EPITOPES OF ANTI-SERINE PROTEASE INHIBITOR KAZAL (SPIK) ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of the filing date of US Provisional Patent Application Serial Number 62/899,024, filed on September 11, 2019, the disclosure of which application is herein incorporated by reference in its entirety.

GOVERNMENT RIGHTS

[0002] This invention was made with government support under grant number 2R44CA165314-02A1 and FAIN number R44CA165314 awarded by the National Institutes of Health (NIH) under the Small Business Innovation Research (SBIR) program. The government has certain rights in the invention.

SEQUENCE LISTING

[0002.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 12, 2020, is named IMC-0003-WO_SL.txt and is 50,298 bytes in size.

FIELD OF THE INVENTION

[0003] The present invention relates to two classes of anti-AS-SPIK antibodies that specifically bind to one of two different conformational epitopes, along with methods of making such antibodies, compositions, including pharmaceutical compositions, comprising such antibodies, and their use to diagnose and/or treat disorders characterized by the expression of AS-SPIK (e.g., liver cancer). Diagnostic methods and kits comprising the anti-AS-SPIK antibodies are also disclosed.

BACKGROUND

[0004] The liver is one of the largest organs in the body. The liver has many functions, including the production of enzymes and bile required for the digestion of food, regulation of glycogen storage, plasma protein synthesis, hormone production, and detoxification of various metabolites. Liver disorders include liver cancers such as Hepatocellular Carcinoma (HCC) and intrahepatic Cholangiocarcinoma (ICC), viral infections, cirrhosis, and other inflammatory disorders of the liver affect millions of people worldwide. For example, over 5 million individuals in the U.S. and over 450

million individuals worldwide suffer from hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, and over 30% of these infected individuals are at a high risk of developing liver cancer. (1-4) Despite advances in diagnosis and treatment, liver cancer remains an important cause of both morbidity and mortality. Primary liver cancer, or cancer that originates in the liver, has a five-year survival rate of less than 10%. However, if liver cancer is detected early and during its most treatable stages, the survival rate increases to almost 40%. Patients with early-stage liver cancer may have few or no symptoms. Current detection methods, such as serological methods, ultrasound, computed tomography (CT) scans, magnetic resonance imaging (MRI), and angiography, can be unreliable

HCC, even in its very early stages, without interference from other liver or non-liver diseases, which will tremendously improve the diagnosis and treatment of HCC.

SUMMARY OF THE INVENTION

[0005a] In one aspect, provided herein is an isolated antibody that specifically binds to a conformational epitope of an AS-SPIK protein (SEQ ID NO: 2), and does not specifically bind to a NS-SPIK protein (SEQ ID NO: 4), wherein the conformational epitope of the AS-SPIK protein comprises two separate regions comprising: one or more amino acids selected from the group consisting of: L14, L15, S16, L17, D24 and S25 of SEQ ID NO: 2; and one or more amino acids selected from the group consisting of: C58, V59, L60, C61, and F62 of SEQ ID NO: 2, wherein the antibody binds to at least one amino acid in each of the two separate regions.

[0005b] In one aspect, provided herein is an isolated antibody that specifically binds to a conformational epitope of an AS-SPIK protein (SEQ ID NO: 2), and does not specifically bind to a NS-SPIK protein (SEQ ID NO: 4), wherein the conformational epitope of the AS-SPIK protein comprises two separate regions comprising: one or more amino acids selected from the group consisting of: L36, N37, I42 and Y43 of SEQ ID NO: 2; and one or more amino acids selected from the group consisting of: R67, Q68, I71 and L72 of SEQ ID NO: 2, wherein the antibody binds to at least one amino acid in each of the two separate regions.

[0006] ~~Aspect~~Examples of the ~~invention~~disclosure include isolated antibodies that specifically bind to a conformational epitope of an AS-SPIK protein, and do not specifically bind to a NS-SPIK protein, wherein the conformational epitope of the AS-SPIK protein comprises: one or more amino acids selected from the group consisting of: L14, L15, S16, L17, D24 and S25 of SEQ ID NO: 2; and one or more amino acids selected from the group consisting of: C58, V59, L60, C61, and F62 of SEQ ID NO: 2.

