the marketed drug Secukinumab for the same target.

Example 9: Detection of the binding activity of the antibodies 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3 to the antigen with an ELISA method

1. The binding activity of the antibodies 2G2 H1L1, 2G2 H2L2 and 2G2
20 H3L3 to the antigen IL17A-His is detected with an indirect ELISA.

Experimental steps: IL17A-His was added to the microplate and incubated at 4 °C overnight; after blocking with 1 % BSA in PBS at 37 °C for 2 h, antibodies were added respectively, and incubated at 37 °C for 30 min; and Goat Anti Human IgG (H + L)-HRP (Jackson,
25 109-035-088) was added and incubated at 37 °C for 30 min; and then the color reaction was performed with TMB (Neogen, 308177) for 5 min, and the absorbance at 450 nm was detected in a microplate reader. The

obtained experimental data were analyzed and processed with SoftMax Pro 6.2.1 software, and the 4-parameter fitted curve was plotted for analysis with the antibody concentration as the abscissa and the absorbance value as the ordinate.

- 5 The experimental results are shown in Figure 16 and Table 7 below. Wherein, the binding EC_{50} of 2G2 H1L1 is 0.177 nM, the binding EC_{50} of 2G2 H2L2 is 0.372 nM, and the binding EC_{50} of 2G2 H3L3 is 0.421 nM.

Table 7: detection results of the binding of 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3 to the antigen IL17A-His

10

Antibody concentration/ gradient	Antigen coating: IL17A-His, 1 μ g/mL							
	2G2 H1L1		2G2 H2L2		2G2 H3L3		Secukinumab	
1 μ g/mL	3.220	3.135	3.030	2.960	2.485	2.384	3.448	3.448
1:3	3.284	3.149	2.899	2.886	2.311	2.314	3.477	3.478
1:9	2.820	2.747	2.199	2.126	1.693	1.703	3.320	3.331
1:27	2.020	1.969	1.348	1.328	0.982	1.002	2.768	2.764
1:81	1.113	1.075	0.638	0.624	0.439	0.440	1.761	1.689
1:243	0.469	0.426	0.261	0.350	0.188	0.186	0.807	0.806
1:729	0.224	0.188	0.131	0.148	0.113	0.102	0.337	0.326
0	0.061	0.057	0.058	0.068	0.063	0.060	0.062	0.060
EC_{50} (nM)	0.177		0.372		0.421		0.090	

The experimental results show that the antibodies 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3 all can effectively bind to the antigen IL17A-His, and their binding efficiency is dose-dependent.

2. The activity of antibodies 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3 competing with the receptor IL17A-His (biotin) for binding to the antigen IL17A-His was detected by competitive ELISA.

Experimental steps: IL17A-His was added to the microplate and incubated at 4 °C overnight; after blocking with 1 % BSA in PBST at 37 °C for 2 h, antibodies were added respectively, and the antigen and the antibody reacted at room temperature for 10 min; then the receptor IL17RA-His (biotin) was added which is mixed well with the antibody at a volume ration of 1:1 and incubated at 37°C for 30 min; and SA-HRP (KPL, 14-30-00) was added and incubated at 37 °C for 30 min; and then the color reaction was performed with TMB (Neogen, 308177) for 5 min, and the absorbance at 450 nm was detected in a microplate reader. The obtained experimental data were analyzed and processed with SoftMax Pro 6.2.1 software, and the 4-parameter fitted curve was plotted for analysis with the antibody concentration as the abscissa and the absorbance value as the ordinate.

The experimental results are shown in Figure 17 and Table 8 below. Wherein, the blocking EC₅₀ of 2G2 H1L1 is 2.264 nM, the blocking EC₅₀ of 2G2 H2L2 is 5.408 nM, and the blocking EC₅₀ of 2G2 H3L3 is 5.911 nM (Figure 17, Table 8).

