

ANTIBODY OF HEPATITIS B VIRUS SURFACE ANTIGEN AND USE THEREOF

TECHNICAL FIELD

The present application belongs to the field of antibody technology, and relates to an antibody or an antigen-binding fragment thereof that binds to a hepatitis B virus surface antigen, and the use of the antibody or the antigen-binding fragment thereof in detecting hepatitis B virus or treating diseases associated with hepatitis B virus infection.

BACKGROUND ART

Hepatitis B virus (HBV) infection is prevalent worldwide, but the prevalence of HBV infection varies greatly in different regions. According to the World Health Organization, approximately 2 billion people worldwide have been infected with HBV, of which 240 million are chronic HBV carriers, and approximately 650,000 people die each year from liver failure, cirrhosis and hepatocellular carcinoma (HCC) caused by HBV infection. Globally, 30% and 45% of patients with cirrhosis and HCC are caused by HBV infection, respectively.

Although hepatitis B patients can reduce the viral load in their bodies and delay the progression of serious diseases after antiviral treatment, many people cannot completely eliminate the hepatitis B virus. Hepatitis B patients need to take long-term medication, which places a heavy financial burden on them. According to data published by the China Centers for Disease Control and Prevention, the average annual medical expenses of chronic hepatitis patients account for 56.24% of the average annual household income, compensated cirrhosis accounts for 81.53%, decompensated cirrhosis accounts for 157.21%, and liver cancer accounts for 96.76%. The heavy economic burden causes many patients to either give up treatment or cause their families to fall back into poverty due to the illness. In addition, judging from the evolution of the cost of diagnosis and treatment of liver disease in the past decade, the cost of diagnosis and treatment of liver disease has shown an increasing trend year by year, with an annual increase of about 10% to 20%, and the portion borne by individuals has also increased accordingly.

Studies have shown that the presence of a large number of subviral particles composed of

HBsAg in the blood of hepatitis B patients is the main reason for suppressing the immune system and making it impossible to eliminate infected liver cells. Relying on a certain technology to reduce the HBsAg level in the blood by 10-100 times can activate the body's own T cell immunity, allowing the body's own immune system to begin to effectively kill infected liver cells. Therefore, effectively reducing the HBsAg level in the blood is not only one of the important cure criteria for hepatitis B, but also a prerequisite for treating hepatitis B and eliminating infected cells.

The latest clinical research results show that for patients who have cleared viral DNA through nucleotide analog therapy, if HBsAg is below a certain level (such as HBsAg <1500 IU/mL), the chance of interferon clearing HBsAg can exceed 50%, which is much higher than the commonly believed interferon cure rate of only 5-10%.

However, due to the lack of effective drugs to reduce HBsAg, the HBsAg levels of most patients (over 90%) exceed this indicator, resulting in interferon being unable to cure these patients (this is why the cure rate of interferon is only 5-10%). These new clinical evidences have created a more realistic and urgent need for treatment methods to reduce HBsAg.

Current studies have found that there are three main ways to reduce surface antigen: inhibiting the expression of HBsAg (siRNA), preventing the release of HBsAg from cells (NAPs), and using HBsAg antibodies.

SUMMARY OF THE INVENTION

One of the purposes of the present application is to provide an antibody or an antigen-binding fragment thereof that specifically binds to the hepatitis B virus surface antigen (HBsAg). In vitro antigen affinity assay has demonstrated that the antibodies of the present application have the ability to specifically bind to HBsAg. In vivo assay has further demonstrated that the antibody of the present application can be used as a neutralizing antibody for hepatitis B virus (HBV), effectively reducing HBsAg levels and inhibiting HBV proliferation.

Specifically, this application relates to:

1. An antibody or an antigen-binding fragment thereof that specifically binds to hepatitis B virus surface antigen (HBsAg), comprising a heavy chain variable region (VH) sequence and a light chain variable region (VL) sequence, wherein:

VH comprises the complementarity determining region (CDR) amino acid sequence as shown

below:

HCDR1: GYTFTGYY (SEQ ID NO:1),

HCDR2: INPNSGGT (SEQ ID NO:2), and

HCDR3: ARDLWX1X2X3X4X5DYGX6DV; and

VL comprises the CDR amino acid sequences as shown below:

LCDR1: X7SIX8TX9,

LCDR2: AAS (SEQ ID NO: 28), and

LCDR3: QQSYSTPLT (SEQ ID NO:29);

wherein:

X1 is selected from N, D, Y, I, S, V or M,

X2 is selected from F, H, E, W, R, Q, M, P or D,

X3 is selected from V, Q, P, R, H, T or Y,

X4 is selected from S, T, D, L, Y, F or H,

X5 is selected from D, Q or E,

X6 is selected from M, I or L,

X7 is selected from Q, S, H, Y, P, L, I or E,

X8 is selected from S or D,

X9 is selected from Y, F, D, T, S or V.

2. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to item 1, wherein the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below:

HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:3, LCDR1 as shown in SEQ ID NO:15, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29; alternatively,

HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:4, LCDR1 as shown in SEQ ID NO:16, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29; alternatively,

HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:5, LCDR1 as shown in SEQ ID NO:17, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29; alternatively,

3. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to item 1 or 2, comprising a human universal framework region (FR). In some embodiments, the VH comprises a human subgroup III universal framework region (FR). In some embodiments, the VL comprises a human subgroup κI common framework. In some embodiments, it comprises a human universal framework region (FR) and one or more amino acid substitutions based on the human universal framework region (FR).

In some embodiments, the framework region is a universal framework region of a fully human antibody and comprises one or more (e.g., 1-20, 1-15, 1-10, 1-5, 1-4, 1-3) amino acid substitutions. In some embodiments, the framework regions are the universal framework regions of a fully human antibody or are 70%, 80%, 90%, 95%, 97%, 98%, 99% identity with the universal framework regions of a fully human antibody.

4. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of items 1 to 3, wherein

VH comprises the FR amino acid sequence as shown below:

HFR1: QVQLVESGAEVKKPGASVKVSCAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: NYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC (SEQ ID NO:63), and

HFR4: WGKGTLVTVSS (SEQ ID NO:64); and

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSASVGDRVITTCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

5. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of items 1 to 3, wherein:

VH comprises the FR amino acid sequence as shown below:

HFR1: QVQLVESGAEVKKPGASVKVSCAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: YYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC (SEQ ID NO:69), and

HFR4: WGKGTLVTVSS (SEQ ID NO:64); and

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

6. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of items 1 to 3, wherein

VH comprises the FR amino acid sequence as shown below:

HFR1: QVQLVESGAIEVKKPGASVKVSKAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: HYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC (SEQ ID NO:70), and

HFR4: WGKGTLLVTVSS (SEQ ID NO:64);

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

7. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of items 1 to 6, wherein the VH sequence and the VL sequence are selected from amino acid sequences in any of the following groups 1) to 14), or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, 99% identity with the VH sequence and the VL sequence in any of the following groups 1) to 14), respectively:

1) SEQ ID NO:31 and SEQ ID NO:46,

2) SEQ ID NO:32 and SEQ ID NO:47,

3) SEQ ID NO:33 and SEQ ID NO:48,

4) SEQ ID NO:34 and SEQ ID NO:49,

5) SEQ ID NO:35 and SEQ ID NO:50,

6) SEQ ID NO:36 and SEQ ID NO:51,

7) SEQ ID NO:37 and SEQ ID NO:52,

8) SEQ ID NO:38 and SEQ ID NO:53,

- 9) SEQ ID NO:39 and SEQ ID NO:54,
- 10) SEQ ID NO:40 and SEQ ID NO:55,
- 11) SEQ ID NO:41 and SEQ ID NO:56,
- 12) SEQ ID NO:42 and SEQ ID NO:57,
- 13) SEQ ID NO:43 and SEQ ID NO:58, or
- 14) SEQ ID NO:44 and SEQ ID NO:58.

8. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of items 1 to 7, wherein the antigen-binding fragment is selected from: Fab, F(ab')₂, Fab', scFv, Fv, Fd, dAb, diabody, or multibody.

9. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of items 1 to 8, wherein the antibody is a fully human antibody, a humanized antibody, a murine antibody, a chimeric antibody or a nanobody.

10. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of items 1 to 9, which is a monospecific antibody, a bispecific antibody, or a multispecific antibody.

11. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of items 1 to 10, wherein the antibody further comprises a heavy chain constant region and a light chain constant region, preferably, the heavy chain constant region is selected from the heavy chain constant region of IgG, and the light chain constant region is selected from the κ chain or the λ chain.

12. A diagnostic reagent for diagnosing HBsAg infection, comprising antibody or an antigen-binding fragment thereof that specifically binds to HBsAg as described in any one of items 1 to 11 that is labeled.

13. A diagnostic reagent according to item 12, wherein the antibody or an antigen-binding fragment thereof that specifically binds to HBsAg is labeled with one or more markers selected from the group consisting of: a radioactive marker, a fluorophore, a chromophore, an imaging agent and a metal ion.

14. An isolated nucleic acid molecule comprising a polynucleotide sequence encoding the antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of items 1 to 11.