[0007] In some ~~embodiment~~examples, the conformational epitope comprises amino acids L14, L15, S16, and L17 of SEQ ID NO: 2. In some ~~embodiment~~examples, the conformational epitope comprises amino acids L60 and C61 of SEQ ID NO: 2. In some ~~embodiment~~examples, the conformational epitope comprises amino acids L14, L15, S16, L17, L60, and C61 of SEQ ID NO: 2. In some ~~embodiment~~examples, the conformational epitope further comprises amino acids D24 and S25 of SEQ ID NO: 2. In some ~~embodiment~~examples, the conformational epitope further comprises amino acids C58, V59 and F62 of SEQ ID NO: 2. In some

~~embodiment~~examples, the conformational epitope comprises amino acids L14, L15, S16, L17, D24, S25, C58, V59, L60, C61 and F62 of SEQ ID NO: 2.

[0008] In some ~~embodiment~~examples, an isolated antibody comprises: a CDRH1 sequence comprising S6; and/or a CDRH2 sequence comprising I2, G5, G6, Y10 and K16; and/or a CDRH3 sequence comprising G4 and Y7; and/or a CDRL1 sequence comprising Q4 and S9; and/or a CDRL2 sequence comprising A2, S3, T4 and S7; and/or a CDRL3 sequence comprising Q1, Q2, Y4 and S5.

[0009] In some ~~embodiment~~examples, an isolated antibody comprises: a CDRH1 sequence comprising S6; a CDRH2 sequence comprising I2, G5, G6, Y10 and K16; a CDRH3 sequence comprising G4 and Y7; a CDRL1 sequence comprising Q4 and S9; a CDRL2 sequence comprising A2, S3, T4 and S7; and a CDRL3 sequence comprising Q1, Q2, Y4 and S5.

[0010] ~~Aspect~~Examples of the ~~invention~~disclosure include isolated antibodies that specifically bind to a conformational epitope of an AS-SPIK protein, and do not specifically bind to a NS-SPIK protein, wherein the conformational epitope of the AS-SPIK protein comprises: one or more amino acids selected from the group consisting of: L36, N37, I42 and Y43 of SEQ ID NO: 2; and one or more amino acids selected from the group consisting of: R67, Q68, I71 and L72 of SEQ ID NO: 2.

[0011] In some ~~embodiment~~examples, the conformational epitope comprises amino acids L36 and N37 of SEQ ID NO: 2. In some ~~embodiment~~examples, the conformational epitope comprises amino acids I42 and Y43 of SEQ ID NO: 2. In some ~~embodiment~~examples, the conformational epitope comprises amino acids L36, N37, I42 and Y43

of SEQ ID NO: 2. In some ~~embodiment~~examples, the conformational epitope comprises amino acids R67, Q68, I71 and L72 of SEQ ID NO: 2. In some ~~embodiment~~examples, the conformational epitope comprises amino acids L36, N37, I42, Y43, R67, Q68, I71 and L72 of SEQ ID NO: 2.

[0012] In some ~~embodiment~~examples, and antibody comprises: a CDRH1 sequence comprising Y3, S7 and W9; and/or a CDRH2 sequence comprising A1, I2, G4, G6 and Y10; and/or a CDRH3 sequence comprising R1 and D7; and/or a CDRL1 sequence comprising A2, S3, Q4, I6, Y9, L10 and S11; and/or a CDRL2 sequence comprising A2, S3, L5 and S7; and/or a CDRL3 sequence comprising Q1, Q2, and T5.

[0013] In some ~~embodiment~~examples, an antibody comprises: a CDRH1 sequence comprising Y3, S7 and W9; a CDRH2 sequence comprising A1, I2, G4, G6 and Y10; a CDRH3 sequence comprising R1 and D7; a CDRL1 sequence comprising A2, S3, Q4, I6, Y9, L10 and S11; a CDRL2 sequence comprising A2, S3, L5 and S7; and a CDRL3 sequence comprising Q1, Q2, and T5.

[0014] In some ~~embodiment~~examples, an antibody is multi-specific. In some ~~embodiment~~examples, an antibody is bispecific. In some ~~embodiment~~examples, an antibody has binding affinity to an effector cell. In some ~~embodiment~~examples, an antibody has binding affinity to a T-cell antigen. In some ~~embodiment~~examples, the T-cell antigen comprises a CD3 protein. In some ~~embodiment~~examples, an antibody is a monoclonal antibody. In some ~~embodiment~~examples, an antibody is in a CAR-T format.

