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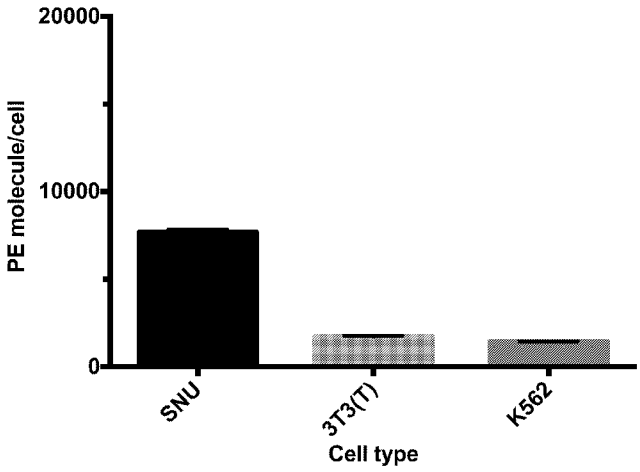
(54) Title: ANTI-SAS1B ANTIBODIES AND METHODS OF USE

Fig. 2
Cell surface density of SAS1B



(57) Abstract: The disclosure provides anti-SAS1 B antibodies, antigen-binding fragments thereof, and antibody-drug conjugates and methods of their use.

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— *with sequence listing part of description (Rule 5.2(a))*

Anti-SAS1B Antibodies and Methods of Use

Priority

This application claims the benefit of U.S. Ser. No. 62/298941 filed on February 23, 2016, which is incorporated by reference in its entirety.

Sequence Listing

This document incorporates by reference herein an electronic sequence listing text file, which is filed in electronic format via EFS-Web. The text file is named "15-1619-WO.SL.txt," is 58,700 bytes, and was created on February 17, 2017.

Background

Methods are needed in the art to identify and inhibit the growth of cells expressing SAS1B.

Summary

One embodiment provides an isolated antibody or antigen-binding portion thereof comprising: (a) a VH CDR1 of SEQ ID NO:1, 7, 9, 14, 25, 31, 37, 43, 48, or 54; a VH CDR2 of SEQ ID NO:2, 10, 15, 26, 32, 38, 44, 49, or 55; a VH CDR3 of SEQ ID NO:3, 8, 16, 27, 33, 39, 45, 50, 56, or GGL; a VL CDR1 of SEQ ID NO:4, 11, 17, 28, 34, 40, 46, 51, or 57; a VL CDR2 of SEQ ID NO:5, 12, 18, 29, 35, 41, 47, 35, or 58; and a VL CDR3 of SEQ ID NO:6, 13, 19, 30, 36, 42, 53, or 59; or (b) a VH CDR1 of SEQ ID NO:20, a VH CDR2 of SEQ ID NO:21, a VH CDR3 of SEQ ID NO:22, a VL CDR1 of SEQ ID NO:23, a VL CDR2 of FAS, a VL CDR3 of SEQ ID NO:24 or 95% identity thereto. In one embodiment, the antibody comprises: (a) a VH CDR1 of SEQ ID NO:1, a VH CDR2 of SEQ ID NO:2, a VH CDR3 of SEQ ID NO:3, a VL CDR1 of SEQ ID NO:4, a VL CDR2 of SEQ ID NO:5, a VL CDR3 of SEQ ID NO:6; (b) a VH CDR1 of SEQ ID NO:7, a VH CDR2 of SEQ ID NO:2, a VH CDR3 of SEQ ID NO:8, a VL CDR1 of SEQ ID NO:4, a VL CDR2 of SEQ ID NO:5, a VL CDR3 of SEQ ID NO:6; or (c) a VH CDR1 of SEQ ID NO:9, a VH CDR2 of SEQ ID NO:10, a VH CDR3 of GLL, a VL CDR1 of SEQ ID NO:11, a VL CDR2 of SEQ ID NO:12, a VL CDR3 of SEQ ID NO:13; (d) a VH CDR1 of SEQ ID NO:14, a VH CDR2 of SEQ ID NO:15, a VH CDR3 of SEQ ID NO:16, a VL CDR1 of SEQ ID NO:17, a VL CDR2 of SEQ ID NO:18, a VL CDR3 of SEQ ID NO:19; (e) a VH CDR1 of SEQ ID NO:25, a VH CDR2 of SEQ ID NO:26, a VH CDR3 of SEQ ID NO:27, a VL CDR1 of SEQ ID NO:28, a VL CDR2 of SEQ ID NO:29, a VL CDR3 of SEQ ID NO:30; (f) a VH CDR1 of SEQ ID NO:31, a VH CDR2 of SEQ ID NO:32, a VH CDR3 of SEQ ID

NO:33, a VL CDR1 of SEQ ID NO:34, a VL CDR2 of SEQ ID NO:35, a VL CDR3 of SEQ ID NO:36; (g) a VH CDR1 of SEQ ID NO:37, a VH CDR2 of SEQ ID NO:38, a VH CDR3 of SEQ ID NO:39, a VL CDR1 of SEQ ID NO:40, a VL CDR2 of SEQ ID NO:41, a VL CDR3 of SEQ ID NO:42; (h) a VH CDR1 of SEQ ID NO:43, a VH CDR2 of SEQ ID NO:44, a VH CDR3 of SEQ ID NO:45, a VL CDR1 of SEQ ID NO:46, a VL CDR2 of SEQ ID NO:47, a VL CDR3 of SEQ ID NO:42; (i) a VH CDR1 of SEQ ID NO:48, a VH CDR2 of SEQ ID NO:49, a VH CDR3 of SEQ ID NO:50, a VL CDR1 of SEQ ID NO:51, a VL CDR2 of SEQ ID NO:35, a VL CDR3 of SEQ ID NO:53; or (j) a VH CDR1 of SEQ ID NO:54, a VH CDR2 of SEQ ID NO:55, a VH CDR3 of SEQ ID NO:56, a VL CDR1 of SEQ ID NO:57, a VL CDR2 of SEQ ID NO:58, a VL CDR3 of SEQ ID NO:59.

Another embodiment provides an isolated antibody or antigen-binding portion thereof that specifically binds human SAS1B, wherein said antibody binds the same human SAS1B epitope recognized by a monoclonal antibody disclosed herein .

One embodiment provides an isolated antibody or antigen-binding portion thereof, wherein said antibody or antigen-binding portion specifically binds human SAS1B, wherein the antibody or antigen-binding portion thereof competes for binding with the antibody or antigen-binding portion thereof discussed herein.

Another embodiment provides an isolated antibody or antigen-binding portion thereof, wherein said antibody or antigen-binding portion thereof inhibits the binding of the isolated antibody or antigen-binding portion thereof of discussed to human SAS1B.

In one embodiment, the antibody or antigen-binding portion thereof specifically binds to a polypeptide consisting of amino acids 55-289 or 280-430 of SEQ ID NO:112.

In another embodiment, the antibody or antigen-binding portion thereof is a monoclonal antibody, a chimeric antibody, a humanized antibody, a synthetic antibody, a single chain antibody, a diabody, or a CDR-grafted antibody.

In another embodiment, the antibody or antigen-binding portion thereof comprises a VL amino acid sequence of SEQ ID NOs:76, 78, 80, 82, 84, 101,103, 105, 107, 109, or 111.

In another embodiment, the antibody or antigen-binding portion thereof comprises the VH amino acid sequence of SEQ ID NOs:75, 77, 79, 81, 83, 100, 102, 104, 106, 108, or 110.

One embodiment provides a composition comprising: (a) the antibody or antigen-binding portion thereof disclosed herein and a pharmaceutically acceptable carrier; or (b) the antibody or antigen-binding disclosed herein, wherein the antibody or antigen-binding portion thereof is conjugated to a therapeutic agent, and a pharmaceutically acceptable carrier.

Another embodiment provides an antibody-drug conjugate (ADC) comprising the antibody or antigen-binding portion described herein, wherein the antibody or antigen-binding portion is conjugated to a therapeutic agent.

In one embodiment, the antibody or antigen-binding portion thereof specifically binds human SAS1B with an affinity (K_d) of at least about 10^{-6} M. In another embodiment, the said antibody or antigen-binding portion thereof binds to cancer cells.

An embodiment provides a radioimmunoconjugate comprising an antibody described herein linked to a radionuclide.

Another embodiment provides an isolated polypeptide consisting of one of SEQ ID NO: 1-51, 53-59, 75-84, or 100-111 or the isolated polynucleotide encoding said polypeptide.

One embodiment provides an isolated polynucleotide encoding an anti-human SAS1B antibody or antigen-binding portion thereof, wherein said isolated polynucleotide encodes a heavy chain and a light chain, wherein: (a) the immunoglobulin heavy chain complementarity determining region (CDR) CDR1 comprises SEQ ID NO:1, 7, 9, 14, 25, 31, 37, 43, 48, or 54, CDR2 comprises SEQ ID NO:2, 10, 15, 26, 32, 38, 44, 49, or 55, and CDR3 comprises SEQ ID NO:3, 8, 16, 27, 33, 39, 45, 50, 56, or GGL, and wherein the immunoglobulin light chain CDR1 comprises SEQ ID NO:4, 11, 17, 28, 34, 40, 46, 51, or 57, CDR2 comprises SEQ ID NO:5, 12, 18, 29, 35, 41, 47, 52, or 58, and CDR3 comprises SEQ ID NO:6, 13, 19, 30, 36, 42, 53, or 59; or (b) the immunoglobulin heavy chain complementarity determining region (CDR) CDR1 comprises SEQ ID NO:20, CDR2 comprises SEQ ID NO:21, and CDR3 comprises SEQ ID NO:22, and wherein the immunoglobulin light chain CDR1 comprises SEQ ID NO:23, CDR2 comprises FAS, and CDR3 comprises SEQ ID NO:24. Another embodiment provides a vector comprising such polynucleotides and yet another embodiment provides a host cell comprising such vectors.

One embodiment provides a method for producing a human SAS1B antibody or

antigen-binding portion thereof, comprising culturing the isolated host cell of claim 19 and recovering said antibody.

In one embodiment, the antibody is a chimeric antibody comprising VL and VH domains obtained from a mouse antibody, wherein said VL and VH domains comprise sequences capable of binding to human SAS1B, and the VL and VH domains are fused to human CL and CH domains, respectively.

One embodiment provides a method of treating a hyperproliferative disorder comprising administering a composition disclosed herein to a mammal in need thereof.

Another embodiment provides a method of detecting a SASB1 polypeptide in a sample comprising: (a) contacting one or more antibodies disclosed with a test sample under conditions that allow polypeptide/antibody complexes to form; and (b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that the human SAS1B polypeptide is present in the sample.