15. A construct comprising the nucleic acid molecule according to item 14.
16. The construct according to item 15, which is selected from a plasmid, a phagemid, a viral vector, or a linear nucleic acid.
17. A cell expressing the antibody or an antigen-binding fragment thereof that specifically binds to HBsAg as described in any one of items 1 to 11, or the nucleic acid according to item 14, or the construct according to item 15 or 16.
18. A pharmaceutical composition comprising the antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of items 1 to 11 or the nucleic acid according to item 14.
19. A diagnostic kit comprising the diagnostic reagent according to item 12 or 13.
20. A method for treating or preventing hepatitis B virus infection, comprising administering an effective amount of the pharmaceutical composition according to item 18 to a patient.
21. A method for treating hepatitis B, comprising administering an effective amount of the pharmaceutical composition according to item 18 to a patient.
22. A method for detecting hepatitis B virus, comprising contacting the diagnostic reagent according to item 12 or 13 with a body fluid from a subject.
23. A method according to claim 22, wherein the body fluid from the subject is plasma or serum of the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the binding activity of the antibody of the present application with the antigen at different pH values.

Figure 2 shows the effect of different antibodies of the present application on the plasma HBsAg content of HBV mice.

Figure 3 shows the effect of the antibodies KW027-021, KW027-2-055, KW027-2-055-HD, and KW027-2-055-YD of the present application on the HBsAg content in the plasma of HBV mice.

DETAIL DESCRIPTION OF THE INVENTION

The present application aims to provide a novel antibody or an antigen-binding fragment thereof that can specifically bind to the surface antigen of hepatitis B virus (HBsAg), which can reduce the

expression level of HBsAg in the patient's blood through the binding, activate the body's own T cell immunity, and enable the body's own immune system to begin to effectively kill infected liver cells and inhibit HBV proliferation. Through the specifically binding antibodies or antigen-binding fragments thereof, the present application provides new options and ideas for the prevention and treatment of HBV, and also provides new tools for the detection and diagnosis of HBV.

Definition

As used herein, the term "antibody", also known as "immunoglobulin", encompasses antibodies having structural features of natural antibodies and antibody-like molecules having structural features different from natural antibodies but exhibiting binding specificity to antigen molecules. The term antibody is intended to include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, i.e., molecules that contain an antigen binding site. The immunoglobulin molecule can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY) or subtype (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2).

As used herein, "antigen-binding fragments" of an antibody can be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen-binding fragments include, inter alia, Fab, Fab', F(ab')₂, Fv, dAb and complementarity determining region (CDR) fragments, single-chain antibodies (scFvs), single-domain antibodies, chimeric antibodies, diabodies and polypeptides that contain at least a portion of an immunoglobulin sufficient to confer specific antigen binding to the polypeptide. Examples of antigen-antibody fragments described herein include, but are not limited to: (i) a Fab fragment having VL, CL, VH and CH1 domains; (ii) a Fab' fragment, i.e., a Fab fragment having one or more cysteine residues at the C-terminus of the CH1 domain; (iii) a Fd fragment having VH and CH1 domains; (iv) a Fd' fragment having VH and CH1 domains and one or more cysteine residues at the C-terminus of the CH1 domain; (v) a Fv fragment having the VL and VH domains of a single antibody arm; (vi) a dAb fragment consisting of a VH domain (Ward et al., Nature 341, 544-546 (1989)); (vii) a fragment consisting of a VH domain. (viii) F(ab')₂ fragments, bivalent fragments comprising two Fab' fragments bridged by a disulfide bond at the hinge region; (ix) single-chain antibody molecules (e.g., single-chain Fv; scFv) (Bird et al., Science 242: 423-426 (1988); and Huston et al., PNAS (USA) 85: 5879-5883 (1988)); (x) "diabodies" with two antigen-binding sites, which comprise a heavy chain variable region (VH)

connected to a light chain variable region (VL) in the same polypeptide chain (see, e.g., EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90: 6444-6448 (1993)); (xi) "linear antibodies", which comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) that, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al., Protein Eng. 8(10): 1057-1062 (1995); and U.S. Pat. No. 5,641,870).

The terms "heavy chain" ("CH"), "light chain" ("CL"), "light chain variable region" ("VL"), "heavy chain variable region" ("VH"), and "framework region" ("FR") refer to domains found in naturally occurring immunoglobulins and the corresponding domains of synthetic (e.g., recombinant) binding proteins (e.g., humanized antibodies). The basic structural unit of naturally occurring immunoglobulins (e.g., IgG) is a tetramer having two light chains and two heavy chains. The amino-terminal ("N") portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxyl terminus (the "C" portion of each chain defines a constant region; light chains have a single constant region, while heavy chains typically have three constant regions and a hinge region. Therefore, the naturally occurring light chain structure of IgG molecules is N'-VL-CL-C', and the IgG heavy chain structure is N'-VH-CH1-H-CH2-CH3-C' (where H is the hinge region). The variable region of an IgG molecule contains the complementarity determining regions (CDRs), which are responsible for recognizing and contacting antigens, and the non-CDR segments, namely the framework regions (FRs), which maintain the variable region structure and determine the positions of the CDR loops. Thus, the VL and VH domains have the structure N'-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-C'. In the present application, the three CDRs of VH are referred to as HCDR1, HCDR2, and HCDR3; the four framework regions of VH are referred to as HFR1, HFR2, HFR3, and HFR4; the three CDRs of VL are referred to as LCDR1, LCDR2, and LCDR3; the four framework regions of VL are referred to as LFR1, LFR2, LFR3, and LFR4.

In this application, the CDRs and FRs are numbered according to the ImMunoGeneTics (IMGT) numbering system (see, e.g., Lefranc M.P., The IMGT unique numbering for immunoglobulins, T-cell receptors, and Ig-like domains. The immunologist 7, 132-136, 1999 (1999))) was determined.

As used herein, the term "nanobody" refers to a single domain antibody consisting only of the variable region of an antibody heavy chain. Because its relative molecular size is at the nanometer level, it is called a nanobody. Nanobodies can bind tightly to antigens like normal antibodies, but

they are not as easy to stick to each other and aggregate into clumps as single-chain antibodies. The term "single domain antibody" is an independent antigen binding unit consisting of one variable region or one engineered constant domain that only assists in target binding. The term "diabody" refers to a dimer of scFv, wherein two scFv fragments are co-expressed through interchain pairing (cross-pairing) of the VH and VL domains to form a diabody. Likewise, a polymer formed by multiple scFvs is called a multimeric antibody. For example, a triabody formed by three scFvs, a tetrabody formed by four scFvs, and the like. Each scFv may have the same antigen specificity or different antigen specificity.

As used herein, "monospecific antibody", "bispecific antibody" and "multispecific antibody" refer to a single antibody molecule that can bind to one, two or more different antigens or one, two or more different antigenic epitopes of the same antigen, respectively.

As used herein, the term "chimeric antibody" refers to an antibody combining antibody fragments from different species. Specifically, for example, the Fc constant region of a monoclonal antibody derived from one species (e.g., mouse) is replaced with an Fc constant region derived from one species (e.g., human) via DNA recombination technology. See, for example, patent applications PCT/US86/02269; EP/173,494.

As used herein, the term "humanized antibody" refers to an antibody comprising a human immunoglobulin framework region and one or more CDRs from a non-human (e.g., mouse, rat, rabbit, or synthetic) immunoglobulin. Except for CDR, all other parts in the humanized antibody are substantially identity with the corresponding parts of natural human immunoglobulin sequences. For methods of constructing humanized antibodies by genetic engineering, see, for example, patent application US/5,585,089.

As used herein, the term "fully human antibody" is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The fully human antibodies of the present application may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "fully human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a rabbit, have been grafted onto human framework sequences. Therefore, as used herein, the term "fully human antibody" means that almost every part of its

protein molecule (e.g., CDR, FR, CL, HC domains (e.g., CH1, CH2, CH3), hinge, VL, VH) is almost non-immunogenic in the human body, and has only small sequence changes or variations relative to human natural immunoglobulins. Thus, fully human antibodies are distinguished from chimeric or humanized antibodies. It should be noted that fully human antibodies can be produced by non-human animals or prokaryotic or eukaryotic cells that are capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes.

As used herein, the term "specific binding" refers to the property of complementary binding with high affinity determined by the antigenic determinant and the spatial conformation of the variable region of the antibody molecule. This high affinity determines that once the antibody molecule binds to the antigen, it can exert its corresponding physiological function. For example, in some embodiments of the present application, the antibody binds to and helps to clear the antigen.

As used herein, the term "human universal framework" is a framework that represents the amino acid residues that most commonly occur in selected the framework sequences of human immunoglobulin VL or VH. Generally, the human immunoglobulin VL or VH sequence is selected from a subgroup of variable domain sequences. Generally, a subgroup of sequences is a subgroup as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th ed., NIH Publication 91-3242, Bethesda MD (1991), Vols. 1-3. In some embodiments, for the VL, the subgroup is subgroup kappa human as described by Kabat et al. (supra). In some embodiments, for VH, the subgroup is subgroup III as described by Kabat et al. (supra).

"Homology" or "identity" between two amino acid sequences or nucleotide sequences refers to the percentage of amino acid residues or nucleotide residues that are exactly the same between the two sequences. If the two sequences to be compared to each other differ in length, sequence "homology" or "identity" preferably refers to the percentage of the amino acid residues or nucleotide residues in the shorter sequence that are exactly the same as the amino acid residues or nucleotide residues in the longer sequence. Sequence identity can be routinely determined by using sequence analysis software commonly used in the art, such as the Wisconsin Sequence Analysis Package and the like.