[0015] ~~Aspect~~Examples of the ~~invention~~disclosure include immunoconjugates comprising an antibody as described herein, covalently attached to a cytotoxic agent. In some ~~embodiment~~examples, the cytotoxic agent is selected from the group consisting of: a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope and a nucleolytic enzyme.

[0016] In some ~~embodiment~~examples, an immunoconjugate has the formula Ab-(L-D)p, wherein: Ab is an antibody as described herein; L is a linker; D is a drug moiety; and p is an integer that ranges from 1 to 8. In some ~~embodiment~~examples, D is selected from the group consisting of: a maytansinoid, an auristatin and dolostatin. In some ~~embodiment~~examples, L comprises one or more linkers selected from the group consisting of 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio)pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1 carboxylate (SMCC), 4-(2-Pyridyldithio)butyric acid-N-hydroxysuccinimide ester (SPDB), and N-Succinimidyl (4-iodo-acetyl)aminobenzoate (SIAB).

[0017] ~~Aspects of the invention include~~In one aspect, provided herein are pharmaceutical compositions comprising and antibody or immunoconjugate as described herein.

[0018] ~~Aspect~~Examples of the ~~invention~~disclosure include methods for the treatment of a disorder characterized by expression of AS-SPIK, comprising administering to a subject with said disorder an antibody or immunoconjugate as described herein, or a pharmaceutical composition as described herein.

[0019] ~~Aspects of the invention include~~In one aspect, provided herein is a use of an antibody or immunoconjugate as described herein, in the preparation of a medicament for the treatment of a disorder characterized by expression of AS-SPIK.

[0020] ~~Aspect~~Examples of the ~~invention~~disclosure include an antibody or immunoconjugate as described herein for use in the treatment of a disorder characterized by expression of AS-SPIK.

[0021] In some ~~embodiment~~examples, the disorder is a liver disorder. In some ~~embodiment~~examples, the liver disorder is hepatocellular carcinoma. In some ~~embodiment~~examples, the liver disorder is intrahepatic cholangiocarcinoma. In some ~~embodiment~~examples, the liver disorder is a viral infection. In some ~~embodiment~~examples, the liver disorder is an inflammatory liver disorder. In some ~~embodiment~~examples, the inflammatory liver disorder is cirrhosis of the liver.

[0022] In one aspect, provided herein is ~~Aspects of the invention include~~ a polynucleotide encoding an antibody as described herein. In one aspect, provided herein is ~~Aspects of the invention include~~ a vector comprising a polynucleotide as described herein. In one aspect, provided herein is ~~Aspects of the invention include~~ a host cell comprising a vector as described herein.

[0023] ~~Aspect~~Examples of the ~~invention~~disclosure include methods of producing an antibody or immunoconjugate as described herein, comprising growing a host cell as described herein under conditions permissive for expression of the antibody, and isolating the antibody from the cell.

[0024] In one aspect, provided herein is ~~Aspects of the invention include~~ a diagnostic method for determining whether a subject has or is at risk of developing a disorder characterized by expression of AS-SPIK, the method comprising: contacting a biological test sample from the subject with an AS-SPIK antibody as described herein to generate an AS-SPIK-antibody complex; detecting a concentration of the AS-SPIK-antibody complex in the biological test sample; and comparing the concentration of the AS-SPIK-antibody complex to a reference value to determine whether the subject has or is at risk of developing the disorder.

[0025] In one aspect, provided herein is ~~Aspects of the invention include~~ a diagnostic method for determining whether a subject has or is at risk of developing a disorder characterized by expression of AS-SPIK, the method comprising: contacting a biological test sample from the subject with a first antibody or antigen-binding fragment that specifically binds to SPIK to form a SPIK-antibody complex; contacting the SPIK-antibody complex with an AS-SPIK antibody or antigen-binding fragment as described herein to generate an AS-SPIK-antibody complex; detecting a concentration of the AS-SPIK-antibody complex in the biological test sample; and comparing the concentration of the

AS-SPIK-antibody complex to a reference value to determine whether the subject has or is at risk of developing the disorder.

[0026] In some embodiments, the antibody or antigen-binding fragment comprises a detectable label. In some ~~embodiment~~examples, the disorder is a liver disorder. In some ~~embodiment~~examples, the liver disorder is selected from the group consisting of: hepatocellular carcinoma, intrahepatic cholangiocarcinoma, viral infection of the liver, inflammatory disorder of the liver, and cirrhosis of the liver.