One embodiment provides a method of detecting SAS1B-positive cells in a test sample comprising: (a) contacting one or more antibodies disclosed herein with the test sample under conditions that allow SAS1B-positive cell/antibody complexes to form; and (b) detecting SAS1B positive cell/antibody complexes; wherein the detection of SAS1B positive cell/antibody complexes is an indication that SAS1B cells are present in the test sample. In one embodiment, the sample is lymph node or tissue aspirate, serum, whole blood, cellular suspension, lymphocytes, whole blood, plasma, ovarian cyst fluid, pap smear, circulating tumor cells, tumor cells or tissue, ascites fluid, urine, or fluid effusion.

An embodiment provides antibodies and antigen-binding portions thereof that specifically bind to the human metallo-endoprotease SAS1B, a product of the ASTL gene. These antibodies and antigen-binding portions thereof have applications in diagnostic assays to measure SAS1B. They also have applications as therapeutic probes, both alone, as “naked” unconjugated antibodies, and conjugated with cytotoxic drugs and radionuclides. These antibodies also have therapeutic use as imaging agents.

Immunochemical, biochemical, morphological, molecular biology, and pharmacological working examples demonstrate several points. Antibodies and antigen-binding fragments have been identified that are specific for at

least two regions of human SAS1B. These antibodies selectively recognize SAS1B proteins in the presence of an array of other proteins. These antibodies can be used to precipitate SAS1B from extracts of cells that express the SAS1B protein, and can bind with SAS1B in fixed and permeabilized cells.

Brief Description of the Drawings

Fig. 1 shows a diagram of the human SAS1B polypeptide. SAS1B amino acid sequence is SEQ ID NO:112.

Fig. 2 shows cell surface density of SAS1B using RCT-7 antibody (also referred to herein as 2H2/2K5).

Fig. 3 shows a cell screening assay with RCT-7 antibody.

Detailed Description

As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. The term “about” in association with a numerical value means that the numerical value can vary plus or minus by 5% or less of the numerical value.

In the following detailed description, numerous specific embodiments are set forth in order to provide a thorough understanding of the compositions and methods disclosed herein. However, as will be apparent to those skilled in the art, the present embodiments may be practiced without these specific details or by using alternate elements or processes. In other instances, well-known processes, procedures, and/or components have not been described in detail so as not to unnecessarily obscure aspects of embodiments disclosed herein. Features regarding exemplary isolated antibodies or antigen-binding portions thereof are described followed by features relating to compositions and methods for detecting and treating cancer.

Polypeptides

A polypeptide is a polymer of three or more amino acids covalently linked by amide bonds. A polypeptide can be post-translationally modified. A purified polypeptide is a polypeptide preparation that is substantially free of cellular material, other types of polypeptides, chemical precursors, chemicals used in synthesis of the polypeptide, or combinations thereof. A polypeptide preparation that is substantially free of cellular material, culture medium, chemical precursors, chemicals used in synthesis of the polypeptide has less than about 30%, 20%, 10%, 5%, 1% or more of

other polypeptides, culture medium, chemical precursors, and/or other chemicals used in synthesis. Therefore, a purified polypeptide is about 70%, 80%, 90%, 95%, 99% or more pure.

A light or heavy chain variable region of an antibody has four framework regions interrupted by three hypervariable regions, known as complementary determining regions (CDRs). CDRs determine the specificity of antigen binding. The heavy chain and light chain each have three CDRs, designated from the N terminus as CDR1, CDR2, and CDR3 with the four framework regions flanking these CDRs. The amino acid sequences of the framework region are highly conserved and CDRs can be transplanted into other antibodies. Therefore, a recombinant antibody can be produced by combining CDRs from one or more antibodies with the framework of one or more other antibodies. In an embodiment antibodies include antibodies that comprise at least one, two, three, four, five, or six (or combinations thereof) of the CDRs of any of the monoclonal antibodies isolated from the hybridomas shown in Table 1, or variant CDRs. Variant CDRs are CDRs comprising amino acid sequences similar to the amino acid sequences of CDRs of any of the monoclonal antibodies produced by the hybridomas shown in Table 1. In an embodiment variant CDRs specifically bind to amino acids 55-289 or 280-430 of SEQ ID NO:112 when present in an appropriate antibody structure (*e.g.*, framework regions and other appropriate CDRs).

Polypeptides can comprise full-length human, mouse, or rabbit anti-SAS1B heavy chain variable regions, full-length human, mouse or rabbit light chain regions, fragments thereof, and combinations thereof.

Table 1.

	Antibody type	Immunogen
7H2	Mouse	SAS1B SVA amino acids 280-430 of SEQ ID NO:112
6C1	Mouse	SAS1B SVA amino acids 280-430 of SEQ ID NO:112
1H2/1K2	Rabbit	SAS1B SVA amino acids 280-430 of SEQ ID NO:112
1H3/1K3	Rabbit	SAS1B SVA amino acids 280-430 of SEQ ID NO:112
2H2/2K5	Rabbit	SAS1B SVA amino acids 280-430 of SEQ ID NO:112
6B1	Mouse	SAS1B SVA amino acids 55-289 of SEQ ID NO:112
3F2	Mouse	SAS1B SVA amino acids 55-289 of SEQ ID NO:112
3H4/3K5	Rabbit	A C-terminal fragment of

		recombinant human SAS1B protein, aa 280-431
5H2/5K1	Rabbit	A C-terminal fragment of recombinant human SAS1B protein, aa 280-431
6H2/6K1	Rabbit	A C-terminal fragment of recombinant human SAS1B protein, aa 280-431
7H1/7K3	Rabbit	A C-terminal fragment of recombinant human SAS1B protein, aa 280-431
9H1/9K5	Rabbit	A C-terminal fragment of recombinant human SAS1B protein, aa 280-431
31H1/ 31K1	Rabbit	A C-terminal fragment of recombinant human SAS1B protein, aa 280-431

Table 2.

	CDR1 HEAVY VARIABLE	CDR2 HEAVY VARIABLE	CDR3 HEAVY VARIABLE	CDR1 LIGHT VARIABLE	CDR2 LIGHT VARIABLE	CDR3 LIGHT VARIABLE
7H2	GFSLSTSG SEQ ID NO:1	AHIWWDDV SEQ ID NO:2	IPTDDYYALDH SEQ ID NO:3	KSVSTSGYSF SEQ ID NO:4	LASNL SEQ ID NO:5	QHSRELPYT SEQ ID NO:6
6C1	GFSLSTST SEQ ID NO:7	AHIWWDDV SEQ ID NO:2	IPNDGYCAMDY SEQ ID NO:8	KSVSTSGYSF SEQ ID NO:4	LASNL SEQ ID NO:5	QHSRELPYT SEQ ID NO:6
1H2/1K2	SNDMK SEQ ID NO:9	YIFSSGSIYY ASWAKG SEQ ID NO:10	GGL	SVYNNNR SEQ ID NO:11	NLDSGVP SEQ ID NO:12	GTYDCNSADCHA SEQ ID NO:13
1H3/1K3	SNDMK SEQ ID NO:9	YIFSSGSIYY ASWAKG SEQ ID NO:10	GGL	SVYNNNR SEQ ID NO:11	NLDSGVP SEQ ID NO:12	GTYDCNSADCHA SEQ ID NO:13
2H2/2K5 (RCT-7)	SYAMV SEQ ID NO:14	AINTGGVTFY ASWAKG SEQ ID NO:15	AFEF SEQ ID NO:16	SVYDNW SEQ ID NO:17	DLASGVP SEQ ID NO:18	LGTYSSTSDMYV SEQ ID NO:19
6B1	GFTFINYW SEQ ID NO:20	IYPGKSDI SEQ ID NO:21	TRGGAMDY SEQ ID NO:22	QSLLYSSDQKNY SEQ ID NO:23	FAS	QQHYNTPLT SEQ ID NO:24
3F2	GFTFINYW SEQ ID NO:20	IYPGKSDI SEQ ID NO:21	TRGGAMDY SEQ ID NO:22	QSLLYSSDQKNY SEQ ID NO:23	FAS	QQHYNTPLT SEQ ID NO:24
3H4/3K5	SYAMS SEQ ID NO:25	IVVSTGDTYY ASWAKG SEQ ID NO:26	NYDGSTYYLDL SEQ ID NO:27	QSSQSVYSN SEQ ID NO:28	GASTLA SEQ ID NO:29	LGTYSSTSDMYV SEQ ID NO:30
5H2/5K1	TYYMS SEQ ID NO:31	IIAISGNTYYA SWAKG SEQ ID NO:32	DPYDDYGDWL SEQ ID NO:33	QSSKSVYNNN SEQ ID NO:34	GASTLAS SEQ ID NO:35	AGGYSSVSDFFA SEQ ID NO:36
6H2/6K1	SYVMG SEQ ID NO:37	FIYSGGSAYY ASWKG SEQ ID NO:38	RYDL SEQ ID NO:39	QSSQSVYDNN SEQ ID NO:40	YASTLYS SEQ ID NO:41	EGEFSCSNGDCVV SEQ ID NO:42
7H1/7K3	LSSFAMS SEQ ID NO:43	IIATSSTYFAT WAKG SEQ ID NO:44	VGGDPAHTYITAFDP SEQ ID NO:45	QSSQSVYDNNELS SEQ ID NO:46	SASKLTS SEQ ID NO:47	EGEFSCSNGDCVV SEQ ID NO:42
9H1/9K5	LSSYYMS SEQ ID NO:48	IWSTADTYYA SWAKG	NYDGSTYYLDL SEQ ID NO:50	QSSQSVFDNN SEQ ID NO:51	GASTLAS SEQ ID	LGTYTSTSDMYV SEQ ID NO:53

		SEQ ID NO:49			NO:35	
31H1/ 31K1	LSNYAMV SEQ ID NO:54	AINTGGVTFY ASWAKG SEQ ID NO:55	AFEL SEQ ID NO:56	QSSQSVYDN SEQ ID NO:57	DASDLAS SEQ ID NO:58	LGGYSGNIYT SEQ ID NO:59

Table 3.