As used herein, the terms "polynucleotide" or "nucleic acid", "nucleic acid molecule" are used interchangeably and include, but are not limited to, DNA, RNA, cDNA (complementary DNA), mRNA (messenger RNA), rRNA (ribosomal RNA), shRNA (small hairpin RNA), snRNA (small

nuclear RNA), snoRNA (short nucleolar RNA), miRNA (microRNA), genomic DNA, synthetic DNA, synthetic RNA and/or tRNA.

As used herein, the term "construct" refers to a vector by which a polynucleotide sequence (e.g., foreign gene) can be introduced into a host cell to transform the host and promote the expression (e.g., transcription and translation) of the introduced sequence, including plasmids, phagemids, viral vectors, etc.

As used herein, the term "phagemid" is a filamentous phage-derived vector, the basic components of which mainly include the replication origin, selection marker, and intergenic region (IG region) of the plasmid, and usually also contains packaging sequences and replication origins of the negative and positive chains, genes for phage coat proteins, restriction endonuclease recognition sites, promoters, and DNA fragments encoding signal peptides. In addition, phagemids can also contain a molecular tag to facilitate screening of phagemid-based libraries. The phagemid cannot independently complete the assembly of progeny phage particles; other structural and functional proteins required to complete its life cycle are provided by helper phage. The helper phage is a mutant filamentous phage with extremely low efficiency of its own DNA replication. Therefore, when the helper phage and the phage formed by phagemid packaging co-infect the host bacteria, a large number of phages containing phagemids can be packaged, while only a small number of helper phages can be packaged.

As used in this article, "plasmid" refers to a DNA molecule other than chromosomes (or nucleoids) in organisms such as bacteria, yeast and actinomycetes, which exists in the cytoplasm or nucleus and has the ability to replicate autonomously, so that it can maintain a constant copy number in daughter cells and express the genetic information it carries.

As used herein, "viral vector" refers to a virus-based gene vector, which is a genetically engineered viral genome that can carry exogenous genes and related gene elements and is packaged into viral particles, which can introduce the exogenous genes they carry into cells through viral infection.

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

Antibodies or antigen-binding fragments thereof

In one aspect, the present application provides an antibody or an antigen-binding fragment thereof that specifically binds to hepatitis B virus surface antigen (HBsAg). Generally, the antibodies of the present application comprise a heavy chain variable region (VH) sequence and a light chain variable region (VL) sequence, wherein:

VH comprises the complementarity determining region (CDR) amino acid sequence as shown below:

HCDR1: GYTFTGYY (SEQ ID NO:1),

HCDR2: INPNSGGT (SEQ ID NO:2), and

HCDR3: ARDLWX1X2X3X4X5DYGX6DV; and

VL comprises the CDR amino acid sequences as shown below:

LCDR1: X7SIX8TX9,

LCDR2: AAS (SEQ ID NO:28), and

LCDR3: QQSYSTPLT (SEQ ID NO:29);

wherein:

X1 is selected from N, D, Y, I, S, V or M,

X2 is selected from F, H, E, W, R, Q, M, P or D,

X3 is selected from V, Q, P, R, H, T or Y,

X4 is selected from S, T, D, L, Y, F or H,

X5 is selected from D, Q or E,

X6 is selected from M, I or L,

X7 is selected from Q, S, H, Y, P, L, I or E,

X8 is selected from S or D, and

X9 is selected from Y, F, D, T, S or V.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:3, LCDR1 as shown in SEQ ID NO:15, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ

ID NO:2, HCDR3 as shown in SEQ ID NO:4, LCDR1 as shown in SEQ ID NO:16, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:5, LCDR1 as shown in SEQ ID NO:17, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:6, LCDR1 as shown in SEQ ID NO:18, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:7, LCDR1 as shown in SEQ ID NO:19, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:8, LCDR1 as shown in SEQ ID NO:20, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:9, LCDR1 as shown in SEQ ID NO:21, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:10, LCDR1 as shown in SEQ ID NO:22, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:11, LCDR1 as shown in SEQ ID NO:23, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:12, LCDR1 as shown in SEQ ID NO:24, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:13, LCDR1 as shown in SEQ ID NO:25, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:14, LCDR1 as shown in SEQ ID NO:26, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below: HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:6, LCDR1 as shown in SEQ ID NO:27, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

In some embodiments, the antibody comprises a human universal framework region (FR).

In some embodiments, VH comprises a FR amino acid sequence as shown below:

HFR1: QVQLVESGAIEVKKPGASVKVCKAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: NYAQQFQGRVTMTSDTSISTAYMELSRSDDTAVYYC (SEQ ID NO:63), and

HFR4: WGKGTLVTVSS (SEQ ID NO:64);

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFLTISLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

In some embodiments, VH comprises a FR amino acid sequence as shown below:

HFR1: QVQLVESGAIEVKKPGASVKVCKAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: YYAQKFQGRVTMTRDTSISTAYMELSRLSDDTAVYYC (SEQ ID NO:69), and

HFR4: WGKGTTLVTVSS (SEQ ID NO:64); and

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

In some specific embodiments, VH comprises the FR amino acid sequence as shown below:

HFR1: QVQLVESGAIEVKKPGASVKVCKAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: YYAQKFQGRVTMTRDTSISTAYMELSRLSDDTAVYYC (SEQ ID NO:70), and

HFR4: WGKGTTLVTVSS (SEQ ID NO:64); and

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

In some specific embodiments, the VH sequence is selected from any one of SEQ ID NOs: 31-44, or an amino acid sequence that has at least 80%, 85%, 90%, 95%, 95%, 96%, 97%, 98%, or 99% identity with any one of SEQ ID NOs: 31-44.

In some specific embodiments, the VL sequence is selected from any one of SEQ ID NOs: 46-58, or an amino acid sequence that has at least 80%, 85%, 90%, 95%, 95%, 96%, 97%, 98%, or 99% identity with any one of SEQ ID NOs: 46-58.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:31 and SEQ ID NO:46, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:32 and SEQ ID NO:47, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:33 and SEQ

ID NO:48, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:34 and SEQ ID NO:49, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:35 and SEQ ID NO:50, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:36 and SEQ ID NO:51, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:37 and SEQ ID NO:52, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:38 and SEQ ID NO:53, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:39 and SEQ ID NO:54, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:40 and SEQ ID NO:55, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:41 and SEQ ID NO:56, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:42 and SEQ ID NO:57, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:43 and SEQ

ID NO:58, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

In some specific embodiments, the VH sequence and VL sequence are SEQ ID NO:44 and SEQ ID NO:58, respectively, or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the above VH sequence or VL sequence.

However, those skilled in the art will be aware that not all antibodies or antigen-binding fragments thereof contain complete heavy chain variable regions and light chain variable regions. Moreover, in many cases, protein molecules containing only antibody or an antigen-binding fragments thereof still have the ability to specifically bind to and/or neutralize antigens. In some embodiments, the antibody may comprise only the VH and/or VL of an antibody, such as a dAb, a single domain antibody containing only the light chain variable region, a nanobody, and the like. In some embodiments, the antibody or an antigen-binding fragment thereof may not contain a constant region, such as a scFv, which comprises the aforementioned HCDR and LCDR. In some embodiments, the antibody or an antigen-binding fragment thereof is selected from the group consisting of Fab, F(ab')₂, Fab', Fv, Fd, diabody, and multibody. The diabody and multibody antibody contain one, two or more of the aforementioned scFvs, and may include other scFvs that specifically bind to HBsAg or other antigens. In some embodiments, the antibody is a monospecific antibody, a bispecific antibody, or a multispecific antibody. In addition, as antigen-binding fragments of the antibodies described in the present application, the various CDRs disclosed in the present application, combinations of the CDRs, and antibodies or antibody-binding fragments comprising the CDRs (e.g., HCDR3 and/or LCDR3, etc.) and retaining the ability to specifically recognize HBsAg are also included in the protection scope of the present application.

In some embodiments, the antibodies described herein are fully human antibodies, humanized antibodies, murine antibodies, chimeric antibodies, or nanobodies.

In some embodiments, the antibodies described herein further comprise a heavy chain constant region and a light chain constant region. In some embodiments, the heavy chain constant region and/or the light chain constant region are derived from a natural immunoglobulin, for example, the heavy chain constant region can be derived from IgG, IgE, IgM, IgD, IgA and IgY, or subtypes, for example, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The light chain constant region may be selected from a kappa chain or a lambda chain.

In some specific embodiments, the heavy chain constant region is the heavy chain constant region of human IgG1, comprising the amino acid sequence as shown in SEQ ID NO:59, or comprising an amino acid sequence having 70% or more sequence identity with SEQ ID NO:59, for example, at least 70%, at least 75%, at least 80%, at least 85%, at least 88%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity with SEQ ID NO:59.

In some specific embodiments, the light chain constant region is derived from a kappa chain and comprises an amino acid sequence as shown in SEQ ID NO:60, or comprises an amino acid sequence having 70% or more sequence identity with SEQ ID NO:60, for example, an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 88%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity with SEQ ID NO:60.