[0027] ~~Aspects of the invention include~~In one aspect, provided herein are kits comprising an antibody or immunoconjugate as described herein. In some ~~embodiment~~examples, a kit further comprises an antibody that specifically binds to SPIK.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] FIG. 1 shows the size of AS-SPIK and NS-SPIK using gel electrophoresis.

[0029] FIG. 2 shows the N-terminal sequence of AS-SPIK by Edman N-terminal analysis. FIG. discloses "KVTGI" as SEQ ID NO: 85.

[0030] FIG. 3 shows a comparison of the amino acid sequences of AS-SPIK and NS-SPIK (SEQ ID NOS 2 and 4, respectively).

[0031] FIG. 4 shows a comparison of the 3D structure of AS-SPIK with that of NS-SPIK.

[0032] FIG. 5 shows the test results of binding activity of antibodies that bind to AS-SPIK and NS-SPIK.

[0033] FIG. 6 shows the results of binding tests of anti-AS-SPIK antibodies to synthesized peptides (SEQ ID NOS 86-89, respectively, in order of appearance).

[0034] FIG. 7 the shows the 3D structure (crystal model) of AS-SPIK and its epitopes.

[0035] FIG. 8 shows the result of inhibition tests for locating the binding site of Class I anti-AS-SPIK antibodies. FIG. discloses SEQ ID NOS 90-103, respectively, in order of appearance.

[0036] FIG. 9 shows the consensus amino acids of CDRs in Class I anti-AS-SPIK antibodies.

[0037] FIG. 10 shows the consensus amino acids of CDRs in Class II anti-AS-SPIK antibodies.

[0038] FIG. 11 provides an illustration of the mechanism for the AS-SPIK detection kit.

[0039] FIG. 12, panels A, is a graph showing that IM-CA22 binds specifically to AS-SPIK but not NS-SPIK, while antibody IM-BA2 binds to both AS-SPIK and NS-SPIK. Panel B is a graph showing that AS-SPIK is elevated in HCC, but not in pancreatitis or healthy patients. Panel C is a graph showing that NS-SPIK is elevated in pancreatitis patients but does not interfere with the performance of the AS-SPIK-based detection kit.

[0040] FIG. 13 is a table (Table 1) that shows the binding characteristics of Class I and Class II anti-AS-SPIK antibodies.

[0041] FIG. 14 is a table (Table 2) that shows the regions for anti-AS-SPIK binding predicted by a CLIPS analysis study, and the amino acid residues that constitute the epitopes therein. FIG. discloses the CLIPS predicted Region 1, Region 4, Region 2, and Region 4 sequences as SEQ ID NOS 104-108 and 7, SEQ ID NOS 109-113 and 8, SEQ ID NOS 114-119 and 9, and SEQ ID NOS 120-125 and 10, all respectively, in order of appearance.

[0042] FIG. 15 is a table (Table 3) that shows the amino acid sequences of four example Class I antibodies that bind to Epitope I, as described further herein.

[0043] FIG. 16 is a table (Table 4) that shows the amino acid sequences of four example Class II antibodies that bind to Epitope II, as described further herein.

[0044] FIG. 17 is a table (Table 5) that shows serum AS-SPIK levels in a clinical study with 512 samples.

[0045] FIG. 18 is a table (Table 6) that provides a performance summary of AS-SPIK v. AFP in the detection of HCC.

[0046] FIG. 19 is a table (Table 7) that shows AS-SPIK levels in early v. late stage HCC.

[00134] An “Fv” fragment is the minimum antibody fragment that contains a complete antigen-recognition and binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, con-covalent association. It is in this configuration that three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. While six hypervariable regions confer antigen-binding specificity, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site. To improve stability, the VH-VL domains may be connected by a flexible peptide linker such as (Gly4Ser)3 (SEQ ID NO: 83) to form a single chain Fv or scFV antibody fragment or may be engineered to form a disulfide bond by introducing two cysteine residues in the framework regions to yield a disulfide stabilized Fv (dsFv). Fragments of antibodies are suitable for use in the methods provided so long as they retain the desired epitope specificity of the full-length antibody and/or sufficient specificity to bind AS-SPIK and not NS-SPIK.

[00135] The antigen-binding domains of the antibodies described herein can be utilized in the production of T-cell engager molecules (e.g., bispecific T-cell engagers, aka BiTE molecules) as well as CAR-T structures. T-cell engager molecules are described, for example, in Huehls et al., Bispecific T cell engagers for cancer immunotherapy, Immunol Cell Biol. 2015 Mar; 93(3):290-296. CAR-T structures comprising single-domain antibodies as a binding (targeting) domain are described, for example, in Iri-Sofla et al., 2011, Experimental Cell Research 317:2630-2641 and Jamnani et al., 2014, Biochim Biophys Acta, 1840:378-386.