	Heavy Variable Chain Nucleic acid	Light Variable Chain Nucleic acid	Heavy Constant Chain Nucleic acid	Light Constant Chain Nucleic acid
7H2	SEQ ID NO:60	SEQ ID NO:61		
6C1	SEQ ID NO:62	SEQ ID NO:63		
1H2/1K2	SEQ ID NO:64	SEQ ID NO:65	SEQ ID NO:71	SEQ ID NO:72
1H3/1K3	SEQ ID NO:64	SEQ ID NO:65	SEQ ID NO:71	SEQ ID NO:72
2H2/2K5	SEQ ID NO:67	SEQ ID NO:68	SEQ ID NO:73	SEQ ID NO:74
6B1	SEQ ID NO:69	SEQ ID NO:70		
3F2	SEQ ID NO:69	SEQ ID NO:70		
3H4/3K5	SEQ ID NO:88	SEQ ID NO:89		
5H2/5K1	SEQ ID NO:90	SEQ ID NO:91		
6H2/6K1	SEQ ID NO:92	SEQ ID NO:93		
7H1/7K3	SEQ ID NO:94	SEQ ID NO:95		
9H1/9K5	SEQ ID NO:96	SEQ ID NO:97		
31H1/ 31K1	SEQ ID NO:98	SEQ ID NO:99		

Table 4.

	Heavy Variable Chain Amino acid	Light Variable Chain Amino acid	Heavy Constant Chain Amino Acid	Light Constant Chain Amino acid
7H2	SEQ ID NO:75	SEQ ID NO:76		
6C1	SEQ ID NO:77	SEQ ID NO:78		
1H2/1K2	SEQ ID NO:79	SEQ ID NO:80	SEQ ID NO:85	SEQ ID NO:86
1H3/1K3	SEQ ID NO:79	SEQ ID NO:80	SEQ ID NO:85	SEQ ID NO:86
2H2/2K5	SEQ ID NO:81	SEQ ID NO:82	SEQ ID NO:87	SEQ ID NO:66
6B1	SEQ ID NO:83	SEQ ID NO:84		
3F2	SEQ ID NO:83	SEQ ID NO:84		
3H4/3K5	SEQ ID NO:100	SEQ ID NO:101		
5H2/5K1	SEQ ID NO:102	SEQ ID NO:103		
6H2/6K1	SEQ ID NO:104	SEQ ID NO:105		
7H1/7K3	SEQ ID NO:106	SEQ ID NO:107		
9H1/9K5	SEQ ID NO:108	SEQ ID NO:109		
31H1/ 31K1	SEQ ID NO:110	SEQ ID NO:111		

An antibody can comprise a VH (variable heavy chain) of SEQ ID NOs:75, 77, 79, 81, 83, 100, 102, 104, 106, 108, or 110. An antibody can comprise a VL (variable light chain) of SEQ ID NOs:76, 78, 80, 82, 84, 101, 103, 105, 107, 109, or 111. An antibody can comprise a VH CDR1 of SEQ ID NO:1, 7, 9, 14, 20, 25, 31,

37, 43, 48, or 54. An antibody can comprise a VH CDR 2 of SEQ ID NOs:2, 10, 15, 21, 26, 32, 38, 44, 49, or 55. An antibody can comprise a VH CDR 3 of SEQ ID NOs:3, 8, 16, 22, 27, 33, 39, 45, 50, 56 or GGL. An antibody can comprise a VL CDR 1 of SEQ ID NOs:4, 11, 17, 23, 28, 34, 40, 46, 51, or 57. An antibody can comprise a VL CDR 2 of SEQ ID NOs:5, 12, 18, 29, 35, 41, 47, 35, 58, or FAS. An antibody can comprise a VL CDR3 of SEQ ID NOs:6, 13, 19, 24, 30, 36, 42, 42, 53, or 59.

An antibody can comprise a VH nucleic acid sequence of SEQ ID NO:60, 62, 64, 67, 69, 88, 90, 92, 94, 96, or 98. An antibody can comprise a VL nucleic acid sequence of SEQ ID NO:61, 63, 65, 68, 70, 89, 91, 93, 95, 97, or 99.

An antibody can comprise a VH of SEQ ID NO:75 and a VL of SEQ ID NO:76. An antibody can comprise a VH of SEQ ID NO:77 and a VL of SEQ ID NO:78. An antibody can comprise a VH of SEQ ID NO:79 and a VL of SEQ ID NO:80. An antibody can comprise a VH of SEQ ID NO:81 and a VL of SEQ ID NO:82. An antibody can comprise a VH of SEQ ID NO:83 and a VL of SEQ ID NO:84. An antibody can comprise a VH of SEQ ID NO:100 and a VL of SEQ ID NO:101. An antibody can comprise a VH of SEQ ID NO:102 and a VL of SEQ ID NO:103. An antibody can comprise a VH of SEQ ID NO:104 and a VL of SEQ ID NO:105. An antibody can comprise a VH of SEQ ID NO:106 and a VL of SEQ ID NO:107. An antibody can comprise a VH of SEQ ID NO:108 and a VL of SEQ ID NO:109. An antibody can comprise a VH of SEQ ID NO:110 and a VL of SEQ ID NO:111.

An antibody can comprise a VH CDR1 of SEQ ID NO:1, a VH CDR2 of SEQ ID NO:2, and a VH CDR3 of SEQ ID NO:3. An antibody can comprise a VL CDR1 of SEQ ID NO:4, a VL CDR2 of SEQ ID NO:5, and a VL CDR3 of SEQ ID NO:6. An antibody can comprise a VH CDR1 of SEQ ID NO:7, a VH CDR2 of SEQ ID NO:2, and a VH CDR3 of SEQ ID NO:8. An antibody can comprise a VH CDR1 of SEQ ID NO:9, a VH CDR2 of SEQ ID NO:10, and a VH CDR3 of GGL. An antibody can comprise a VL CDR1 of SEQ ID NO:11, a VL CDR2 of SEQ ID NO:12, and a VL CDR3 of SEQ ID NO:13. An antibody can comprise a VH CDR1 of SEQ ID NO:14, a VH CDR2 of SEQ ID NO:15, and a VH CDR3 of SEQ ID NO:16. An antibody can comprise a VL CDR1 of SEQ ID NO:17, a VL CDR2 of SEQ ID NO:18, and a VL CDR3 of SEQ ID NO:19. An antibody can comprise a VH CDR1 of SEQ ID NO:20, a VH CDR2 of SEQ ID NO:21, and a VH CDR3 of SEQ ID NO:22. An antibody can

comprise a VL CDR1 of SEQ ID NO:23, a VL CDR2 of FAS, and a VL CDR3 of SEQ ID NO:24. An antibody can comprise a VH CDR1 of SEQ ID NO:25, a VH CDR2 of SEQ ID NO:26, and a VH CDR3 of SEQ ID NO:27. An antibody can comprise a VL CDR1 of SEQ ID NO:28, a VL CDR2 of SEQ ID NO:29, and a VL CDR3 of SEQ ID NO:30. An antibody can comprise a VH CDR1 of SEQ ID NO:31, a VH CDR2 of SEQ ID NO:32, and a VH CDR3 of SEQ ID NO:33. An antibody can comprise a VL CDR1 of SEQ ID NO:34, a VL CDR2 of SEQ ID NO:35, and a VL CDR3 of SEQ ID NO:36. An antibody can comprise a VH CDR1 of SEQ ID NO:37, a VH CDR2 of SEQ ID NO:38, and a VH CDR3 of SEQ ID NO:39. An antibody can comprise a VL CDR1 of SEQ ID NO:40, a VL CDR2 of SEQ ID NO:41, and a VL CDR3 of SEQ ID NO:42. An antibody can comprise a VH CDR1 of SEQ ID NO:43, a VH CDR2 of SEQ ID NO:44, and a VH CDR3 of SEQ ID NO:45. An antibody can comprise a VL CDR1 of SEQ ID NO:46, a VL CDR2 of SEQ ID NO:47, and a VL CDR3 of SEQ ID NO:42. An antibody can comprise a VH CDR1 of SEQ ID NO:48, a VH CDR2 of SEQ ID NO:49, and a VH CDR3 of SEQ ID NO:50. An antibody can comprise a VL CDR1 of SEQ ID NO:51, a VL CDR2 of SEQ ID NO:35, and a VL CDR3 of SEQ ID NO:53. An antibody can comprise a VH CDR1 of SEQ ID NO:54, a VH CDR2 of SEQ ID NO:55, and a VH CDR3 of SEQ ID NO:56. An antibody can comprise a VL CDR1 of SEQ ID NO:57, a VL CDR2 of SEQ ID NO:58, and a VL CDR3 of SEQ ID NO:59.

An antibody can have any of the above VHs combined with any of the above VLs. An antibody can have any combination of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, VL CDR3, variant VH CDR1, variant VH CDR2, variant VH CDR3, variant VL CDR1, variant VL CDR2, or variant VL CDR3. In one embodiment an antibody comprises a VH of SEQ ID NOs:75, 77, 79, 81, 83, 100, 102, 104, 106, 108, or 110 (or a variant thereof) and at least one, two or three VL CDRs of SEQ ID NOs:4, 5, 6, 11, 12, 13, 17, 18, 19, 23, 24, 28, 29, 30, 34, 35, 36, 40, 41, 42, 46, 47, 51, 35, 53, 57, 58, 59, FAS (or a variant thereof). In one embodiment an antibody comprises a VL of SEQ ID NOs:76, 78, 80, 82, 84, 101, 103, 105, 107, 109, 111 (or a variant thereof) and at least one, two or three VH CDRs of SEQ ID NOs:1, 2, 3, 7, 8, 9, 10, 14, 15, 16, 20, 21, 22, 25, 26, 27, 31, 32, 33, 37, 38, 39, 43, 44, 45, 48, 49, 50, 54, 55, 56, GGL (or a variant thereof).

An antibody can comprise the variable heavy chain CDRs from antibody 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1,

7H1/7K3, 9H1/9K5, or 31H1/31K1. An antibody can comprise the variable light chain CDRs from antibody 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1. An antibody can comprise a variable light chain that comprises the amino acid sequence of at least one or at least two or at least 3 CDRs of the 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1 antibody variable light chains. An antibody can comprise a variable heavy chain that comprises the amino acid sequence of at least one or at least two or at least 3 CDRs of the 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1 antibody variable heavy chains.

Heavy chain CDRs can be combined with appropriate variable regions of an antibody light chain. Light chain CDRs optionally combined with heavy chain CDRs are, for example, CDRs comprising SEQ ID NOs: 4, 5, 6, 11, 12, 13, 17, 18, 19, 23, 24, 28, 29, 30, 34, 35, 36, 40, 41, 42, 46, 47, 51, 35, 53, 57, 58, 59, FAS, or CDRs functionally equivalent to these CDRs. The respective amino acid sequences correspond to CDR1 (SEQ ID NOs:4,11, 17, 23, 28, 34, 40, 46, 51, 57), CDR2 (SEQ ID NOs:5, 12, 18, 29, 35, 41, 47, 52, 58, FAS), and CDR3 (SEQ ID NO:6, 13, 19, 24, 30, 36, 42, 53, 59) of an antibody light chain. Alternatively, these light chain CDRs may be used independently of the heavy chains described above. The CDRs are substituted for the corresponding CDR1, CDR2, and CDR3, between the framework of a desired light chain variable region.