In certain embodiments, the antibodies or antigen-binding fragments disclosed herein can bind to the antigenic loop region of HBsAg. The envelope of hepatitis B virus generally contains three "HBV envelope proteins" (also called "HBsAg", "hepatitis B surface antigen"): S protein (also called S-HBsAg), M protein (also called M-HBsAg), and L protein (also called L-HBsAg). S-HBsAg, M-HBsAg and L-HBsAg share the same C-terminus (also called "S domain", 226 amino acids, Salisse J. and Sureau C., 2009, Journal of Virology 83:9321-9328)), which corresponds to S-HBsAg and is essential for viral assembly and infectivity.

When referring herein to positions in the amino acid sequence of the S domain of HBsAg, such positions are obtained with reference to the amino acid sequence as set forth in SEQ ID NO:73 or a natural or artificial sequence variant thereof. However, it should be understood by those skilled in the art that mutations or variations (including but not limited to substitutions, deletions and/or additions, such as HBsAg of different genotypes or different HBsAg mutants) may occur naturally in the amino acid sequence of the S domain of HBsAg or be artificially introduced into the amino acid sequence of the S domain of HBsAg without affecting its biological properties. Therefore, as used herein, the term "HBsAg" encompasses all such polypeptides, including, for example, the polypeptide according to SEQ ID NO:73 and natural or artificial mutants thereof.

MENVTSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLGQNSQSPTS
NH
SPTSCPPTCPGYRWMCLRRFIIFLLCLIFLLVLLDYQGMLPVCPLIPGSSTTGTGPCRTCTT

PAQGTSMYPSCCCTKPSDGNCTCIPISSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPT
VWLSVIWMMWYWGPSLYSTLSPFLPLLPIFFCLWVYI (SEQ ID NO:73)

In a specific embodiment, the antibodies or antigen-binding fragments of the present application can specifically bind to S-HBsAg, M-HBsAg or L-HBsAg. In some specific embodiments, the antibodies or antigen-binding fragments of the present application specifically bind to S-HBsAg. In some specific embodiments, the antibodies or antigen-binding fragments of the present application specifically bind to M-HBsAg. In some specific embodiments, the antibodies or antigen-binding fragments of the present application specifically bind to L-HBsAg. In some specific embodiments, the antibodies or antigen-binding fragments of the present application specifically bind to the S domain. In some specific embodiments, the antibodies or antigen-binding fragments of the present application specifically binds to HBsAg comprising the amino acid sequence shown in SEQ ID NO:73. In some specific embodiments, the antibodies or antigen-binding fragments of the present application specifically bind to HBsAg comprising the amino acid sequence shown in SEQ ID NO: 73, or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% identity with SEQ ID NO:73.

Diagnostic reagents

On the one hand, the present application also provides a diagnostic reagent, which comprises the labeled aforementioned antibody or an antigen-binding fragment thereof that specifically binds to HBsAg. The antibody can be labeled using methods known in the art. In some specific embodiments, the label used for labeling is selected from the group consisting of radioactive labels, fluorophores, chromophores, imaging agents and metal ions.

Nucleic acids, constructs, cells

On the one hand, the present application also provides an isolated nucleic acid molecule comprising a polynucleotide sequence encoding the aforementioned antibody or an antigen-binding fragment thereof that specifically binds to HBsAg. In some embodiments, the nucleic acid molecule is an RNA or DNA molecule. In some embodiments, the nucleic acid is single-stranded or double-stranded. In some embodiments, the nucleic acid is linear or circular. The nucleic acid may be synthesized by cells or chemically synthesized. In some embodiments, the nucleic acid is a

chemically modified nucleic acid molecule.

On the one hand, the present application also provides a construct comprising the aforementioned nucleic acid molecule, wherein the construct is selected from: a plasmid, a phagemid, a viral vector, or a linear nucleic acid. The present application also provides a construct composition, comprising the aforementioned constructs, wherein each construct in the composition may respectively comprise a portion of a nucleic acid molecule encoding the aforementioned antibody or an antigen-binding fragment thereof that specifically binds to HBsAg. For example, the present application provides a construct composition, wherein the construct is a plasmid, and the construct composition comprises plasmids encoding the heavy chain and light chain of the aforementioned antibody molecule, respectively.

On the one hand, the present application also provides a cell that expresses or contains the aforementioned antibody or an antigen-binding fragment thereof that specifically binds to HBsAg, or comprises the aforementioned nucleic acid, the aforementioned construct, or the aforementioned construct composition. The cell can be selected from, for example, mammalian cells, insect cells, bacteria, fungi, and the like.

In addition, the present application also includes the use of nucleic acids, constructs, and cells for producing the antibody or an antigen-binding fragment thereof that specifically binds to HBsAg.

Pharmaceutical compositions and therapies

The present application also provides a pharmaceutical composition comprising the aforementioned antibody or an antigen-binding fragment thereof that specifically binds to HBsAg or the aforementioned nucleic acid. In some embodiments, the pharmaceutical composition further comprises an appropriate amount of a pharmaceutically acceptable excipient. These agents are incorporated into the formulation to improve delivery and tolerability, among other things. Large numbers of suitable formulations can be found in the pharmacopoeia well known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (Mack Publishing Company, Easton, PA). As used herein, pharmaceutically acceptable excipients refer to non-toxic fillers, stabilizers, diluents, carriers, solvents or other formulation excipients. Among them, diluents, excipients, such as microcrystalline cellulose, mannitol, etc.; fillers, such as starch, sucrose, etc.; binders, such as starch, cellulose derivatives, alginate, gelatin and/or polyvinyl pyrrolidone; disintegrants, such as calcium

carbonate and/or sodium bicarbonate; absorption promoters, such as quaternary ammonium compounds; surfactants, such as cetyl alcohol; carriers, solvents, such as water, saline, kaolin, soap clay, etc.; lubricants, such as talc, calcium/magnesium stearate, polyethylene glycol, etc. In addition, the pharmaceutical composition of the present application is preferably an injection.

In some embodiments of the present application, the antibody or an antigen-binding fragment thereof in the pharmaceutical composition of the present application is present in a concentration of 1 mg/ml to 1000 mg/ml, preferably 10 mg/ml to 1000 mg/ml, more preferably 50 mg/ml to 500 mg/ml, and more preferably 100 mg/ml to 300 mg/ml.

The pharmaceutical composition of the present application preferably has a pH of 3.0 to 9.0. Among them, it may further contain a buffer system, a preservative, a surface tension agent, a chelating agent, a stabilizer and a surfactant. In one embodiment of the present application, the pharmaceutical composition of the present application is an aqueous preparation. Such preparations are usually solutions or suspensions. In a specific embodiment of the present application, the pharmaceutical composition is a stable aqueous solution. In another specific embodiment of the present application, the pharmaceutical composition is a lyophilized preparation, and the physician or the patient adds a solvent and/or a diluent to dissolve the lyophilized preparation before use.

On the one hand, the present application also provides a method for treating hepatitis B, comprising administering an effective amount of the aforementioned pharmaceutical composition to a patient.

In one aspect, the present application also provides a method for treating or preventing hepatitis B virus infection, comprising administering an effective amount of the aforementioned pharmaceutical composition to a patient.

The effective amount is adjusted depending on the age and body size of the subject, the target disease, symptoms, administration route, and the like. When the pharmaceutical composition of the present application comprises the aforementioned antibody or an antigen-binding fragment thereof that specifically binds to HBsAg and is used to treat various conditions and diseases associated with hepatitis B virus infection in adults, the pharmaceutical composition can be administered intravenously, usually with a single dose of about 0.01 to about 20 mg of antibody per kilogram of body weight, for example, about 0.1 to about 15, about 1 to about 10, about 3 to about 10 mg/kg body weight (mpk), about 12 mpk body weight. Depending on the severity of the condition, the

frequency and duration of treatment may be adjusted. For example, the drug is administered to the patient once a week.

It is known that various drug delivery systems can be used to administer the pharmaceutical compositions of the present application, such as encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing mutant viruses, receptor-mediated endocytosis (see, e.g., Wu et al. (1987), *J. Biol. Chem.* 262: 4429-4432) etc. Methods of administering the drug include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, intraepithelial, and oral routes. The pharmaceutical composition may be administered by any convenient route, such as by infusion or intravenous bolus, absorption through epithelial and mucosal layers (e.g., oral mucosa, rectal and intestinal mucosa), and may be administered together with other biologically active agents. Administration can be systemic or local.

The pharmaceutical composition can also be delivered by vesicles, in particular by liposome vesicles (see Langer (1990) *Science* 249: 1527-1533; Treat et al. (1989) in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez Berestein and Fidler (Eds.), Liss, New York, pp. 353-365; Lopez-Berestein, *supra*, pp. 317-327).

In certain cases, the pharmaceutical composition may be delivered by a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton (1987) *CRC Crit. Ref. Biomed. Eng.* 14:201). In another embodiment, polymeric materials may be used (see *Medical Applications of Controlled Release*, Langer and Wise (Eds.), CRC Pres., Boca Raton, Florida (1974)). For a discussion of other controlled release systems, see Langer (1990) *Science* 249: 1527-1533.