Table 8: detection results of the activity of 2G2 H1L1, 2G2 H2L2 and 2G2 H3L3 competing with the receptor IL17RA-His (biotin) for binding to the antigen IL17A-His

Antibody concentration/ gradient	Antigen coating: IL17A-His 0.5 µg/mL							
	2G2 H1L1		2G2 H2L2		2G2 H3L3		Secukinumab	
10 µg/mL	0.285	0.272	0.337	0.366	0.680	0.718	0.247	0.267

1:3	0.417	0.485	0.500	0.623	0.971	0.876	0.333	0.361
1:9	0.506	0.502	0.785	0.751	0.971	0.954	0.478	0.507
1:27	0.891	0.763	1.087	1.038	1.242	1.178	0.758	0.784
1:81	1.162	1.167	1.286	1.309	1.246	1.312	1.088	1.135
1:243	1.231	1.281	1.423	1.402	1.398	1.466	1.272	1.184
1:729	1.453	1.472	1.464	1.596	1.607	1.466	1.392	1.324
0	1.455	1.425	1.494	1.424	1.470	1.340	1.170	1.230
Receptor	IL17RA-His (biotin) :0.1 µg/mL							
EC ₅₀ (nM)	2.264		5.408		5.911		2.749	

The experimental results show that the antibodies 2G2 H1L1, 2G2 H2L2, and 2G2 H3L3 all can effectively block the binding of the receptor IL17RA-His (biotin) to the antigen IL17A-His, and the blocking efficiency is dose-dependent.

5

Example 10: Mixed human embryonic fibroblast reaction: secretion of cytokine IL-6

MRC 5 (purchased from the Cell Center of the Chinese Academy of Sciences) cells were plated into a 96-well plate with 5000 cells/well and cultured overnight. A mixture of IL-17 and antibody (hIgG as a control) incubated at 37 °C for 20 min was added to the MRC 5 cells and cultured for 48 h. After 48 h of culturing, the cell supernatant was collected, and the amount of IL-6 secreted was detected by an ELISA kit (purchased from Dakewe Corporation).

MRC 5 cells were mixed and cultured with 13E9 H1L1, 13E9 H2L2, 13E9 H3L2, 2G2 H1L1, 2G2 H2L2 or 2G2 H3L3 (1 nM, 10 nM, 100 nM) and Secukinumab (1 nM, 10 nM, 100 nM), respectively, and the detection results of secreted IL-6 are shown in Figure 18.

It can be seen from Figure 18 that 13E9 H1L1, 13E9 H2L2, 13E9 H3L2, and 2G2 H2L2 all can effectively reduce the IL-6 secretion of MRC 5 cells induced by IL-17, wherein:

5 the effect of 13E9 H1L1 antibody on inhibiting IL-6 secretion at a concentration of 100 nM is better than that of the control antibody Secukinumab at the same dose, and the inhibitory effect on IL-6 secretion at a concentration of 10 nM is equivalent to that of the control antibody Secukinumab at a concentration of 100 nM;

10 the effects of 13E9 H2L2 antibody on inhibiting IL-6 secretion at concentrations of 1 nM, 10 nM and 100 nM all are equivalent to that of the control antibody Secukinumab at the same dose;

15 the inhibitory effects of 13E9 H3L2 antibody on IL-6 secretion at concentrations of 10 nM and 100 nM all are better than that of the control antibody Secukinumab at the same dose, and the effect at a concentration of 1 nM is equivalent to that of the control antibody Secukinumab;

the effects of 2G2 H2L2 antibody on inhibiting IL-6 secretion at concentrations of 1 nM and 100 nM all are equivalent to that of the control antibody Secukinumab;

20 the effect of 2G2 H3L3 on inhibiting IL-6 secretion at a concentration of 1 nM is better than that of the control antibody Secukinumab at the same dose.

25 The above results show that in a mixed human embryonic fibroblast reaction in vitro, the biological activity of the antibodies of the present invention in blocking the IL-17A-mediated secretion of IL-6 is better than or at least equivalent to that of the marketed drug Secukinumab for the same target.