Light chain CDRs can be combined with appropriate variable regions of an antibody heavy chain. Heavy chain CDRs optionally combined with light chain CDRs are, for example, CDRs comprising SEQ ID NOs:1, 2, 3, 7, 8, 9, 10, 14, 15, 16, 20, 21, 22, 25, 26, 27, 31, 32, 33, 37, 38, 39, 43, 44, 45, 48, 49, 50, 54, 55, 56, GGL, or CDRs functionally equivalent to these CDRs. The respective amino acid sequences correspond to CDR1 (SEQ ID NO:1, 7, 9, 14, 20, 25, 31, 37, 43, 48, 54), CDR2 (SEQ ID NO:2, 10, 15, 21, 26, 32, 38, 44, 49, 55), and CDR3 (SEQ ID NOs:3, 8, 16, 22, 27, 33, 39, 45, 50, 56, GGL) of an antibody light chain. Alternatively, these heavy chain CDRs may be used independently of the light chains described above. The CDRs are substituted for the corresponding CDR1, CDR2, and CDR3 regions, between the framework of a desired heavy chain variable region.

A polypeptide variant or variant CDR differs by about, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60 or more amino acid residues (*e.g.*, amino acid

additions, substitutions or deletions) from a polypeptide shown in SEQ ID NOs:1-51, 53-59, 75-84, 100-111 or a fragment thereof. Where this comparison requires alignment the sequences are aligned for maximum homology. The site of variation can occur anywhere in the polypeptide. In one embodiment a variant polypeptide has activity substantially similar to a polypeptide shown in SEQ ID NOs:1-51, 53-59, 75-84, and 100-111. Activity substantially similar means that when the polypeptide is used to construct an antibody, the antibody has the same or substantially the same activity as an antibody shown in Table 1.

Methods of introducing a mutation into an amino acid sequence are well known to those skilled in the art. *See, e.g.,* Ausubel (ed.), Current Protocols in Molecular Biology, John Wiley and Sons, Inc. (1994); Maniatis *et al.,* Molecular Cloning: A Laboratory Manual, Cold Spring Harbor laboratory, Cold Spring Harbor, N.Y. (1989)). Mutations can also be introduced using commercially available kits such as "QuikChange™ Site-Directed Mutagenesis Kit" (Stratagene). The generation of a functionally active variant polypeptide by replacing an amino acid that does not influence the function of a polypeptide can be accomplished by one skilled in the art.

The variant polypeptides can have conservative amino acid substitutions at one or more predicted non-essential amino acid residues. A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

A variant polypeptide can also be isolated using a hybridization technique. Briefly, DNA having a high homology to the whole or part of a nucleic acid molecule of SEQ ID NOs:60-65, 67-70, 88-99 or a nucleic acid molecule encoding a polypeptide shown in SEQ ID NOs:1-51, 53-59, 75-84, or 100-111 is used to prepare a polypeptide. Therefore, a polypeptide also includes polypeptides that are variants of SEQ ID NOs:1-51, 53-59, 75-84, 100-111, and polypeptides that are encoded by a nucleic acid molecule that hybridizes under high stringency with a nucleic acid molecule encoding SEQ ID NOs:60-65, 67-70, 88-99, or a complement thereof. One of skill in the art can easily determine nucleic acid sequences that encode

polypeptides using readily available codon tables. As such, these nucleic acid sequences are not presented herein.

As used herein, percent identity of two amino acid sequences (or of two nucleic acid sequences) is determined using the algorithm of Karlin and Altschul (PNAS USA 87:2264-2268, 1990), modified as in Karlin and Altschul, PNAS USA 90:5873-5877, 1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (J. Mol. Biol. 215:403-410, 1990). BLAST nucleotide searches are performed with the NBLAST program, score=100, wordlength=12. BLAST protein searches are performed with the XBLAST program, score=50, wordlength=3. To obtain gapped alignment for comparison purposes GappedBLAST is utilized as described in Altschul *et al.* (Nucleic Acids Res. 25:3389-3402, 1997). When utilizing BLAST and GappedBLAST programs the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) are used to obtain nucleotide sequences homologous to a nucleic acid molecule.

Identity or identical means amino acid sequence (or nucleic acid sequence) similarity and has an art recognized meaning. Sequences with identity share identical or similar amino acids (or nucleic acids). Sequence identity is the percentage of amino acids identical to those in the antibody's original amino acid sequence, determined after the sequences are aligned and gaps are appropriately introduced to maximize the sequence identity as necessary. Thus, a candidate sequence sharing 85% amino acid sequence identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 85% of the amino acids in the candidate sequence are identical to the corresponding amino acids in the reference sequence, and/or constitute conservative amino acid changes.

Antibodies can comprise CDRs of 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1 antibodies or variant antibodies comprising one or more variant CDRs. These variant antibodies can have an activity equivalent (*e.g.*, binding to human SAS1B with the same or substantially similar K_d as an antibody produced by a hybridoma of Table 1) to that of 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1. Antibody variants retain substantially the same functional activity of 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1

antibodies. Naturally-occurring functionally active variant antibodies such as allelic variants and species variants and non-naturally occurring functionally active variants are included herein and can be produced by, for example, mutagenesis techniques or by direct synthesis. Antibody variants are encoded by variant polypeptides and variant CDRs of SEQ ID NOs:1-51, 53-59, 75-84, and 100-111.

Polypeptide variants or CDR variants of SEQ ID NOs:1-51, 53-59, 75-84, 100-111 are part of this disclosure. Polypeptide variants or CDR variants of SEQ ID NOs:1-51, 53-59, 75-84, 100-111 can comprise one or more amino acid substitutions, additions or deletions. In one embodiment, a variant polypeptide or variant CDR includes an amino acid sequence at least about 75% identical to a sequence shown as SEQ ID NOs:1-51, 53-59, 75-84, and 100-111. In one embodiment, the variant polypeptide or CDR is at least about 75%, 80%, 85%, 90%, 95%, 98%, 99%, 99.5% or more identical to SEQ ID NOs:1-51, 53-59, 75-84, and 100-111. Variant polypeptides or variant CDRs encode a variant antibody, which is an antibody comprising an amino acid sequence of SEQ ID NOs:1-51, 53-59, 75-84, 100-111 in which one or more amino acid residues have been added, substituted or deleted. For example, the variable region of an antibody can be modified to improve its biological properties, such as antigen binding. Such modifications can be achieved by *e.g.*, site-directed mutagenesis, PCR-based mutagenesis, cassette mutagenesis. Variant antibodies comprise an amino acid sequence which is at least about 75%, 80%, 85%, 90%, 95%, 98%, 99%, 99.5% or more identical to the amino acid sequence of a heavy or light chain variable region of 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1. In one embodiment, a variant antibody retains the same function of a 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1antibody (*e.g.*, binds human SAS1B, in particular a C-terminal region of human SAS1B such as amino acids 280-430 of SEQ ID NO:112) or at an N-terminal region of human SAS1B such as amino acids 55-289 of SEQ ID NO:112 at the same or substantially similar K_d as an antibody produced by the hybridomas shown in Table 1, *e.g.* within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20% of the K_d of an antibody produced by the hybridomas shown in Table 1). In another embodiment, a variant antibody may have a function that is somewhat altered from a 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1 antibody (*e.g.*, binding human

SAS1B with a K_d that is higher or lower than an 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1 antibody).

Polypeptide sequences can be modified, for example, by synthesizing multiple polynucleotides encoding the amino acid sequence of a variable region, and preparing nucleic acids encoding the variable region by PCR using the polynucleotides. Antibodies that comprise one or more CDRs can be prepared by inserting the polynucleotide into an appropriate expression vector and expressing the polynucleotide. For example, polynucleotides can be synthesized using mixed nucleotides to prepare a DNA library that encodes a variety of antibodies comprising CDRs with various amino acids introduced at certain positions. An antibody can be isolated by selecting from the library a clone encoding an antibody that binds to human SAS1B with a K_d that is the same or substantially similar to the K_d of an antibody produced by a hybridoma shown in Table 1.

A polypeptide or antibody can be covalently or non-covalently linked to an amino acid sequence to which the polypeptide or antibody is not normally associated with in nature. Additionally, a polypeptide or antibody can be covalently or non-covalently linked to compounds or molecules other than amino acids. For example, a polypeptide or antibody can be linked to an indicator reagent, an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, a transmembrane domain, a protein purification ligand, or a combination thereof. In one embodiment a protein purification ligand can be one or more C amino acid residues at, for example, the amino terminus or carboxy terminus of a polypeptide. An amino acid spacer is a sequence of amino acids that are not usually associated with a polypeptide or antibody in nature. An amino acid spacer can comprise about 1, 5, 10, 20, 100, or 1,000 amino acids.

If desired, a polypeptide can be a fusion protein, which can also contain other amino acid sequences, such as amino acid linkers, amino acid spacers, signal sequences, TMR stop transfer sequences, transmembrane domains, as well as ligands useful in protein purification, such as glutathione-S-transferase, histidine tag, and staphylococcal protein A, or combinations thereof. A fusion protein is two or more different amino acid sequences operably linked to each other. A fusion protein construct can be synthesized chemically using organic compound synthesis techniques by joining individual polypeptide fragments together in fixed sequence. A

fusion protein construct can also be expressed by a genetically modified host cell (such as *E. coli*) cultured *in vitro*, which carries an introduced expression vector bearing specified recombinant DNA sequences encoding the amino acids residues in proper sequence. The heterologous polypeptide can be fused, for example, to the N-terminus or C-terminus of a polypeptide. A polypeptide can also comprise homologous amino acid sequences, *i.e.*, other immunoglobulin-derived sequences. More than one polypeptide can be present in a fusion protein. Fragments of polypeptides can be present in a fusion protein. A fusion protein can comprise, *e.g.*, one or more of SEQ ID NOs:1-51, 53-59, 75-84, 100-111, fragments thereof, or combinations thereof. Polypeptides can be in a multimeric form. That is, a polypeptide can comprise two or more copies of SEQ ID NOs:1-51, 53-59, 75-84, 100-111 or a combination thereof.

In one embodiment, a polypeptide is derived from a human, rabbit, mouse, other mammal, or combinations thereof. A polypeptide can be isolated from cells or tissue sources using standard protein purification techniques. Polypeptides can also be synthesized chemically or produced by recombinant DNA techniques. For example, a polypeptide can be synthesized using conventional peptide synthesizers.