The pharmaceutical composition can be administered alone or in combination, and can improve the patient's symptoms by reducing the load of hepatitis B virus surface antigen. In some embodiments, the administration is a combined administration, wherein the anti-HBsAg antibody or an antigen-binding fragment thereof is administered in combination with one or more therapeutic agents (or second therapeutic agents). Co-administration and combination therapy are not limited to simultaneous administration, but also include a therapeutic regimen in which the anti-HBsAg antibody or an antigen-binding fragment thereof is administered at least once during a course of treatment involving administration of at least one other therapeutic agent to the patient. The second therapeutic agent may be another HBV therapeutic drug, such as another antibody/antibody fragment,

or a soluble cytokine receptor (such as interferon, cytokine, and thymosin, etc.), a nucleoside analog (Tenofovir, Entecavir, and Adefovir, etc.).

The present application also provides the use of any anti-HBsAg antibody or an antigen-binding fragment thereof described herein in the preparation of a medicament for treating or preventing hepatitis B virus infection.

The present application also provides the use of any anti-HBsAg antibody or an antigen-binding fragment thereof described herein in the preparation of a medicament for treating hepatitis B.

The present application also includes the use of any anti-HBsAg antibody or an antigen-binding fragment thereof described herein in the preparation of a medicament for treating a disease or symptom, wherein the disease or symptom is improved by reducing the level of HBsAg in the human body.

Diagnostic reagents, kits, and detection methods

On the one hand, the present application provides a diagnostic reagent comprising the labeled aforementioned antibody or an antigen-binding fragment thereof that specifically binds to HBsAg, a complementary chain of the aforementioned nucleic acid or a fragment of the complementary chain, or the aforementioned construct, virus, phage or cell.

In some embodiments, the labeling is performed using a label selected from one or more of the following: a radioactive label, a fluorophore, a chromophore, an imaging agent, and a metal ion. In some embodiments, the label can be selected from one or more of the following: enzyme labeling molecules such as horseradish peroxidase and alkaline phosphatase, fluorescent protein molecules, fluorescein molecules, biotin molecules, isotopes and other developing agents. In some embodiments, the label is coupled to the antibody or an antigen-binding fragment thereof that specifically binds to HBsAg, the complementary chain of the aforementioned nucleic acid, or a fragment of the complementary chain to form a complex.

On the other hand, the present application also provides a diagnostic kit. In some embodiments, the kit comprises the aforementioned antibody or an antigen-binding fragment thereof that specifically binds to HBsAg. In some embodiments, the kit comprises the aforementioned nucleic acid, the nucleic acid fragment, or the complementary strand of the nucleic acid or a fragment of the complementary strand. In some embodiments, the kit comprises the aforementioned diagnostic

reagents. In some embodiments, the kit is an in vitro detection kit. In some embodiments, the kit is an immunoassay kit, such as an ELISA kit or an immunohistochemistry kit, which comprises the aforementioned antibody or an antigen-binding fragment thereof that specifically binds to HBsAg. In some embodiments, the kit is an in vivo non-invasive diagnostic kit, which comprises the aforementioned antibody or an antigen-binding fragment that specifically binds to HBsAg, and the antibody or an antigen-binding fragment that specifically binds to HBsAg forms a complex with a label molecule. In some embodiments, the in vivo non-invasive diagnostic kit can be used for an imaging method selected from the following: radioimmunoassay or targeted ultrasound angiography. When used in radioimmunoassay, the labeling molecule is a radionuclide. When used for targeted ultrasound contrast imaging, the labeling molecule is an ultrasound contrast agent.

In addition, the present application also provides a method for detecting HBV, diagnosing HBV infection or judging the course of HBV-related diseases using the aforementioned antibody or an antigen-binding fragment thereof that specifically binds to HBsAg, the aforementioned diagnostic reagent or kit, which comprises contacting the aforementioned diagnostic reagent with body fluids from a subject.

In some embodiments, the body fluid from the subject is plasma or serum from the subject.

The preferred embodiments of the present application are described in detail above; however, the present application is not limited thereto. Within the technical concept of the present application, a variety of simple modifications can be made to the technical solution of the present application, including combining various technical features in any other suitable manner. These simple modifications and combinations should also be regarded as the contents disclosed in the present application and belong to the protection scope of the present application.

It should be understood that the above description as well as the following examples are intended to illustrate rather than limit the scope of the present application. Other aspects, advantages and modifications within the scope of the present application will be apparent to those skilled in the art to which the present application pertains. Based on the embodiments of the present application, all other embodiments obtained by ordinary technicians in the field without making any creative work shall fall within the scope of protection of the present application.

EXAMPLE

Example 1: Construction of HBsAg antibody expression vector

The HindIII restriction site, Kozak sequence, secretion signal peptide gene and anti-HBsAg antibody heavy chain encoding gene (including the VH amino acid sequence encoding gene and the humanized IgG1 constant region encoding gene), termination code and EcoRI encoding gene were sequentially fused in series, and the gene fragment was obtained by chemical synthesis. The above fragment was inserted into the eukaryotic expression plasmid pCDNA 3.1(+) (purchased from Invitrogen Corporation, catalog number V790-20) through the EcoRI and HindIII sites and sequenced to obtain the expression plasmid pCDNA3.1(+)-DH for the anti-HBsAg antibody heavy chain.

The HindIII restriction site, Kozak sequence, secretion signal peptide gene and HBsAg light chain coding gene (including the coding gene of the VL amino acid sequence and the coding gene of the kappa chain constant region), the termination code and the EcoRI coding gene were sequentially fused in series and the gene fragments were obtained by chemical synthesis. The above fragment was inserted into the eukaryotic expression plasmid pCDNA 3.1(+) through the EcoRI and HindIII sites and sequenced to obtain the expression plasmid pCDNA3.1(+)-DL for the antibody light chain.

Among the HBsAg antibodies tested in the examples of the present application, for the antibodies numbered KW027-021, KW027-2-032, KW027-2-033, KW027-2-042, KW027-2-055, KW027-2-060, KW027-2-085, KW027-2-088, KW027-2-094, KW027-2-100, KW027-2-112, KW027-2-113, KW027-2-116, KW027-2-055-YD, and KW027-2-055-HD, the sequences of HCDR1 are all as shown in SEQ ID NO:1, the sequences of HCDR2 are all as shown in SEQ ID NO:2, and the sequences of LCDR2 are all as shown in SEQ ID NO: 28, and LCDR3 are all shown in SEQ ID NO:29. The sequences of HCDR3 and LCDR1 are shown in Table 1, wherein HCDR3 conforms to the general formula ARDLWX1X2X3X4X5DYGX6DV, and LCDR1 conforms to the general formula X7SIX8TX9.

Table 1

Combination Name	HCDR3	LCDR1
KW027-021	ARDLWNDDVDY YGMDV (SEQ ID NO:71)	QSISTY (SEQ ID NO:72)

KW027-2-032	X1 is N, X2 is F, X3 is V, X4 is S, X5 is D, X6 is I (ARDLWNFVSDDYGIDV, SEQ ID NO:3)	X7 is S, X8 is S, X9 is F (SSISTF, SEQ ID NO:15)
KW027-2-033	X1 is D, X2 is H, X3 is Q, X4 is S, X5 is Q, X6 is I (ARDLWDHQSQDYGIDV, SEQ ID NO:4)	X7 is H, X8 is S, X9 is D (HSISTD, SEQ ID NO:16)
KW027-2-042	X1 is Y, X2 is F, X3 is P, X4 is S, X5 is E, X6 is M (ARDLWYFPSEDYGM DV, SEQ ID NO:5)	X7 is Y, X8 is S, X9 is D (YSISTD, SEQ ID NO:17)
KW027-2-055	X1 is I, X2 is E, X3 is R, X4 is T, X5 is E, X6 is M (ARDLWIERTEDYGM DV, SEQ ID NO:6)	X7 is S, X8 is S, X9 is D (SSISTD, SEQ ID NO:18)
KW027-2-060	X1 is S, X2 is W, X3 is H, X4 is D, X5 is E, X6 is M (ARDLWSWHDEDYGM DV, SEQ ID NO:7)	X7 is P, X8 is S, X9 is T (PSISTT, SEQ ID NO:19)
KW027-2-085	X1 is D, X2 is R, X3 is Q, X4 is L, X5 is D, X6 is M (ARDLWDRQLDDYGM DV, SEQ ID NO:8)	X7 is Y, X8 is S, X9 is S (YSISTS, SEQ ID NO:20)
KW027-2-088	X1 is D, X2 is Q, X3 is T, X4 is Y, X5 is D, X6 is L (ARDLWDQTYDDYGLDV, SEQ ID NO:9)	X7 is Q, X8 is S, X9 is V (QSISTV, SEQ ID NO:21)
KW027-2-094	X1 is D, X2 is Q, X3 is T, X4 is F, X5 is D, X6 is L (ARDLWDQTFDDYGLDV, SEQ ID NO:10)	X7 is Q, X8 is S, X9 is F (QSISTF, SEQ ID NO:22)
KW027-2-100	X1 is V, X2 is M, X3 is Q, X4 is S, X5 is D, X6 is M (ARDLWVMQSDDYGM DV, SEQ ID NO:11)	X7 is Y, X8 is S, X9 is T (YSISTT, SEQ ID NO:23)
KW027-2-112	X1 is S, X2 is P, X3 is Y, X4 is S, X5 is D, X6 is M (ARDLWSPYSDDYGM DV, SEQ ID NO:12)	X7 is L, X8 is S, X9 is Y (LSISTY, SEQ ID NO:24)
KW027-2-113	X1 is M, X2 is E, X3 is R, X4 is T, X5 is D, X6 is M (ARDLWMERTDDYGM DV, SEQ ID NO:13)	X7 is I, X8 is S, X9 is D (ISISTD, SEQ ID NO:25)
KW027-2-116	X1 is S, X2 is D, X3 is Q, X4 is H, X5 is D, X6 is I (WSDQHDDYGIDV, SEQ ID NO:14)	X7 is E, X8 is S, X9 is Y (ESISTY, SEQ ID NO:26)
KW027-2-055-YD	X1 is I, X2 is E, X3 is R, X4 is T, X5 is E, X6 is M (SEQ ID NO:6)	X7 is S, X8 is D, X9 is D (SSIDTD, SEQ ID NO:27)
KW027-2-055-HD	X1 is I, X2 is E, X3 is R, X4 is T, X5 is E, X6 is M (SEQ ID NO:6)	X7 is S, X8 is D, X9 is D (SEQ ID NO:27)