Example 11: Effect of the antibody drug 13E9 H3L2 on epidermal thickness of the C57BL/6 mouse psoriasis model

C57BL/6 mice were divided into 5 groups with 8 mice in each.

5 (1) Modeling:

normal group, C57BL/6 mice were injected intradermally with normal saline on the smooth back for 6 consecutive days from day 1 to day 6, 25 μ L/mouse;

10 the remaining groups of mice were injected intradermally with a recombinant human IL-17A from day 1 to day 4, 2 μ g/25 μ L/mouse, and were injected intradermally with the recombinant human IL-17A from day 5 to day 6, 5 μ g/25 μ L/mouse.

(2) Specific grouping and administration:

15 normal group: normal saline, administered at a dose of 0 mg/kg, 3 times a week for a total of 3 times;

model group: negative isotype control, administered at a dose of 50 mg/kg, 3 times a week for a total of 3 times;

Secukinumab group: Secukinumab, administered at a dose of 50 mg/kg, 3 times a week for a total of 3 times;

20 13E9 H3L2 high dose group: administered at a dose of 50 mg/kg, 3 times a week for a total of 3 times;

13E9 H3L2 low dose group: administered at a dose of 10 mg/kg, 3 times a week for a total of 3 times.

25 Each group was administered subcutaneously 3 times, namely 1 day before the modeling, modeling day 3 and modeling day 6, respectively.

On day 7, the skins of the injection sites on the backs of the mice were fixed to make pathological sections and measure the epidermal thickness.

The experimental results are shown in Figure 19.

- 5 The results show that statistically, the epidermal thickness of the Secukinumab group (50 mg/kg), the 13E9 H3L2 high dose group (50 mg/kg) and the 13E9 H3L2 low dose group (10 mg/kg) was significantly smaller than that of the model group ($P < 0.01$).

- 10 The results show that the antibody 13E9 H3L2 (50 mg/kg) shows statistically significant inhibitory effect on epidermal thickness in a C57BL/6 mouse psoriasis model, and has the same efficacy as Secukinumab (50 mg/kg); the inhibitory effect of 13E9 H3L2 (10 mg/kg) on epidermal thickness is also statistically significant.

15

- Although specific embodiments of the present invention have been described in detail, those skilled in the art will understand. Various modifications and substitutions can be made to those details according to all the teachings that have been disclosed, and these changes are all within the protection scope of the present invention. The full scope of the present invention is given by the appended claims and any equivalent thereof.
- 20

CLAIMS

1. A monoclonal antibody or an antigen binding fragment thereof, wherein,

5 a heavy chain variable region of the monoclonal antibody comprises: HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs: 31-33 respectively, or HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs: 37-39 respectively, and

a light chain variable region of the monoclonal antibody comprises:

10 LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 34-36 respectively, or LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 40-42 respectively.

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

the heavy chain variable region of the monoclonal antibody comprises HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs:

31-33 respectively, and the light chain variable region of the monoclonal antibody comprises LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 34-36 respectively;

or

the heavy chain variable region of the monoclonal antibody comprises HCDR1-HCDR3 with amino acid sequences set forth in SEQ ID NOs:

25 37-39 respectively, and the light chain variable region of the monoclonal antibody comprises LCDR1-LCDR3 with amino acid sequences set forth in SEQ ID NOs: 40-42 respectively.