[00136] Methods for preparing antibody fragments encompass both biochemical methods (e.g. proteolytic digestion of intact antibodies which may be followed by chemical cross-linking) and recombinant DNA-based methods in which immunoglobulin sequences are genetically engineered to direct the synthesis of the desired fragments. Antibody fragments can be obtained by proteolysis of the whole immunoglobulin by the non-specific thiolprotease, papain. Papain digestion yields two identical antigen-binding fragments, termed “Fab fragments,” each with a single antigen-binding site, and a residual “Fc fragment.” The various fractions can be separated by protein A-Sepharose or ion exchange chromatography. The usual procedure for preparation of F(ab')2 fragments from IgG of rabbit and human origin is limited proteolysis by the enzyme pepsin. Pepsin treatment of intact antibodies yields an F(ab')2 fragment that has two antigen-combining sites and is still capable of cross-linking antigen. A Fab fragment contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain

data confirms that antibodies which selectively bind to AS-SPIK and not NS-SPIK can be developed in multiple animal models.

[00234] As a control, we simultaneously developed a series of monoclonal antibodies in mice, using a peptide containing the sequence $_{36}$ELNGCTKIYDPV$_{47}$ (SEQ ID NO: 84), which is a common sequence in both AS-SPIK and NS-SPIK (SEQ ID NO: 4) and is colored green in FIG. 4. This led to the production of antibody IM-BA2, which binds to both AS-SPIK and NS-SPIK (data not shown). However, we were unable to generate an antibody that binds specifically to AS-SPIK but not NS-SPIK using this method, suggesting that epitopes specific to AS-SPIK are conformational and not linear.

Example 4. The anti-AS-SPIK antibodies are conformation-dependent

[00235] In order to further support the observation that epitopes specific to AS-SPIK and not NS-SPIK are conformation-dependent, additional tests to evaluate antibody binding to various synthesized peptides was completed. Four peptides, each containing sequences from different regions of AS-SPIK were synthesized by BioMatik (Wilmington DE). Peptide A contains the entire sequence of AS-SPIK, but without the formation of any disulfide bonds via the inactivation of cysteine side-groups. Peptide B contains the sequence of AS-SPIK fragment from M_1- G_{50}, Peptide C contains the sequence of AS-SPIK fragment from D_{23}-G_{50} and Peptide D contains the sequence of AS-SPIK fragment from N_{51}-C_{79}. The binding activity of our monoclonal antibodies to AS-SPIK and the other peptides was tested by ELISA and results for IM-CA22 are shown in FIG. 6. Similar results were obtained from all other monoclonal anti-AS-SPIK antibodies we have developed, including IM-CA18, IM-CA46, IM-CA77, IM-A1, IM-A6, IM-B10, IM-C6, IM-D3, IM-D5, IM-E2, IM-F5, IM-G6 and IM-G7. Briefly, a 96-well plate was coated with Native AS-SPIK and different peptides, the plate then reacted with anti-AS-SPIK antibody (IM-CA22 herein) labeled with HRP. The OD450nm was determined after incubating the plate with the substrate, TMB. FIG. 6 shows that antibody IM-CA22 only recognizes native protein, but does not recognize any of the synthesized peptides, including Peptide A, which contains the entire native AS-SPIK sequence. This result further supports the observation that anti-AS-SPIK antibodies are conformation-dependent antibodies. In contrast, our polyclonal antibody Poly S recognizes all synthesized peptides as well as native AS-SPIK (FIG. 6, Poly S), suggesting there are both linear epitope antibodies as well as conformation-dependent epitope antibodies in Poly S. However, the differences in conformation that we identified above, in conjunction with these test results, strongly demonstrate that the monoclonal antibodies described herein have conformation-dependent epitopes.

separate regions, implying that all anti-AS-SPIK antibodies described herein bind to discontinuous, conformational epitopes. This is consistent with our previous observation that anti-AS-SPIK antibodies are exclusively conformational dependent antibodies.