The FR amino acid sequences of antibodies KW027-021, KW027-2-032, KW027-2-033, KW027-2-042, KW027-2-055, KW027-2-060, KW027-2-085, KW027-2-088, KW027-2-094, KW027-2-100, KW027-2-112, KW027-2-113, and KW027-2-116 are:

VH comprises the FR amino acid sequence as shown below:

HFR1: QVQLVESGAIEVKKPGASVKVCKAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: NYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC (SEQ ID NO:63), and

HFR4: WGKGTLVTVSS (SEQ ID NO:64); and

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

The FR amino acid sequence of antibody KW027-2-055-YD is:

VH comprises the FR amino acid sequence as shown below:

HFR1: QVQLVESGAIEVKKPGASVKVCKAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: YYAQQKFQGRVTMTRDTSISTAYMELSRSDDTAVYYC (SEQ ID NO:69), and

HFR4: WGKGTLVTVSS (SEQ ID NO:64); and

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

The FR amino acid sequence of antibody KW027-2-055-HD is:

VH comprises the FR amino acid sequence as shown below:

HFR1: QVQLVESGAIEVKKPGASVKVCKAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: HYAQQKFQGRVTMTRDTSISTAYMELSRSDDTAVYYC (SEQ ID NO:70), and

HFR4: WGKGTLVTVSS (SEQ ID NO:64); and

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

The VH and VL sequences of the above antibodies are shown in Table 2.

Table 2

Combination Name	VH	VL
KW027-021	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYYM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARDLWN DDVDYYGMDVWGKGTLLTVSS (SEQ ID NO:30)	DIQLTQSPSSLSASVGDRVITTC RASQSISTYLNWYQQKP GKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFAT YYCQSYSTPLTFGGGTKVDI KR(SEQ ID NO:45)
KW027-2-032	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYYM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARDLWN FVSDDYGIDVWGKGTLLTVSS (SEQ ID NO:31)	DIQLTQSPSSLSASVGDRVITTC RASSISTFLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSG SGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR (SEQ ID NO:46)
KW027-2-033	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYYM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARDLWD HQSQDYGIDVWGKGTLLTVSS(SEQ ID NO:32)	DIQLTQSPSSLSASVGDRVITTC RASHSISTDLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSG SGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR(S EQ ID NO:47)
KW027-2-042	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYYM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARDLWY FPSEDYGMDVWGKGTLLTVSS(SEQ ID NO:33)	DIQLTQSPSSLSASVGDRVITTC RASYSISTDLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSG SGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR(S EQ ID NO:48)
KW027-2-055	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYYM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARDLWIE RTEDYGMDVWGKGTLLTVSS (SEQ ID NO:34)	DIQLTQSPSSLSASVGDRVITTC RASSISTDLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSG SGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR (SEQ ID NO:49)
KW027-2-060	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYYM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARDLWS WHDEDYGMDVWGKGTLLTVSS (SEQ ID NO:35)	DIQLTQSPSSLSASVGDRVITTC RASPSISTTLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSG SGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR (SEQ ID NO:50)
KW027-2-085	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYYM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSRLRSDDTAVYYCARDLWD RQLDDYGMDVWGKGTLLTVSS (SEQ ID NO:36)	DIQLTQSPSSLSASVGDRVITTC RASYSISTSLNWYQQKPGKAP KLLIYAASSLQSGVPSRFSGSG SGTDFTLTISSLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR(S

		EQ ID NO:51)
KW027-2-088	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYIM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSLRSDDTAVYYCARDLWD QTYDDYGLDVWGKGTLLTVSS (SEQ ID NO:37)	DIQLTQSPSSLSASVGDRVITIC RASQSISTVLNWIYQKPGKAP KLLIYAASSLQSGVPSRFGSG SGTDFTLTISLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR(S EQ ID NO:52)
KW027-2-094	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYIM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSLRSDDTAVYYCARDLWD QTFDDYGLDVWGKGTLLTVSS (SEQ ID NO:38)	DIQLTQSPSSLSASVGDRVITIC RASQSISTFLNWIYQKPGKAP KLLIYAASSLQSGVPSRFGSG SGTDFTLTISLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR(S EQ ID NO:53)
KW027-2-100	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYIM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSLRSDDTAVYYCARDLWV MQSDDYGMDEVWGKGTLLTVSS (SEQ ID NO:39)	DIQLTQSPSSLSASVGDRVITIC RASYSISTTLNWIYQKPGKAP KLLIYAASSLQSGVPSRFGSG SGTDFTLTISLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR(S EQ ID NO:54)
KW027-2-112	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYIM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSLRSDDTAVYYCARDLWS PYSDDYGMDEVWGKGTLLTVSS (SEQ ID NO:40)	DIQLTQSPSSLSASVGDRVITIC RASLSISTYLNWIYQKPGKAP KLLIYAASSLQSGVPSRFGSG SGTDFTLTISLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR (SEQ ID NO:55)
KW027-2-113	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYIM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSLRSDDTAVYYCARDLWM ERTDDYGMDEVWGKGTLLTVSS (SEQ ID NO:41)	DIQLTQSPSSLSASVGDRVITIC RASISISTDLNWIYQKPGKAP KLLIYAASSLQSGVPSRFGSG SGTDFTLTISLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR (SEQ ID NO:56)
KW027-2-116	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYIM HWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGR VTMTRDTSISTAYMELSLRSDDTAVYYCARDLWS DQHDDYGIDVWGKGTLLTVSS (SEQ ID NO:42)	DIQLTQSPSSLSASVGDRVITIC RASESISTYLNWIYQKPGKAP KLLIYAASSLQSGVPSRFGSG SGTDFTLTISLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR (SEQ ID NO:57)
KW027-2-055- YD	QVQLVESGAIEVKKPGASVKVSCASGYTFTGYIM HWVRQAPGQGLEWMGWINPNSGGTYAQKFQGR VTMTRDTSISTAYMELSLRSDDTAVYYCARDLWIE	DIQLTQSPSSLSASVGDRVITIC RASSIDTDLNWIYQKPGKAP KLLIYAASSLQSGVPSRFGSG

	RTEDYGMDVWGKGLTVTVSS (SEQ ID NO:43)	SGTDFTLTISLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR (SEQ ID NO:58)
KW027-2-055- HD	QVQLVESGAEVKKPGASVKVSKASGYTFTGYM HWVRQAPGQGLEWMGWINPNSGGTHYAQKFQGR VTMTRDTSISTAYMELSRLSDDTAVYYCARDLWIE RTEDYGMDVWGKGLTVTVSS (SEQ ID NO:44)	DIQLTQSPSSLSASVGDRVITC RASSIDTDLNWDYQQKPGKAP KLLIYAASSLQSGVPSRFGSG SGTDFTLTISLQPEDFATYYC QQSYSTPLTFGGGTKVDIKR (SEQ ID NO:58)

The heavy chain constant region of each antibody is shown in SEQ ID NO:59, and the light chain constant region is shown in SEQ ID NO:60.

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPGK (SEQ ID NO:59)

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:60)

Example 2: Expression and purification of anti-HBsAg antibodies

The expression plasmids pCDNA3.1(+)-DH and pCDNA3.1(+)-DL described in Example 1 were used to express the target antibody via eukaryotic expression cells ExpiCHO-S™ cells (ThermoFisher, A29127). According to the ExpiCHO-S ExpressionSystem manual, the cell density was adjusted to 1×10^6 cells/mL one day before plasmid transfection. On the day of plasmid transfection, the plasmids were combined according to the corresponding relationship between the heavy chain and the light chain (i.e., the antibody light chain was combined with the antibody heavy chain with the same number), mixed with the transfection reagent, and added to the cell culture medium. After culturing for about 22 hours at 37°C and 8% CO₂, the cells were fed and cultured for 10-12 days at 32°C and 5% CO₂. The cell culture supernatant was collected for antibody purification.