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

**the amino acid sequence of the heavy chain variable region is selected
5 from SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 14,
SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 24 and SEQ ID NO: 28,
and**

**the amino acid sequence of the light chain variable region is selected
from SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 18,
10 SEQ ID NO: 22, SEQ ID NO: 26 and SEQ ID NO: 30.**

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

**the amino acid sequence of the heavy chain variable region is selected
15 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 light chain variable region is
selected from SEQ ID NO: 4, SEQ ID NO: 8, and SEQ ID NO: 12;
or**

**the amino acid sequence of the heavy chain variable region is selected
20 from SEQ ID NO: 16, SEQ ID NO: 20, SEQ ID NO: 24 and SEQ ID NO:
28, and the amino acid sequence of the light chain variable region is
selected from SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 26 and
SEQ ID NO: 30.**

25 5. The monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 4, wherein the heavy chain variable

region and the light chain variable region are selected from any one of (1)-(8) below:

(1) a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 2, and a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 4;

(2) a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 6, and a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 8;

(3) a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 10, and a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 12;

(4) a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 14, and a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 12;

(5) a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 16, and a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 18;

(6) a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 20, and a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 22;

(7) a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 24, and a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 26; and

5 **(8) a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 28, and a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 30.**

10 **6. The monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 5, wherein the monoclonal antibody or the antigen binding fragment thereof is selected from a Fab, a Fab', an F(ab')₂, an Fd, an Fv, a dAb, a complementarity determining region fragment, a single chain antibody, a humanized antibody, a chimeric**
15 **antibody, and a diabody.**

7. The monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 6, wherein the monoclonal antibody binds to IL-17A with an EC₅₀ of less than about 100 nM, such as less
20 **than about 10 nM, 5 nM, 4 nM, 3 nM, 2.5 nM, 2 nM, or less; preferably, the EC₅₀ is measured by competitive ELISA.**

8. The monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 7, wherein the monoclonal antibody
25 **comprises a non-CDR region derived from species other than murine, such as from a human antibody.**

9. The monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 8, wherein:

the monoclonal antibody is a monoclonal antibody produced by hybridoma cell line LT006, which was deposited at China Center for Type Culture Collection (CCTCC) with the accession number of CCTCC NO: C2017102; or

the monoclonal antibody is a monoclonal antibody produced by hybridoma cell line LT007, which was deposited at China Center for Type Culture Collection (CCTCC) with the accession number of CCTCC NO: C2017165.

10. An isolated nucleic acid molecule, comprising a nucleic acid encoding the heavy chain variable region and the light chain variable region of the monoclonal antibody according to any one of claims 1 to 9.

11. The isolated nucleic acid molecule according to claim 10, wherein the nucleic acid encoding the heavy chain variable region and the light chain variable region is selected from any one of (1)-(8) below:

(1) SEQ ID NO: 1, SEQ ID NO: 3;

(2) SEQ ID NO: 5, SEQ ID NO: 7;

(3) SEQ ID NO: 9, SEQ ID NO: 11;

(4) SEQ ID NO: 13, SEQ ID NO: 11;

(5) SEQ ID NO: 15, SEQ ID NO: 17;

(6) SEQ ID NO: 19, SEQ ID NO: 21;

(7) SEQ ID NO: 23, SEQ ID NO: 25; and

(8) SEQ ID NO: 27, SEQ ID NO: 29.

12. A recombinant vector, comprising the isolated nucleic acid molecule according to claim 10 or 11, wherein, preferably, the recombinant vector is a recombinant expression vector, such as a recombinant prokaryotic expression vector or a recombinant eukaryotic expression
5 vector.

13. A host cell, comprising the recombinant vector according to claim 12.

14. A method for preparing the monoclonal antibody or the antigen
10 binding fragment thereof according to any one of claims 1 to 9, comprising cultivating the host cell according to claim 13 under appropriate conditions and isolating the monoclonal antibody or the antigen binding fragment thereof from cell cultures.

15 15. A hybridoma cell line selected from:
hybridoma cell line LT006, which was deposited at China Center for Type Culture Collection (CCTCC) with the accession number of CCTCC NO: C2017102; and
hybridoma cell line LT007, which was deposited at China Center for
20 Type Culture Collection (CCTCC) with the accession number of CCTCC NO: C2017165.