Example 7. The critical residues of epitope I and epitope II

[00241] In addition to identifying the potential binding regions of AS-SPIK for both classes of antibodies, the CLIPS study also evaluates the relative importance of amino acids within each region to its binding affinity. Region 1 ($_{7}$FLLSALALLSLSGNTGADSLGREA$_{29,}$ SEQ ID NO: 7) and region 4 ($_{58}$CVLCFENRKRQ$_{68,}$ SEQ ID NO: 8) comprise the essential binding sites for all Class I antibodies. The most critical amino acids for binding functionality in region 1 are $_{14}$LLSL$_{17}$ (SEQ ID NO: 12), and to a lesser degree $_{24}$DS$_{25}$ (SEQ ID NO: 13). In region 4, the critical residues are $_{58}$CVLCF$_{26}$ (SEQ ID NO: 14). Together, these amino acids comprise a discontinuous conformational epitope for all Class I antibodies, called Epitope I (Table 2 (FIG. 14) and FIG. 7). The discontinuous nature of this epitope is further supported by an inhibition test we performed to confirm these findings (shown in FIG. 8). Here, we synthesized a short peptide with 9 amino acids containing $_{14}$LLSL$_{17}$ (SEQ ID NO: 12), which can inhibit the binding between native AS-SPIK and Class I antibody in this region. However, the inhibition of binding is incomplete, suggesting that there is a distinct second region of AS-SPIK that is responsible for the binding. This is consistent with our hypothesis that the anti-AS-SPIK binds a discontinuous conformational dependent epitope which should involve at least two separate regions in AS-SPIK. The CLIPS study also shows that the amino acids in region 2 ($_{36}$LNGCTKIYD$_{44,}$ SEQ ID NO: 9) and region 4 ($_{64}$NRKRQTSILIQ$_{75,}$ SEQ ID NO: 10) comprise the essential binding sites for all Class II antibodies. The most critical amino acids for binding functionality in region 2 are $_{36}$LN$_{37}$ (SEQ ID NO: 15) and $_{42}$IY$_{43}$ (SEQ ID NO: 16). In region 4, the critical residues are $_{67}$RQ$_{68}$ (SEQ ID NO: 17) and $_{71}$IL$_{72}$ (SEQ ID NO: 18). Together, these amino acids comprise a discontinuous conformational epitope for all Class II antibodies, called Epitope II (Table 2 (FIG. 14) and FIG. 7). Results from the CLIPS study on binding epitopes for Poly S suggests that the dominant antibody in poly S can be categorized as a Class II anti-AS-SPIK antibody, because it binds to Epitope II, similar to other Class II antibodies.

Example 8. Consensus sequences within Class I and Class II antibody CDRs

[00242] Now that we have defined Epitopes I and II and identified examples of antibodies that bind to them, the next step is to identify structural similarities between antibodies within each class. The CDRs in 4 Class I antibodies (IM-A (rabbit), IM-B10 (rabbit), IM-CA22 (mouse) and IM-CA18 (mouse)) were compared and analyzed. Consensus sequences of their CDRs were determined using the software BioEdit (North

CLAIMS

What is claimed is:

1. An isolated antibody that specifically binds to a conformational epitope of an AS-SPIK protein (SEQ ID NO: 2), and does not specifically bind to a NS-SPIK protein (SEQ ID NO: 4), wherein the conformational epitope of the AS-SPIK protein comprises two separate regions comprising:

one or more amino acids selected from the group consisting of: L14, L15, S16, L17, D24 and S25 of SEQ ID NO: 2; and

one or more amino acids selected from the group consisting of: C58, V59, L60, C61, and F62 of SEQ ID NO: 2,

wherein the antibody binds to at least one amino acid in each of the two separate regions.

2. The isolated antibody of claim 1, wherein the conformational epitope comprises amino acids L14, L15, S16, and L17 of SEQ ID NO: 2.

3. The isolated antibody of claim 1, wherein the conformational epitope comprises amino acids L60 and C61 of SEQ ID NO: 2.

4. The isolated antibody of claim 1, wherein the conformational epitope comprises amino acids L14, L15, S16, L17, L60, and C61 of SEQ ID NO: 2.

5. The isolated antibody of claim 2, wherein the conformational epitope further comprises amino acids D24 and S25 of SEQ ID NO: 2.

6. The isolated antibody of claim 3, wherein the conformational epitope further comprises amino acids C58, V59 and F62 of SEQ ID NO: 2.

7. The isolated antibody of claim 1, wherein the conformational epitope comprises amino acids L14, L15, S16, L17, D24, S25, C58, V59, L60, C61 and F62 of SEQ ID NO: 2.