The expression supernatant was filtered with a 0.22 µm filter membrane, and the expressed antibody was captured from the expression supernatant using Protein A affinity chromatography

(purchased from Sepax, 65008). After equilibrating the chromatography column with an equilibrium buffer (20 mM Tris + 100 mM NaCl, pH 7.5), the antibody was passed through the affinity chromatography column and eluted with an elution buffer (0.15 M glacial acetic acid, pH 3.0). The purified antibody was tested by SDS PAGE and SEC, and the purity was above 95%.

Example 3: Determination of antigen binding activity at different pH

An ELISA plate coated with HBsAg antigen (500 ng/mL, HBsAg antigen sequence as shown in SEQ ID NO:74) was taken and blocked, added with 50 μ L of the antibody to be tested (concentration of 2.5 μ g/mL) to each well, and incubated at room temperature for 2 h (two wells for each antibody). Subsequently, the plate was washed three times with PBS at pH 7.4 for odd columns and with PBS at pH 6.0 for even columns, for 3 min each time; then the plate was washed three times with PBST at the corresponding pH, for 3 min each time; finally, the entire plate was washed three times with PBS at pH 7.4, for 3 min each time. 50 μ L of anti M13-HRP diluted at 1:5000 was added, and incubated at 37°C for 30 min. Subsequently, the ELISA plate was washed 4 times with PBS, and substrate TMB solution was added. It took about 15 min for the positive control well to develop color, and the color development reaction was terminated with H₂SO₄, and the reading was measured at OD₄₅₀.

MENTTSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGAPTCPGQNSQSPTSNH
SPTSCPPICPGYRWMCLRRFIIFLLFILLCLIFLLVLLDYQGMLPVCPLLPGTSTTSTGPCKTCTI
PAQGTSMFPSCCCTKPSDGNCTCIPISSWAFARFLWEWASVRFSWLSLLVPFVQWFVGLSPT
VWLSVIWMMWYWGPSLYNILSPFLPLLPIFFCLWVYIHSHHHHHH (SEQ ID NO:74)

The results are shown in Figure 1. The results show that the antigen binding activity of antibodies 032, 033, 042, 055, 060, 085, 088, 094, 100, 112, 113, and 116 under neutral conditions (i.e., pH 7.4) is not weaker than that of the positive control antibody 021, and the difference in binding activity between neutral conditions and acidic conditions (i.e., pH 6.0) is not less than that of 021.

Example 4: In vivo efficacy assay

The constant region of the antibody in this example is human IgG1.

Animal:

Male C57BL/6 mice, 5 weeks old, specific pathogen-free, were purchased from Shanghai Slack

Laboratory Animal Co., Ltd. and housed in individually ventilated cages. Mice were housed and used according to the experimental protocol approved by IACUC (Institutional Animal Care and Use Committee) of WuXi AppTec Co., Ltd. (IACUC#: ID01-013-2021v1.0). After the mice had spent 4 days adapting to the environment, they were injected with AAV/HBV virus (i.e., recombinant adeno-associated virus type 8 vector carrying 1.3 copies of hepatitis B virus rAAV8-1.3HBV, "AAV/HBV virus" and "rAAV8-1.3HBV" can be used interchangeably herein).

Solvent: PBS.

Dose of tested antibody: 15 mpk.

Recombinant rAAV8-1.3HBV: rAAV8-1.3HBV (type D, ayw) was provided by WuXi AppTec Co., Ltd., with batch number awy1-P4-200805, 1×10^{12} viral genome (v.g.)/mL, and was diluted to 1×10^{10} v.g./200 μ L with sterile PBS before the experiment. Each mouse was injected with 200 μ L, i.e., 1×10^{10} v.g. was injected into each mouse.

Assay method:

Establishment of AAV/HBV mouse model

AAV/HBV injection. rAAV8-1.3HBV was prepared in sterile PBS to a concentration of 1×10^{11} v.g./200 μ L solution before injection. 33 mice were injected with 200 μ L rAAV8-1.3HBV solution via the tail vein.

Blood collection before grouping: On days 14 and 21 after virus injection, blood (approximately 60 μ L) was collected from the submandibular vein of all infected mice for plasma isolation. The collected venous blood was anticoagulated with K2-EDTA, centrifuged at 4°C, 7000 g/min for 10 min, and the plasma was collected. Plasma was tested for HBsAg by ELISA. Plasma samples were stored at -80°C until sent to the in vitro laboratory of Biological Department of WuXi AppTec Co., Ltd. for testing related projects.

The first administration was recorded as day 0, and the 24th day after infection was day 0. The mice were grouped according to the plasma HBsAg levels collected on days 14 and 21 after virus injection. 33 mice for formal experiments were selected from 45 mice and randomly divided into 11 groups, labeled Group 1 to Group 11, with 3 mice in each group. All mice were injected 15 mpk once via tail vein with PBS or test antibody. All mice were weighed before administration.

Blood collection:

On days -14, -3, 1, 3, 5, 7, 10, 14, 17, 21, 24 and 28 after administration, all mice were bled

through the submandibular vein and plasma was collected for HBsAg detection.

Experimental endpoint: On day 28, all mice were first subjected to euthanasia after ~60 μ L of blood was collected from the submandibular vein for plasma collection and for HBsAg detection.

Body weight record: During the in vivo experiment, the status of mice was observed every day, and the body weight of mice was recorded on the day of drug administration and infection, as well as on the day of euthanasia.

Sample storage and transfer: All plasma samples were stored at -80°C and transferred on dry ice to the in vitro laboratory of Biological Department of WuXi AppTec Co., Ltd. for corresponding test.

ELISA was used to detect the HBsAg content in mouse plasma. The experimental steps were referred to the instructions of the HBsAg ELISA kit (Autobio Diagnostics Co., Ltd., CL 0310). The method was briefly described as follows: the plasma sample was diluted 20 or 600 times, added to the coated plate, and incubated with the enzyme conjugate (37°C , 60 minutes). The plate was washed 5 times, and the luminescent substrate was added to react at room temperature in the dark for 10 min. The luminescence intensity was detected with a Microplate Reader.

The inhibitory activity of each antibody against HBV replication in the AAV/HBV mouse model was evaluated by detecting the expression of HBsAg in mouse plasma. The results are shown in Figure 2.

The results show the effects of the test compounds on plasma HBsAg content in AAV/HBV mice.

The plasma HBsAg content of mice in the solvent group (Group 1) remains relatively stable during the experiment, fluctuating between 4.27 to 4.73 $\log_{10}$ IU/mL (fluctuation does not exceed 0.46 $\log_{10}$ IU/mL); compared with the solvent group, the plasma HBsAg content of mice in Treatment Group 2 (antibody 021, 15mpk) is significantly reduced on day 1 after administration, with an average decrease of 1.03 $\log_{10}$ IU/mL ($p < 0.01$), and then shows a gradual upward trend, and the plasma HBsAg content of mice is 4.23 $\log_{10}$ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 3 (antibody 033, 15mpk) is significantly reduced on the day 1 after administration, with an average decrease of 2.40 $\log_{10}$ IU/mL ($p < 0.01$), and then shows a gradual upward trend, and the plasma HBsAg content of mice is 4.23 $\log_{10}$ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 4 (antibody 055, 15mpk) is significantly reduced on day after 1 administration, with an average decrease of 2.61 $\log_{10}$ IU/mL ($p < 0.01$), and then shows a

gradual upward trend, and the plasma HBsAg content of mice is 2.97 log₁₀ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 5 (antibody 060, 15mpk) is significantly reduced on day 1 after administration, with an average decrease of 1.00 log₁₀ IU/mL ($p < 0.01$), and the plasma HBsAg content of mice is 4.02 log₁₀ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 6 (antibody 085, 15mpk) is significantly reduced on the day 1 after administration, with an average decrease of 1.27 log₁₀ IU/mL ($p < 0.01$), and the plasma HBsAg content of mice is 4.12 log₁₀ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 7 (antibody 088, 15mpk) is significantly reduced on the first day after administration, with an average decrease of 1.98 log₁₀ IU/mL ($p < 0.01$), and the plasma HBsAg content of mice is 4.23 log₁₀ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 8 (antibody 094, 15mpk) is significantly reduced on the first day after administration, with an average decrease of 1.93 log₁₀ IU/mL ($p < 0.01$), and the plasma HBsAg content of mice is 3.75 log₁₀ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 9 (antibody 100, 15mpk) is significantly reduced on the first day after administration, with an average decrease of 2.46 log₁₀ IU/mL ($p < 0.01$), and the plasma HBsAg content of mice is 4.53 log₁₀ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 10 (antibody 113, 15mpk) is significantly reduced on day 1 after administration, with an average decrease of 2.40 log₁₀ IU/mL ($p < 0.01$), and the plasma HBsAg content of mice is 4.52 log₁₀ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 11 (antibody 116, 15mpk) is significantly reduced on day 1 after administration, with an average decrease of 1.05 log₁₀ IU/mL ($p < 0.01$), and the plasma HBsAg content of mice is 4.04 log₁₀ IU/mL on day 28. At the same time, compared with the Treatment Group 2, the tested antibodies can significantly reduce the HBsAg content in plasma, wherein the Treatment Group 4 (antibody 055, 15mpk) has the most significant effect. One day after administration, the average decrease is 1.58 log₁₀ IU/mL, and 28 days after administration, the average decrease is 1.26 log₁₀ IU/mL.