A polypeptide can be produced recombinantly. A polynucleotide encoding a polypeptide can be introduced into a recombinant expression vector, which can be expressed in a suitable expression host cell system using techniques well known in the art. A variety of bacterial, yeast, plant, mammalian, and insect expression systems are available in the art and any such expression system can be used. Optionally, a polynucleotide encoding a polypeptide can be translated in a cell-free translation system.

Antibodies

The term “antibodies” refers to an intact antibody or an antigen-binding portion or fragment thereof that competes with the intact antibody for antigen binding. The term “antibodies” also includes any type of antibody molecule or specific binding molecule that specifically binds SAS1B. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide, glycoprotein or immunoglobulin that specifically binds SAS1B to form a complex.

Antigen-binding fragments of an antibody may be derived, e.g., from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of nucleic acids encoding antibody variable and optionally constant domains.

An antibody can be any isotype including IgG (IgG1, IgG2, IgG2a, Ig2b, IgG3, IgG4), IgM, IgA (IgA1 and IgA2), IgD, and IgE.

A monoclonal antibody is an antibody obtained from a group of substantially homogeneous antibodies. A group of substantially homogeneous antibodies can contain a small amount of mutants or variants. Monoclonal antibodies are highly specific and interact with a single antigenic site. Each monoclonal antibody typically targets a single epitope, while polyclonal antibody populations typically contain various antibodies that target a group of diverse epitopes. Monoclonal antibodies can be produced by many methods including, for example, hybridoma methods (Kohler and Milstein, Nature 256:495, 1975), recombination methods (U.S. Pat. No. 4,816,567), and isolation from phage antibody libraries (Clackson *et al.*, Nature 352:624-628, 1991; Marks *et al.*, J. Mol. Biol. 222:581-597, 1991).

A “humanized antibody or antigen-binding fragment” thereof is an antibody or fragment thereof that has been engineered to comprise one or more human framework regions in the variable region together with non-human (*e.g.,* mouse, rabbit, rat, or hamster) complementarity-determining regions (CDRs) of the heavy and/or light chain. In some embodiments, a humanized antibody comprises sequences that are entirely human except for the CDR regions. Humanized antibodies are typically less immunogenic to humans, relative to non-humanized antibodies, and thus offer therapeutic benefits in certain situations.

A “human antibody or antigen binding fragment thereof" is an antibody or antigen binding fragment thereof that contains only human-derived amino acid sequences. For example, a fully human antibody may be produced from a human B-cell or a human hybridoma cell. In additional embodiments, the antibody may be produced from a transgenic animal that contains the locus for a human heavy chain immunoglobulin and a human light chain immunoglobulin, or contains a nucleic acid that encodes the heavy and light chains of a specific human antibody. A human antibody or antigen binding fragment thereof is still considered a "human antibody or antigen binding fragment thereof" even if the framework and/or CDRs of the heavy

chain variable domain or light chain variable domain of the antibody isolated or obtained from a human cell, human cell line, or other methodology are mutated (e.g., by amino acid substitution(s), addition(s), and/or deletion(s)) to improve the affinity or other properties of the antibody. In certain embodiments, after the human antibody isolated or obtained from a human cell or human cell line is mutated so that it has at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to the amino acid sequence of the antibody isolated or obtained from a human cell or human cell line. In some embodiments, six, five, four, three, two, or one amino acid substitutions are made in one, two, three, four, five, and/ or six of the CDRs. In some embodiments, six, five, four, three, two, or one amino acid substitutions are made in one, two, three, or four framework regions of the heavy chain variable region, one, two, three, or four framework regions of the light chain variable region of the antibody, Fc, hinge region, or combinations thereof. In one embodiment, a human antibody has an amino acid sequence that is substantially identical to an antibody isolated or obtained from a human cell or human cell line, but is not naturally occurring. The non-naturally occurring human antibody has one or more mutations in the amino acid sequence that do not occur in the variable heavy or light CDR regions, and do not affect the binding or therapeutic characteristics of the human antibody.

Chimeric antibodies or antigen-binding portions thereof have a part of a heavy chain and/or light chain that is derived from a specific species or a specific antibody class or subclass, and the remaining portion of the chain is derived from another species, or another antibody class or subclass. *See e.g.*, Morrison, Science 229:1202 (1985); Oi *et al.,* BioTechniques 4:214 (1986); Gillies *et al.*, J. Immunol. Methods 125:191-202 (1989); U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397.

Chimeric antibodies can be produced using a variety of techniques including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28:489-498 (1991); Studnicka *et al.,* Protein Engineering 7(6):805-814 (1994); Roguska *et al.*, PNAS 96:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332).

In one embodiment, a chimeric antibody can comprise variable and constant regions of species that are different from each other, for example, an antibody can comprise the heavy chain and light chain variable regions of a non-human mammal

such as a mouse or rabbit, and the heavy chain and light chain constant regions of a human. Such an antibody can be obtained by ligating a polynucleotide encoding a variable region of a mouse or rabbit antibody to a polynucleotide encoding a constant region of a human antibody; incorporating the ligated polynucleotides into an expression vector; and introducing the vector into a host cell for production of the antibody. *See* WO 96/02576. The host cells can be eukaryotic cells, such as mammalian cells, including, *e.g.*, CHO cells, lymphocytes, and myeloma cells. The chimeric antibody can comprise additional amino acid acids that are not included in the CDRs introduced into the recipient antibody, nor in the framework sequences. These amino acids can be introduced to more accurately optimize the antibody's ability to recognize and bind to an antigen. For example, as necessary, amino acids in the framework region of an antibody variable region can be substituted such that the CDR of a reshaped antibody forms an appropriate antigen-binding site. *See* Sato *et al.*, Cancer Res. (1993) 53:851-856.

Non-limiting examples of antigen-binding fragments of antibodies include: Fab fragments; Fab' fragments, Fab'-SH fragments, $F(ab')_2$ fragments; Fd fragments; Fv fragments; single-chain Fv (scFv) molecules; sdAb fragments (nanobodies); Fab-like antibodies (an antigen-binding fragment containing variable regions of a heavy chain and light chain that is equivalent to Fab fragments that are obtained by papain digestion); $F(ab')_2$-like antibodies (an antigen-binding fragment containing two antigen-binding domains that is equivalent to $F(ab')_2$ fragments that are obtained by pepsin digestion), multispecific antibodies prepared from antibody fragments, diabody, bispecific antibody, multifunctional antibody, chimeric antibody, humanized antibody, human antibody, murine antibody, rabbit antibody synthetic antibody, CDR-grafted antibody, and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g., an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (e.g, monovalent nanobodies, bivalent nanobodies), single-chain $(Fv)_2$ $(sc(Fv)_2)$; divalent $(sc(Fv)_2)$; tetravalent $([sc(Fv)_2]_2)$ scFV antibodies, and small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are

also encompassed within the expression "antigen-binding fragment," as used herein.

An antigen-binding fragment of an antibody will typically comprise at least 1 variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least 1, 2 or 3 CDRs, which are adjacent to or in frame with 1, 2, 3, or 4 framework sequences, in antigen-binding fragments having a VH domain associated with a VL domain, the VH and VL domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain VH-VH, VH-VL or VL-VL dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric VH or VL domain.

Antigen-binding fragments can be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies.

A "diabody" is a bivalent minibody constructed by gene fusion (*see, e.g.,* Holliger *et al.,* Proc. Natl. Acad. Sci. U. S. A., 90:6444 (1993); EP 404,097; WO 93/11161). Diabodies are dimers composed of two polypeptide chains. The VL and VH domain of each polypeptide chain of the diabody are bound by linkers. The number of amino acid residues that constitute a linker can be between about 2 to 12 residues (e.g., about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12). The linkers of the polypeptides in a diabody are typically too short to allow the VL and VH to bind to each other. Diabody technology provides an alternative mechanism for making bispecific antibody fragments. The fragments comprise a VH connected to a VL by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites.

A scFv is a single-chain polypeptide antibody obtained by linking the VH and VL with a linker (*see e.g.*, Huston *et al.,* PNAS USA, 85:5879 (1988); Pluckthun, "The Pharmacology of Monoclonal Antibodies" Vol.113, Ed Resenburg & Moore, Springer Verlag, New York, pp.269-315, (1994)). The order of VHs and VLs to be linked is not particularly limited, and they may be arranged in any order. Examples of arrangements include: VH-linker-VL; or VL-linker-VH. The H chain V region and L chain V region in a scFv may be derived from any anti-SAS1B antibody or antigen-binding fragment thereof described herein.

A $sc(Fv)_2$ is a fragment where two VHs and two VLs are linked by a linker to form a single chain (Hudson *et al.,* J. Immunol. Methods, 231:177 (1999)). A $sc(Fv)_2$ molecule can be prepared, for example, by connecting scFvs with a linker. $sc(Fv)_2$ molecules can include antibodies where two VHs and two VLs are arranged in the order of: VH, VL, VH, and VL (VH- linker-VL-linker-VH-linker-VL), beginning from the N terminus of a single-chain polypeptide; however the order of the two VHs and two VLs is not limited to this arrangement, and they may be arranged in any order. Examples of arrangements are listed below:

VL-linker-VH-linker-VH-linker-VL;

VH-linker-VL-linker-VL-linker-VH;

VH-linker-VH-linker-VL-linker-VL;

VL-linker-VL-linker-VH-linker-VH; or

VL-linker-VH-linker-VL-linker-VH.

Three linkers are usually required when four antibody variable regions are linked; the linkers used may be identical or different. There is no limitation on the linkers that link the VH and VL regions of the antibody fragments. In some embodiments, the linker is a peptide linker. Any arbitrary single-chain peptide comprising about three to 25 residues (e.g., 5, 6, 7, 8, 9, 10, 1 1, 12, 13, 14, 15, 16, 17, 18 amino acids) can be used as a linker. Examples of such peptide linkers include: Ser; Gly Ser; Gly Gly Ser; Ser Gly Gly; Gly Gly Gly Ser (SEQ ID NO:113); Ser Gly Gly Gly (SEQ ID NO:114); Gly Gly Gly Gly Ser (SEQ ID NO:115); Ser Gly Gly Gly Gly (SEQ ID NO:116); Gly Gly Gly Gly Gly Ser (SEQ ID NO:117); Ser Gly Gly Gly Gly Gly (SEQ ID NO:118); Gly Gly Gly Gly Gly Gly Ser (SEQ ID NO:119); Ser Gly Gly Gly Gly Gly Gly (SEQ ID NO:120); (Gly Gly Gly Gly Ser (SEQ ID NO:121)$_n$, wherein n is an integer of one or more; and (Ser Gly Gly Gly Gly (SEQ ID NO:52)$_n$, wherein n is an integer of one or more.