8. The isolated antibody of any one of claims 1-7, comprising:

a CDRH1 sequence comprising S6; and/or

a CDRH2 sequence comprising I2, G5, G6, Y10 and K16; and/or

a CDRH3 sequence comprising G4 and Y7; and/or

a CDRL1 sequence comprising Q4 and S9; and/or

a CDRL2 sequence comprising A2, S3, T4 and S7; and/or

a CDRL3 sequence comprising Q1, Q2, Y4 and S5.

9. The isolated antibody of claim 8, comprising:

a CDRH1 sequence comprising S6;

a CDRH2 sequence comprising I2, G5, G6, Y10 and K16;

a CDRH3 sequence comprising G4 and Y7;

a CDRL1 sequence comprising Q4 and S9;

a CDRL2 sequence comprising A2, S3, T4 and S7; and

a CDRL3 sequence comprising Q1, Q2, Y4 and S5.

10. An isolated antibody that specifically binds to a conformational epitope of an AS-SPIK protein (SEQ ID NO: 2), and does not specifically bind to a NS-SPIK protein (SEQ ID NO: 4), wherein the conformational epitope of the AS-SPIK protein comprises two separate regions comprising:

one or more amino acids selected from the group consisting of: L36, N37, I42 and Y43 of SEQ ID NO: 2; and

one or more amino acids selected from the group consisting of: R67, Q68, I71 and L72 of SEQ ID NO: 2,

wherein the antibody binds to at least one amino acid in each of the two separate regions.

11. The isolated antibody of claim 10, wherein the conformational epitope comprises amino acids L36 and N37 of SEQ ID NO: 2.

12. The isolated antibody of claim 10, wherein the conformational epitope comprises amino acids I42 and Y43 of SEQ ID NO: 2.

13. The isolated antibody of claim 10, wherein the conformational epitope comprises amino acids L36, N37, I42 and Y43 of SEQ ID NO: 2.

14. The isolated antibody of claim 10, wherein the conformational epitope comprises amino acids R67, Q68, I71 and L72 of SEQ ID NO: 2.

15. The isolated antibody of claim 10, wherein the conformational epitope comprises amino acids L36, N37, I42, Y43, R67, Q68, I71 and L72 of SEQ ID NO: 2.

16. The isolated antibody of any one of claims 10-15, wherein the antibody comprises:
a CDRH1 sequence comprising Y3, S7 and W9; and/or
a CDRH2 sequence comprising A1, I2, G4, G6 and Y10; and/or
a CDRH3 sequence comprising R1 and D7; and/or
a CDRL1 sequence comprising A2, S3, Q4, I6, Y9, L10 and S11; and/or
a CDRL2 sequence comprising A2, S3, L5 and S7; and/or
a CDRL3 sequence comprising Q1, Q2, and T5.

17. The isolated antibody of claim 16, wherein the antibody comprises:
a CDRH1 sequence comprising Y3, S7 and W9;
a CDRH2 sequence comprising A1, I2, G4, G6 and Y10;
a CDRH3 sequence comprising R1 and D7;
a CDRL1 sequence comprising A2, S3, Q4, I6, Y9, L10 and S11;
a CDRL2 sequence comprising A2, S3, L5 and S7; and
a CDRL3 sequence comprising Q1, Q2, and T5.

~~18. The antibody of any one of claims 1-17, which is multi-specific.~~

~~19. The antibody of claim 18, which is bispecific.~~

~~20. The antibody of claim 19, having binding affinity to an effector cell.~~

~~21. The antibody of claim 19, having binding affinity to a T-cell antigen.~~

~~22. The antibody of claim 21, wherein the T-cell antigen comprises a CD3 protein.~~

18~~23~~. The antibody of any one of claims 1-17~~22~~, which is a monoclonal antibody.