Example 5: In vivo activity assay of antibodies 021, 055, 055-YD, and 055-HD

The experimental scheme was the same as that of Example 4, and the mice were divided into 5 groups, each with 4 mice, and the IgG1 used was mouse IgG1. G2 and G3 groups were injected with antibodies 021 and 055 (dosage: 9 mpk), G4 and G5 groups were injected with antibodies 055-HD

and 055-YD (dosage: 9 mpk) through the tail vein. Each group was administrated once, and the solvent group was injected with 15 mpk PBS. The HBsAg test results are shown in Figure 3. The specific results are as follows:

The contents of plasma HBsAg in mice of each group.

The plasma HBsAg content of mice in the solvent group (Group 1) remains relatively stable during the experiment, fluctuating between 4.41 to 4.61 log₁₀ IU/mL (fluctuation does not exceed 0.20 log₁₀ IU/mL). Compared with the solvent group, the plasma HBsAg content of mice in Treatment Group 2 (antibody 021m, 9mpk) is significantly decreased on day 1 after administration, with an average decrease of 1.65 log₁₀ IU/mL ($p < 0.01$), and then shows a gradual upward trend, and the plasma HBsAg content of mice is 3.62 log₁₀ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 3 (antibody 055, 9mpk) is significantly decreased on day 1 after administration, with an average decrease of 3.47 log₁₀ IU/mL ($p < 0.01$), and then shows a gradual upward trend, and the plasma HBsAg content of mice is 2.78 log₁₀ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 4 (antibody 055HD, 9mpk) is significantly decreased on day 1 after administration, with an average decrease of 3.25 log₁₀ IU/mL ($p < 0.01$), and then shows a gradual upward trend, and the plasma HBsAg content of mice is 2.68 log₁₀ IU/mL on day 28; the plasma HBsAg content of mice in Treatment Group 5 (antibody 055YD, 9mpk) is significantly decreased on day 1 after administration, with an average decrease of 3.21 log₁₀ IU/mL ($p < 0.01$), and then shows a gradual upward trend, and the plasma HBsAg content of mice is 3.09 log₁₀ IU/mL on day 28. At the same time, compared with the Treatment Group 2, the tested antibodies can significantly reduce the HBsAg content in plasma, wherein the Treatment Group 3 (antibody 055, 9mpk) has the most significant effect. One day after administration, the average decrease is 1.82 log₁₀ IU/mL, and 28 days after administration, the average decrease is 0.84 log₁₀ IU/mL.

CLAIMS

1. An antibody or an antigen-binding fragment thereof that specifically binds to hepatitis B virus surface antigen (HBsAg), wherein the antibody comprises a heavy chain variable region (VH) and a light chain variable region (VL), wherein:

VH comprises the complementary determining region (CDR) amino acid sequence as shown below:

HCDR1: GYTFTGYY (SEQ ID NO:1),

HCDR2: INPNSGGT (SEQ ID NO:2), and

HCDR3: ARDLWX1X2X3X4X5DYGX6DV; and

VL comprises the CDR amino acid sequences as shown below:

LCDR1: X7SIX8TX9,

LCDR2: AAS (SEQ ID NO:28), and

LCDR3: QQSYSTPLT (SEQ ID NO:29);

wherein:

X1 is selected from N, D, Y, I, S, V or M,

X2 is selected from F, H, E, W, R, Q, M, P or D,

X3 is selected from V, Q, P, R, H, T or Y,

X4 is selected from S, T, D, L, Y, F or H,

X5 is selected from D, Q or E,

X6 is selected from M, I or L,

X7 is selected from Q, S, H, Y, P, L, I or E,

X8 is selected from S or D, and

X9 is selected from Y, F, D, T, S or V.

2. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to claim 1, wherein the antibody comprises a complementary determining region (CDR) amino acid sequence as shown below:

HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:3, LCDR1 as shown in SEQ ID NO:15, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29; alternatively,

HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in

SEQ ID NO:14, LCDR1 as shown in SEQ ID NO:26, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29; alternatively

HCDR1 as shown in SEQ ID NO:1, HCDR2 as shown in SEQ ID NO:2, HCDR3 as shown in SEQ ID NO:6, LCDR1 as shown in SEQ ID NO:27, LCDR2 as shown in SEQ ID NO:28, and LCDR3 as shown in SEQ ID NO:29.

3. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to claim 1 or 2, which comprises a human universal framework region (FR).

4. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 3, wherein:

VH comprises the FR amino acid sequence as shown below:

HFR1: QVQLVESGAIEVKKPGASVKVSCAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: NYAQKFQGRVTMTRDTSISTAYMELSRSDDTAVYYC (SEQ ID NO:63), and

HFR4: WGKGTLVTVSS (SEQ ID NO:64); and

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFLTISLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

5. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 3, wherein:

VH comprises the FR amino acid sequence as shown below:

HFR1: QVQLVESGAIEVKKPGASVKVSCAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: YYAQKFQGRVTMTRDTSISTAYMELSRSDDTAVYYC (SEQ ID NO:69), and

HFR4: WGKGTLVTVSS (SEQ ID NO:64); and

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFLTISLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

6. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 3, wherein:

VH comprises the FR amino acid sequence as shown below:

HFR1: QVQLVESGAIEVKKPGASVKVSCAS (SEQ ID NO:61),

HFR2: MHWVRQAPGQGLEWMGW (SEQ ID NO:62),

HFR3: HYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC (SEQ ID NO:70), and

HFR4: WGKGTLLVTVSS (SEQ ID NO:64);

VL comprises the FR amino acid sequence as shown below:

LFR1: DIQLTQSPSSLSASVGDRVTITCRAS (SEQ ID NO:65),

LFR2: LNWYQQKPGKAPKLLIY (SEQ ID NO:66),

LFR3: SLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC (SEQ ID NO:67), and

LFR4: FGGGTKVDIKR (SEQ ID NO:68).

7. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 6, wherein the VH sequence and the VL sequence are selected from amino acid sequences in any of the following groups 1) to 14), or amino acid sequences that have at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity with the VH sequence and VL sequence in any of the following groups 1) to 14), respectively:

1) SEQ ID NO:31 and SEQ ID NO:46,

2) SEQ ID NO:32 and SEQ ID NO:47,

3) SEQ ID NO:33 and SEQ ID NO:48,

4) SEQ ID NO:34 and SEQ ID NO:49,

5) SEQ ID NO:35 and SEQ ID NO:50,

6) SEQ ID NO:36 and SEQ ID NO:51,

7) SEQ ID NO:37 and SEQ ID NO:52,

8) SEQ ID NO:38 and SEQ ID NO:53,

9) SEQ ID NO:39 and SEQ ID NO:54,

10) SEQ ID NO:40 and SEQ ID NO:55,

11) SEQ ID NO:41 and SEQ ID NO:56,

12) SEQ ID NO:42 and SEQ ID NO:57,

13) SEQ ID NO:43 and SEQ ID NO:58, or

14) SEQ ID NO:44 and SEQ ID NO:58.

8. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 7, wherein the antigen-binding fragment is selected from: Fab, F(ab')₂, Fab', scFv, Fv, Fd, dAb, diabody, or multibody.

9. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 8, wherein the antibody is a fully human antibody, a humanized antibody, a murine antibody, a chimeric antibody or a nanobody.

10. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 9, which is a monospecific antibody, a bispecific antibody, or a multispecific antibody.

11. The antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 10, wherein the antibody further comprises a heavy chain constant region and a light chain constant region; preferably the heavy chain constant region is selected from the heavy chain constant region of IgG, and the light chain constant region is selected from a κ chain or a λ chain.

12. A diagnostic reagent for diagnosing HBsAg infection, comprising the antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 11 that is labeled.

13. The diagnostic reagent according to claim 12, wherein the antibody or an antigen-binding fragment thereof that specifically binds to HBsAg is labeled with one or more labels selected from the group consisting of: a radioactive label, a fluorophore, a chromophore, an imaging agent, and a metal ion.

14. An isolated nucleic acid molecule, comprising a polynucleotide sequence encoding the antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 11.

15. A construct, comprising the nucleic acid molecule according to claim 14.

16. The construct according to claim 15, which is selected from a plasmid, a phagemid, a viral vector, or a linear nucleic acid.

17. A cell, expressing the antibody or an antigen-binding fragment thereof that specifically

binds to HBsAg according to any one of claims 1 to 11, or the nucleic acid according to claim 14, or the construct according to claim 15 or 16.

18. A pharmaceutical composition, comprising the antibody or an antigen-binding fragment thereof that specifically binds to HBsAg according to any one of claims 1 to 11, or the nucleic acid according to claim 14.

19. A diagnostic kit, comprising the diagnostic reagent according to claim 12 or 13.

20. A method for treating or preventing hepatitis B virus infection, comprising administering an effective amount of the pharmaceutical composition according to claim 18 to a patient.

21. A method for treating hepatitis B, comprising administering an effective amount of the pharmaceutical composition according to claim 18 to a patient.

22. A method for detecting hepatitis B virus, comprising contacting the diagnostic reagent according to claim 12 or 13 with a body fluid from a subject.

23. The method according to claim 22, wherein the body fluid from the subject is plasma or serum of the subject.