In certain embodiments, the linker is a synthetic compound linker (chemical cross- linking agent). Examples of cross-linking agents include, for example, N-hydroxysuccinimide (NHS), disuccinimidylsuberate (DSS), bis(sulfosuccinimidyl)suberate (BS3), dithiobis(succinimidylpropionate) (DSP), dithiobis(sulfosuccinimidylpropionate) (DTSSP), ethyleneglycol bis(succinimidylsuccinate) (EGS), ethyleneglycol bis(sulfosuccinimidylsuccinate) (sulfo-EGS), disuccinimidyl tartrate (DST), disulfosuccinimidyl tartrate (sulfo-DST),

bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone (BSOCOES), and bis[2 (sulfosuccinimidooxycarbonyloxy)ethyl]sulfone (sulfo-BSOCOES).

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the SAS1B protein. Other such antibodies may combine an SAS1B binding site with a binding site for another protein. Bispecific antibodies can be prepared as full length antibodies or low molecular weight forms thereof (e.g., $F(ab')_2$ bispecific antibodies, $sc(Fv)_2$ bispecific antibodies, diabody bispecific antibodies). Full length bispecific antibodies can be produced based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.,* Nature, 305:537-539 (1983)). Alternatively, antibody variable domains with the desired binding specificities are fused to immunoglobulin constant domain sequences. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the proportions of the three polypeptide fragments. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields. Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Heteroconjugate antibodies may be made using any convenient cross-linking methods.

Antibodies can be multivalent antibodies with three or more antigen binding sites (e.g., tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. An exemplary dimerization domain comprises an Fc region or a hinge region. A multivalent antibody can comprise about 3, 4, 5, 6, 7, 8, or more antigen binding sites. The multivalent antibody optionally comprises at least one, two, three or more polypeptide chains, wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise $VDI\text{-}(XI)_n\text{-}VD2\text{-}(X2)_n\text{-}Fc$, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is a polypeptide chain of an Fc region, XI and X2 represent an amino acid or peptide spacer, and n is 0 or 1.

Epitopes

One embodiment provides binding molecules (e.g., antibodies or antigen-binding fragments) that specifically bind to human SAS1B. In one embodiment, the antibodies or antigen-binding fragments thereof specifically bind to an epitope within the C-terminal domain (e.g., amino acids 280-430 of human SAS1B (SEQ ID NO:112) or with the N-terminal domain (e.g., amino acids 55-289 of SEQ ID NO:112). In one embodiment, the antibodies or antigen-binding fragments thereof specifically bind to an epitope within amino acids of 280-290, 290-300, 300-310, 310-320, 320-330, 330-340, 340-350, 350-360, 360-370, 370-380, 380-390, 390-400, 400-410, 410-420, 420-430, 280-310, 310-340, 340-370, 370-400, 400-430, 280-300, or 300 to 430 of SEQ ID NO:112 or combinations thereof. In one embodiment, the antibodies or antigen-binding fragments thereof specifically bind to an epitope within amino acids 55-65, 65-75, 75-85, 85-95, 95-105, 105-115, 115-125, 125-135, 135-145, 145-155, 155-165, 165-175, 175-185, 185-195, 195-205, 205-215, 225-235, 235-245, 245-255, 255-265, 265-275, 275-289, 55-85, 85-115, 115-145, 145-175, 175-205, 205-235, 235-265, 265-289, or 80-163 of SEQ ID NO:112 or combinations thereof. The antibody or antigen-binding portion thereof may bind to conformational epitope which comprises 2 or more of these regions.

An antibody or fragment thereof binds to an epitope that overlaps with or is the same (*i.e.*, a substantially identical epitope) as any of the monoclonal antibodies shown in Table 1. An antibody that binds to an epitope substantially identical to an epitope of human SAS1B to which a monoclonal antibody of Table 1 binds, can be obtained by analyzing epitopes of the monoclonal antibodies of Table 1 using well known epitope mapping methods. Competitive assays can be used to determine if two antibodies bind to a substantially identical epitope of SAS1B. Where the binding of a first anti-SAS1B antibody with SAS1B is competitively inhibited by a second anti-SAS1B antibody, the first antibody and the second antibody can be considered to bind to a substantially identical epitope on SAS1B. Competitively inhibits means that an antibody or antigen-binding fragment thereof can specifically bind an epitope that a monoclonal antibody produced by a hybridoma cell line shown in Table 1 is directed to, using conventional reciprocal antibody competition assays. *See e.g.,* Belanger *et al.* (1973), Clinica Chimica Acta 48:15.

Antibodies that competitively inhibit binding of one or more of 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3,

9H1/9K5, or 31H1/31K1 or antigen-binding fragments thereof, reduce the binding of one or more of 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1or antigen-binding fragments thereof to a SAS1B polypeptide (*e.g.,* a full-length SAS1B polypeptide or amino acids 280-430 or amino acids 55-289 of SEQ ID NO:112) or to cancer cells by about 40%, 50%, 75%, 90% or 100% in any type of competitive inhibition assay (*see, e.g.,* Harlow and Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, NY (1988)) are also antibodies of the disclosure. Antibodies and antigen-binding fragments thereof can inhibit the binding 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, or 31H1/31K1 to human SAS1B.

Therefore, antibodies that bind to an epitope that is substantially identical to or the same as an epitope of SAS1B to which an antibody produced by a hybridoma of Table 1 binds, and that can also comprise the activity of binding to SAS1B or fragments thereof (*e.g.* amino acids 280-430 or 55-289 of SEQ ID NO:112) are disclosed herein.

Amount of Binding

Antibodies can specifically bind SAS1B (e.g. human SAS1B). "Specifically binds" means that the antibody recognizes and binds to SAS1B with greater affinity than to other, non-specific molecules that are not SAS1B. For example, an antibody raised against an antigen (polypeptide) to which it binds more efficiently than to a non-specific antigen (*e.g.*, a protein that is not related to or homologous to SAS1B) can be described as specifically binding to the antigen. Binding specificity can be tested using, for example, an enzyme-linked immunosorbant assay (ELISA), a radioimmunoassay (RIA), or a western blot assay using methodology well known in the art.

Antibodies, antigen-binding fragments thereof, or variants thereof can specifically bind SAS1B with a wide range of disassociation constants (K_d). For example, an antibody can bind human SAS1B with a K_d equal to or less than about 10^{-7} M, such as but not limited to, 0.1-9.9 x 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}, 10^{-13}, 10^{-14}, 10^{-15} or any range or value therein, as determined by *e.g.*, surface plasmon resonance or the Kinexa method. An embodiment encompasses antibodies that bind human SAS1B polypeptides with a disassociation constant or K_d that is within any one of the ranges that are between each of the individual recited

values. An antibody has the same or substantially identical activity as antibodies produced by the hybridomas shown in Table 1 when the K_d for binding to SAS1B (*e.g.*, amino acids 55-289 or 280-430 of SEQ ID NO:112) is within about 0.1., 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20% (or any range or particular value between 0.1 and 20%) of the K_d for binding to SAS1B (*e.g.*, amino acids 55-289 or 280-430 of SEQ ID NO:112) of an antibody produced by the hybridomas shown in Table 1.

Antibodies, antigen-binding fragments thereof or variants thereof can specifically bind human SAS1B polypeptides with an off rate (K_{off}) of less than or equal to 01.-9.9 x 10^{-3} sec^{-1}, 10^{-4} sec^{-1}, 10^{-5} sec^{-1}, 10^{-6} sec^{-1}, 10^{-7} sec^{-1}. An embodiment encompasses antibodies that specifically bind SAS1B polypeptides with an off rate that is within any one of the ranges that are between each of the individual recited values. An antibody has the same or substantially identical activity as antibodies produced by the hybridomas shown in Table 1 when the K_{off} for binding to SAS1B (*e.g.,* amino acids 55-289 or 280-430 of SEQ ID NO:112) is within about 0.1., 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20% (or any range or particular value between 0.1 and 20%) of the K_{off} for binding to SAS1B (*e.g.,* amino acids 55-289 or 280-430 of SEQ ID NO:112) of an antibody produced by the hybridomas shown in Table 1.

Antibodies, antigen-binding fragments thereof, or variants thereof can specifically bind SAS1B polypeptides with an on rate (K_{on}) greater than or equal to 0.1-9.9 x 10^3 $M^{-1}sec^{-1}$, 10^4 $M^{-1}sec^{-1}$, 10^5 $M^{-1}sec^{-1}$, 10^6 $M^{-1}sec^{-1}$, 10^7 $M^{-1}sec^{-1}$, 10^8 $M^{-1}sec^{-1}$. An embodiment encompasses antibodies that bind SAS1B polypeptides with on rate that is within any one of the ranges that are between each of the individual recited values. An antibody has the same or substantially identical activity as antibodies produced by the hybridomas shown in Table 1 when the K_{on} for binding to SAS1B (*e.g.,* amino acids 55-289 or 280-430 of SEQ ID NO:112) is within about 0.1., 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20% (or any range or particular value between 0.1 and 20%) of the K_{on} for binding to SAS1B (*e.g.,* amino acids 55-289 or 280-430 of SEQ ID NO:112) of an antibody produced by the hybridomas shown in Table 1.

Methods of Making Antibodies

Antibodies can be produced using methods known to those of skill in the art. For example, an SAS1B antigen or a fragment thereof (*e.g.,* amino acids 55-289 or

280-430 of SEQ ID NO:112) can be used to immunize animals. SAS1B or a fragment thereof can be conjugated to a carrier protein and/or administered to the animals with an adjuvant. An SAS1B antigen can comprise one or more epitopes (*i.e.*, antigenic determinants). An epitope can be a linear epitope, sequential epitope or a conformational epitope. Epitopes within a polypeptide can be identified by several methods. *See, e.g.,* U.S. Patent No. 4,554,101; Jameson & Wolf, *CABIOS* 4:181-186 (1988). For example, SAS1B can be isolated and screened. A series of short peptides, which together span the entire SAS1B polypeptide sequence, can be prepared by proteolytic cleavage. By starting with, for example, 100-mer polypeptide fragments, each fragment can be tested for the presence of epitopes recognized in an ELISA. For example, in an ELISA assay an SAS1B antigen, such as a 100-mer polypeptide fragment, is attached to a solid support, such as the wells of a plastic multi-well plate. A population of antibodies are labeled, added to the solid support and allowed to bind to the unlabeled antigen, under conditions where non-specific absorption is blocked, and any unbound antibody and other proteins are washed away. Antibody binding is detected by, for example, a reaction that converts a colorless substrate into a colored reaction product. Progressively smaller and overlapping fragments can then be tested from an identified 100-mer to map the epitope of interest.