~~24. The antibody of any one of claims 1-22, which is in a CAR-T format.~~

~~25. An immunoconjugate comprising an antibody according to any one of claims 1-23, covalently attached to a cytotoxic agent.~~

~~26. The immunoconjugate of claim 25, wherein the cytotoxic agent is selected from the group consisting of: a toxin, a chemotherapeutic agent, a drug moiety, an antibiotic, a radioactive isotope and a nucleolytic enzyme.~~

~~27. An immunoconjugate having the formula Ab-(L-D)p, wherein:~~

~~(a) Ab is an antibody according to any one of claims 1-23;~~

~~(b) L is a linker;~~

~~(c) D is a drug moiety; and~~

~~(d) p is an integer that ranges from 1 to 8.~~

~~28. The immunoconjugate of claim 27, wherein D is selected from the group consisting of: a maytansinoid, an auristatin and dolostatin.~~

~~29. The immunoconjugate of claim 27, wherein L comprises one or more linkers selected from the group consisting of 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio)pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), 4-(2-Pyridyldithio)butyric-acid-N-hydroxysuccinimide-ester (SPDB), and N-Succinimidyl (4-iodo-acetyl)aminobenzoate (SIAB).~~

19~~30~~. A pharmaceutical composition comprising the antibody or immunoconjugate of any one of claims 1-18~~29~~.

~~31. A method for the treatment of a disorder characterized by expression of AS-SPIK, comprising administering to a subject with said disorder an antibody or immunoconjugate of any one of claims 1-29, or the pharmaceutical composition of claim 30.~~

20~~32~~. Use of an antibody or immunoconjugate of any one of claims 1-18~~29~~, in the preparation of a medicament for the treatment of a disorder characterized by expression of AS-SPIK.

~~33. An antibody or immunoconjugate of any one of claims 1-29 for use in the treatment of a disorder characterized by expression of AS-SPIK.~~

2134. The ~~method or~~ use of ~~any one of~~ claim~~s~~ 2031-33, wherein the disorder is a liver disorder.

2235. The ~~method or~~ use of claim 2134, wherein the liver disorder is selected from the group consisting of: hepatocellular carcinoma, intrahepatic cholangiocarcinoma, viral infection of the liver, inflammatory disorder of the liver, and cirrhosis of the liver~~hepatocellular carcinoma~~.

~~36. The method or use of claim 34, wherein the liver disorder is intrahepatic cholangiocarcinoma.~~

~~37. The method or use of claim 34, wherein the liver disorder is a viral infection.~~

~~38. The method or use of claim 34, wherein the liver disorder is an inflammatory liver disorder.~~

~~39. The method or use of claim 38, wherein the inflammatory liver disorder is cirrhosis of the liver.~~

2340. A polynucleotide encoding an antibody of any one of claims 1-1824.

2441. A vector comprising the polynucleotide of claim 2340.

2542. A host cell comprising the vector of claim 2441.

~~43. A method of producing an antibody or immunoconjugate of any one of claims 1-29, comprising growing a host cell according to claim 42 under conditions permissive for expression of the antibody, and isolating the antibody from the cell.~~

2644. A diagnostic method for determining whether a subject has or is at risk of developing a disorder characterized by expression of AS-SPIK, the method comprising:

(a) contacting a biological test sample from the subject with an AS-SPIK antibody according to any one of claims 1-1823 to generate an AS-SPIK-antibody complex;

(b) detecting a concentration of the AS-SPIK-antibody complex in the biological test sample; and

(c) comparing the concentration of the AS-SPIK-antibody complex to a reference value to determine whether the subject has or is at risk of developing the disorder.

2745. A diagnostic method for determining whether a subject has or is at risk of developing a disorder characterized by expression of AS-SPIK, the method comprising:

(a) contacting a biological test sample from the subject with a first antibody or antigen-binding fragment that specifically binds to SPIK to form a SPIK-antibody complex;

(b) contacting the SPIK-antibody complex with an AS-SPIK antibody or antigen-binding fragment according to any one of claims 1-1823 to generate an AS-SPIK-antibody complex;

(c) detecting a concentration of the AS-SPIK-antibody complex in the biological test sample; and

(d) comparing the concentration of the AS-SPIK-antibody complex to a reference value to determine whether the subject has or is at risk of developing the disorder.

2846. The diagnostic method according to claim 2644 or 2745, wherein the antibody or antigen-binding fragment comprises a detectable label.

2947. The diagnostic method according to claim 2644 or 2745, wherein the disorder is a liver disorder.

3048. The diagnostic method according to claim 2747, wherein the liver disorder is selected from the group consisting of: hepatocellular carcinoma, intrahepatic cholangiocarcinoma, viral infection of the liver, inflammatory disorder of the liver, and cirrhosis of the liver.

3149. A kit comprising an antibody or immunoconjugate according to any one of claims 1-1829.

3250. The kit according to claim 3149, further comprising an antibody that specifically binds to SPIK.