16. A conjugate, comprising the monoclonal antibody or the antigen
binding fragment thereof according to any one of claims 1 to 9, and a
25 conjugated portion, wherein the conjugated portion is a detectable label; preferably, the detectable label is a radioactive isotope, a luminescent substance such as a fluorescent substance, a colored substance, or an enzyme.

17. A kit, comprising the monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 9, or the conjugate according to claim 16, wherein:

5 **preferably, the kit further comprises a second antibody which specifically recognizes the monoclonal antibody or the antigen binding fragment thereof; optionally, the second antibody further comprises a detectable label; preferably, the detectable label is a radioactive isotope, a luminescent substance such as a fluorescent substance, a colored**
10 **substance, or an enzyme.**

18. Use of the monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 9 or the conjugate according to claim 16 in preparing a kit for qualitative or quantitative detection of
15 **IL-17A.**

19. A pharmaceutical composition, comprising the monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 9 or the conjugate according to claim 16, wherein, optionally, the
20 **pharmaceutical composition further comprises pharmaceutically acceptable carriers or excipients.**

20. Use of the monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 9 or the conjugate according to claim 16 in preparing a drug for preventing and/or treating tumors or autoimmune diseases, or in preparing a drug for diagnosing autoimmune diseases, wherein, preferably, the autoimmune disease is
25 **selected from psoriasis, rheumatoid arthritis, psoriatic arthritis,**

ankylosing spondylitis, and systemic lupus erythematosus; preferably, the psoriasis is a moderate to severe plaque psoriasis.

21. Use of the monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 9 or the conjugate according to claim 16 in preparing:

**a drug for blocking the binding of IL-17A to IL17RA,
a drug for regulating (e.g., down-regulating) IL-17A activity or level, or
a drug for inhibiting IL-6 expression in cells.**

10

22. An in vivo or in vitro method, comprising administering to a cell or a subject in need an effective amount of the monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 9 or the conjugate according to claim 16, wherein the method is selected from:

**a method for blocking the binding of IL-17A to IL17RA,
a method for regulating (e.g., down-regulating) IL-17A activity or level,
or
a method for inhibiting IL-6 expression in fibroblasts.**

20

23. A method for treating and/or preventing tumors or autoimmune diseases, comprising administering to a subject in need an effective amount of the monoclonal antibody or the antigen binding fragment thereof according to any one of claims 1 to 9 or the conjugate according to claim 16, wherein, preferably, the autoimmune disease is selected from psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing

spondylitis, and systemic lupus erythematosus; preferably, the psoriasis is a moderate to severe plaque psoriasis.

24. The monoclonal antibody or the antigen binding fragment thereof
5 according to any one of claims 1 to 9, which is used to prevent and/or
treat tumors or autoimmune diseases, or to diagnose autoimmune
diseases, wherein, preferably, the autoimmune disease is selected from
psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing
spondylitis, and systemic lupus erythematosus; preferably, the psoriasis
10 is a moderate to severe plaque psoriasis.

25. The monoclonal antibody or the antigen binding fragment thereof
according to any one of claims 1 to 9, which is used for:
blocking the binding of IL-17A to IL-17RA,
15 regulating (e.g., down-regulating) IL-17A activity or level, or
inhibiting IL-6 expression in cells.

26. A method for diagnosing autoimmune diseases, comprising
administering to a sample to be tested (such as a tissue sample, a cell
20 sample, or a blood sample) or a subject in need an effective amount of
the monoclonal antibody or the antigen binding fragment thereof
according to any one of claims 1 to 9 or the conjugate according to claim
16, wherein, preferably, the autoimmune disease is selected from
psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing
25 spondylitis, and systemic lupus erythematosus; preferably, the psoriasis
is a moderate to severe plaque psoriasis;

preferably, if the measured IL-17A expression level is higher than that in healthy subjects and reaches a pathogenic level, the diagnosis result is positive; otherwise, the diagnosis result is negative.