Methods for preparing monoclonal antibodies from hybridomas are well known to those of skill in the art and include, *e.g.*, standard cell culture methods and ascites production methods. Recombinant antibodies or fragments thereof produced by gene engineering can be made using the polynucleotide sequences disclosed herein. Genes encoding antibodies or fragments thereof can be isolated from hybridomas described herein or other hybridomas. The genes can be inserted into an appropriate vector and introduced into a host cell. *See, e.g.*, Borrebaeck & Larrick, Therapeutic Monoclonal Antibodies, Macmillan Publ. Ltd, 1990.

Antibodies can be produced using immunospot array assay on a chip (ISAAC) to obtain an antibody gene by screening single B cells, which secrete a specific monoclonal antibody, within several weeks (Jin *et al.,* 2009 *Nat. Med.* 15, 1088-1092). Whole antibodies can also be made using PCR primers having VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site to amplify the VH or VL sequences in scFv clones. Using well known cloning techniques, the PCR amplified VH domains can be cloned into vectors

expressing a VH constant region, *e.g.*, a human, rabbit or mouse constant region, and the PCR amplified VL domains can be cloned into vectors expressing a VL constant region, *e.g.*, a human VL constant region or rabbit or murine light constant regions. The vectors for expressing the VH or VL domains can comprise, *e.g.*, a promoter suitable to direct expression of the heavy and light chains in the chosen expression system, a secretion signal, a cloning site for the immunoglobulin variable domain, immunoglobulin constant domains, and a selection marker. The VH and VL domains can also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

The nucleic acid sequences for human, rabbit, and mouse IgG constant regions have been cloned and sequenced and can be used to construct antibodies.

Human antibodies can be made by sensitizing human lymphocytes with antigens of interest or cells expressing antigens of interest *in vitro*; and fusing the sensitized lymphocytes with human myeloma cells. Alternatively, a human antibody can be made by using an antigen to immunize a transgenic animal that comprises a partial or entire repertoire of human antibody genes. *See* Green *et al.*, Nature Genetics 7:13-21 (1994); Mandez *et al.*, Nature Genetics 15:146-156 (1997); Lonberg *et al.,* Nature 368:856-859 (1994); WO 93/12227; WO 92/03918; WO 94/02602, WO 94/25585, WO 96/34096, and WO 96/33735).

Human antibodies can also be made by panning with a human antibody library. For example, the variable region of a human antibody is expressed as a single chain antibody (scFv) on the surface of a phage, using phage display method, and phages that bind to the antigen are selected. By analyzing the polynucleotides of selected phages, the polynucleotides encoding the variable regions of human antibodies that bind to the antigen can be determined. If the polynucleotide sequences of scFvs that bind to the antigen are identified, appropriate expression vectors comprising these sequences can be constructed, and then introduced into appropriate hosts and expressed to obtain human antibodies. *See* WO 92/01047, WO 92/20791, WO 93/06213, WO 93/11236, WO 93/19172, WO 95/01438, and WO 95/15388. These same human antibody production methods can be used to make mouse or rabbit antibodies.

Antibodies and fragments thereof can be purified by any method, including, *e.g.,* protein A-Sepharose methods, hydroxyapatite chromatography, salting-out methods with sulfate, ion exchange chromatography, affinity chromatography, filtration, ultrafiltration, dialysis, preparative polyacrylamide gel electrophoresis, isoelectrofocusing or combinations thereof.

Antibodies can be dried or lyophilized ("freeze-dried) for more ready formulation into a desired vehicle/carrier where appropriate and for increased shelf-life.

Conjugates

Antibodies can be covalently attached to other molecules such that covalent attachment does not affect the ability of the antibody to bind to SAS1B or cells expressing SAS1B. For example, antibodies can be modified by, *e.g.,* glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups (e.g., methyl group, group ethyl group, carbohydrate group), proteolytic cleavage, linkage to a cellular ligand or other protein.

Conjugated antibodies can be bound to various molecules including, for example, polymers, hyaluronic acid, fluorescent substances, luminescent substances, haptens, enzymes, metal chelates, cytotoxic agents, radionuclides, and drugs.

An anti-SAS1B antibody or antigen-binding fragment thereof can be modified with a moiety that improves its binding, stabilization, and/or retention in circulation, e.g., in blood, serum, or other tissues, e.g., by at least 1.5, 2, 5, 10, or 50 fold. For example, the anti-SAS1B antibody or antigen-binding fragment thereof can be associated with (e.g., conjugated to) a polymer, e.g., a substantially non-antigenic polymer, such as a polyalkylene oxide, a polyethylene oxide, polyethylene glycol (PEG), polyethylenimine (PEI) modified with PEG (PEI-PEG), polyglutamic acid (PGA) (N-(2-Hydroxypropyl) methacrylamide (HPMA) copolymers. Suitable polymers will vary substantially by weight.

Polymers having molecular number average weights ranging from about 200 to about 35,000 Daltons (or about 1,000 to about 15,000, and 2,000 to about 12,500) can be used. For example, the anti-SAS1B antibody or antigen-binding fragment thereof can be conjugated to a water soluble polymer, a hydrophilic polyvinyl polymer, polyvinylalcohol or polyvinylpyrrolidone. Examples of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) (*see e.g.,*

Chapman *et al.,* Nature Biotechnology, 17: 780 (1999), or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained. Additional useful polymers include polyoxyalkylenes such as polyoxyethylene, polyoxypropylene, and block copolymers of polyoxyethylene and polyoxypropylene; polymethacrylates; carbomers; and branched or unbranched polysaccharides. The antibodies or antigen-binding fragments thereof can also be conjugated to small molecules and other chemical moieties. Conjugated antibodies can be prepared by performing chemical modifications on the antibodies or fragments thereof. *See e.g.,* US 5057313 and US 5156840.

Variants of Antibodies

The constant region of an antibody or antigen-binding fragment thereof can be a human Fc region, e.g., a wild-type Fc region, or an Fc region that includes one or more amino acid substitutions. The constant region can have substitutions that modify the properties of the antibody (e.g., increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function). Antibodies may have mutations in the CH2 region of the heavy chain that reduce or alter effector function, e.g., Fc receptor binding and complement activation. For example, antibodies may have mutations such as those described in U.S. Patent Nos. 5,624,821 and 5,648,260. Antibodies can also have mutations that stabilize the disulfide bond between the two heavy chains of an immunoglobulin, such as mutations in the hinge region of IgG4, as disclosed in the art (e.g., Angal *et al.* (1993) Mol. Immunol. 30: 105-08). *See also, e.g.,* U.S. 2005/0037000.

The amino acid sequence of the heavy chain variable region (VH) or the light chain variable region (VL) in the antibody or antibody fragments can include modifications such as amino acid substitutions, deletions, additions, and/or insertions. For example, the modification may be in one or more of the CDRs of the anti-SAS1B antibody or antigen-binding fragment thereof. In certain embodiments, the modification involves one, two, or three amino acid substitutions in one or more CDRs and/or framework regions of the VH and/or VL domain of the anti-SAS1B antibody. Such substitutions are made to improve the binding, functional activity and/or reduce immunogenicity of the anti-SAS1B antibody. The amino acid substitutions can be conservative amino acid substitutions. In one embodiment, one,

two, or three amino acids of the CDRs of the anti-SAS1B antibody or antigen-binding fragment thereof may be deleted or added as long as there is SAS1B binding and/or functional activity when VH and VL are associated.

The amino acid sequences of the CDRs are of primary importance for epitope recognition and antibody binding. Changes may be made to the amino acids that comprise the CDRs without interfering with the ability of the antibody to recognize and bind its cognate epitope. For example, changes that do not affect epitope recognition, yet increase the binding affinity of the antibody for the epitope may be made. Thus, also included in the scope of the present disclosure are improved versions of the disclosed antibodies, which also specifically recognize and bind SAS1B, optionally with increased affinity.

The effects of introducing one or more amino acid changes at various positions in the sequence of an antibody has been studied based on the knowledge of the primary antibody sequence, on its properties such as binding and level of expression. *See, e.g.,* Yang *et al.,* 1995, J. Mol. Biol., 254: 392; Rader *et al.,* 1998, Proc. Natl. Acad. Sci. U.S.A., 95: 8910; Vaughan *et al.,* 1998, Nature Biotechnology, 16:535.

For example, equivalents of a primary antibody have been generated by changing the sequences of the heavy and light chain genes in the CDR1, CDR2, CDR3, or framework regions, using methods such as oligonucleotide-mediated site-directed mutagenesis, cassette mutagenesis, error-prone PCR, DNA shuffling, or mutator-strains of *E. coli.* *See* Vaughan *et al.,* 1998, Nature Biotechnology, 16: 535; Adey *et al.,* 1996, Chapter 16, pp. 277-291, in "Phage Display of Peptides and Proteins", Eds. Kay *et al.,* Academic Press). These methods of altering the sequence of a primary antibody have resulted in improved affinities of the newly generated antibodies. Gram *et al.,* 1992, Proc. Natl. Acad. Sci. U.S.A., 89: 3576; Boder *et al.,* 2000, Proc. Natl. Acad. Sci. U.S.A., 97:10701; Davies & Riechmann, 1996, Immunotechnology, 2:169; Thompson *et al.,* 1996, J. Mol. Biol., 256:77; Short *et al.*, 2002, J. Biol. Chem., 277:16365; Furukawa, *et al.,* 2001, J. Biol. Chem., 276:27622.

Using similar directed strategies of changing one or more amino acid residues of the antibody, the antibody sequences described herein can be used to develop anti-SAS1B antibodies with improved functions, including improved affinity for SAS1B.

An embodiment encompasses antibodies, fragments thereof, or variants thereof that have one or more of the same or substantially similar biological characteristics as the antibodies shown in Table 1. Biological characteristics are the *in vitro* or *in vivo* activities or properties of the antibodies shown in Table 1, including, for example, the ability to bind to SAS1B (*e.g.,* amino acids 280-430 or amino acids 55-289 of SEQ ID NO:112) with a substantially similar K_d, K_{off}, and/or K_{on} rate, bind cancer cells, cause death of cancer cells, or combinations thereof.

Antibodies can be used to generate anti-idiotype antibodies that "mimic" human SAS1B polypeptides using techniques well known to those skilled in the art. *See*, Greenspan & Bona, FASEB 17:437-444 (1993); Nissinoff, J. Immunol. 147:2429-2438 (1991).

One embodiment provides mixtures of antibodies, antigen-binding fragments thereof, or variants thereof that bind to SAS1B, wherein the mixture has at least two, three, four, five or more different antibodies.

An embodiment also provides for panels of antibodies that have different affinities for SASB1, different specificities for SAS1B, or different dissociation rates. An embodiment provides panels of at least about 2, 3, 4, 5, 6, 7, 10, 20, 50, 100, 250, 500, 750, or 1,000 antibodies.

In one embodiment, the antibodies or antigen-binding fragments thereof are not naturally occurring due to one or more amino acid mutations in one or more constant regions or one or more framework regions or other mutations.

Polynucleotides

Polynucleotides contain less than an entire human, mouse or rabbit genome and can be single- or double-stranded nucleic acids. A polynucleotide can be RNA, DNA, cDNA, genomic DNA, chemically synthesized RNA or DNA or combinations thereof. The polynucleotides can be purified free of other components, such as proteins, lipids and other polynucleotides. For example, the polynucleotide can be 50%, 75%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% pure by dry weight. Purity can be measured by a method such as column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. The polynucleotides encode the polypeptides described above. In one embodiment the polynucleotides encode polypeptides shown in, *e.g.,* SEQ ID NOs:60-65, 67-74, 88-99 or portions or combinations thereof.

The polynucleotides encode the polypeptides and antibodies disclosed herein, as well as fragments thereof. A polynucleotide fragment can be about 9, 18, 21, 27,

30, 33, 39, 48, 51, 75, 100, 120, 130, 140, 150, 200 or more polynucleotides. One of skill in the art can obtain the polynucleotide sequences using the polypeptide sequences and codon tables known to those of skill in the art. Polynucleotides can contain naturally occurring polynucleotides or sequences that differ from those of any naturally occurring sequences or polynucleotides (e.g., non-naturally occurring polynucleotides). Polynucleotides can differ from naturally occurring nucleic acids, but still encode naturally occurring amino acids due to the degeneracy of the genetic code. Polynucleotides can also comprise other heterologous nucleotide sequences, such as sequences coding for linkers, signal sequences, amino acid spacers, heterologous signal sequences, TMR stop transfer sequences, transmembrane domains, or ligands useful in protein purification such as glutathione-S-transferase, histidine tag, and staphylococcal protein A. Polynucleotides can also comprise other nucleotide sequences.

Methods for introducing polynucleotides (*e.g.*, vectors comprising the polynucleotides or naked polynucleotides) into cells, either transiently or stably, are well known in the art. For example, transformation methods using standard $CaCl_2$, $MgCl_2$, or RbCl methods, protoplast fusion methods or transfection of naked or encapsulated nucleic acids using calcium phosphate precipitation, microinjection, viral infection, and electroporation.

In one embodiment, a polynucleotide is derived from a mammal, such as a human. Polynucleotides can also be synthesized in the laboratory, for example, using an automatic synthesizer. An amplification method such as PCR can be used to amplify polynucleotides from either genomic DNA or cDNA encoding the polypeptides. Polynucleotide molecules encoding a variant polypeptide can also be isolated by a gene amplification method such as PCR using a portion of a nucleic acid molecule DNA encoding a polypeptide shown in SEQ ID NOs: 60-65, 67-74, 88-99 or fragments thereof as the probe.

Polynucleotides and fragments thereof can be used, for example, as probes or primers to detect the presence of SAS1B polynucleotides in a sample, such as a biological sample. A biological sample can be, *e.g.*, lymph node or tissue aspirate, serum, lymphocytes, whole blood, cellular suspension, ovarian cyst fluid, pap smear, plasma, circulating tumor cells, tumor cells or tissue, ascites fluid, urine, or fluid effusion. The ability of such probes to specifically hybridize to polynucleotide sequences will enable them to be of use in detecting the presence of complementary

sequences in a given sample. Polynucleotide probes can hybridize to complementary sequences in a sample such as a biological sample, for example, lymph tissue. Polynucleotides from the sample can be, for example, subjected to gel electrophoresis or other size separation techniques or can be dot blotted without size separation. The polynucleotide probes can be labeled. Suitable labels, and methods for labeling probes are known in the art, and include, for example, radioactive labels incorporated by nick translation or by kinase, biotin, fluorescent probes, and chemiluminescent probes. The polynucleotides from the sample are then treated with the probe under hybridization conditions of suitable stringencies.

The stringency of hybridization conditions for a polynucleotide encoding a variant polypeptide to a polynucleotide encoding polypeptides shown in SEQ ID NOs:1-51, 53-59, 66, 75-87, 100-111 can be, for example, 10% formamide, 5 x SSPE, 1 x Denhart's solution, and 1 x salmon sperm DNA (low stringency conditions). Such conditions include 25% formamide, 5 x SSPE, 1 x Denhart's solution, and 1 x salmon sperm DNA (moderate stringency conditions), including 50% formamide, 5 x SSPE, 1 x Denhart's solution, and 1 x salmon sperm DNA (high stringency conditions). However, several factors influence the stringency of hybridization other than the above-described formamide concentration, and one skilled in the art can suitably select these factors to accomplish a similar stringency. *See e.g.*, Ausubel (ed.), Current Protocols in Molecular Biology, John Wiley and Sons, Inc. (1994); Maniatis *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor laboratory, Cold Spring Harbor, N.Y. (1989)). That is, a polynucleotide encoding a variant polypeptide will hybridize to a polynucleotide encoding SEQ ID NOs:1-51, 53-59, 66, 75-87, 100-111 under low or high or both stringency conditions.

An isolated polynucleotide is a nucleic acid molecule that is not immediately contiguous with one or both of the 5' and 3' flanking sequences with which it is normally contiguous when present in a naturally occurring genome. Therefore, an isolated polynucleotide can be, for example, a polynucleotide that is incorporated into a vector, such as a plasmid or viral vector, a polynucleotide that is incorporated into the genome of a heterologous cell (or the genome of a homologous cell, but at a site different from that where it naturally occurs); and a polynucleotide that exists as a separate molecule such as a polynucleotide produced by PCR amplification, chemically synthesis, restriction enzyme digestion, or *in vitro* transcription. An

isolated polynucleotide is also a nucleic acid molecule, such as a recombinant nucleic acid molecule that forms part of hybrid polynucleotide encoding additional polypeptide sequences that can be used for example, in the production of a fusion protein.

A polynucleotide can also comprise one or more expression control sequences such as promoters or enhancers, for example. A polynucleotide can be present in a vector, such as, for example, an expression vector. If desired, polynucleotides can be cloned into an expression vector comprising, for example, promoters, enhancers, or other expression control sequences that drive expression of the polynucleotides in host cells. The polynucleotides can be operably linked to the expression control sequences.

Methods of Detection

One embodiment provides methods of detecting SAS1B polypeptides in a sample. The methods comprise contacting the sample suspected of containing SAS1B polypeptides with an antibody or antigen binding portion thereof (e.g., antibodies shown in Table 1 or antibodies and antigen binding portions thereof described herein) to form SAS1B/antibody complexes. The presence of the SAS1B/antibody complexes are detected, thereby detecting the presence of the SAS1B polypeptides.

Another embodiment provides a method of detection of SAS1B-positive cells (i.e., cells that express SAS1B) in a test sample comprising contacting one or more antibodies or antigen-binding portions thereof (e.g., antibodies 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, 31H1/31K1, antigen-binding fragments thereof, and antibody molecules that compete with 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, 31H1/31K1 for binding to SAS1B) with the test sample under conditions that allow SAS1B-positive cell/antibody complexes to form. The cells can be permeabilized or cell lysates. The SAS1B positive cell/antibody complexes are then detected. The detection of SAS1B positive cell/antibody complexes is an indication that SAS1B cells are present in the test sample. The test sample can be, e.g., lymph node or tissue aspirate, ovarian cyst fluid, pap smear, serum, whole blood, cellular suspension, lymphocytes, plasma, circulating tumor cells, tumor cells or tissue, ascites fluid, urine, or fluid effusion. Polypeptide/antibody or SAS1B-positive cell/antibody complexes can be detected by any method known in

the art, enzyme-linked immunosorbent assay (ELISA), multiplex fluorescent immunoassay (MFI or MFIA), radioimmunoassay (RIA), sandwich assay, western blotting, immunoblotting analysis, an immunohistochemistry method, immunofluorescence assay, In situ hybridization, fluorescence-activated cell sorting (FACS) or a combination thereof.

An immunoassay for SAS1B can utilize one antibody or several different antibodies. Immunoassay protocols can be based upon, for example, competition, direct reaction, or sandwich type assays using, for example, labeled antibody. Antibodies can be labeled with any type of label known in the art, including, for example, fluorescent, chemiluminescent, radioactive, enzyme, colloidal metal, radioisotope and bioluminescent labels.

Antibodies or antigen-binding portions thereof can be bound to a support and used to detect the presence of SAS1B. Supports include, for example, glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magletite.

Antibodies can be used in a method of the diagnosis of a hyperproliferative disorder by obtaining a test sample from, *e.g.*, a human or animal suspected of having a hyperproliferative disorder. The test sample is contacted with antibodies or antigen-binding portions thereof under conditions enabling the formation of antibody-antigen complexes (*i.e.,* immunocomplexes). One of skill in the art is aware of conditions that enable and are appropriate for formation of antigen/antibody complexes. The amount of antibody-antigen complexes (including, for example, a complex of an antibody or antigen-binding portion thereof and a cell expressing SAS1B on its surface) can be determined by methodology known in the art. A level that is higher than that formed in a control sample indicates the presence of a hyperproliferative disorder. A control sample is a sample that does not comprise any SAS1B polypeptides, SAS1B-positive cells, or antibodies specific for SAS1B. The amount of antibody/antigen complexes or antibodies bound to SAS1B-positive cells or cell lysates can be determined by methods known in the art.

A hyperproliferative disorder can be a neoplastic disorder (e.g., cancer, sarcoma, adenocarcinoma, adenocarcinoma of the lung, squamous carcinoma of the lung, malignant mixed mullerian tumor, lymphoma, breast cancer, ovarian cancer, colorectal cancer, liver cancer, uterine cancer, endometrial cancer, pancreatic cancer, lung cancer, etc.) or a hematologic malignancy (e.g., leukemia, etc.). In one

embodiment, a hyperproliferative disorder can be detected in a subject. A biological sample is obtained from the subject. One or more antibodies or antigen-binding portions thereof (e.g., antibodies 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, 31H1/31K1, antigen-binding fragments thereof, and antibody molecules that compete with 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, 31H1/31K1for binding to SAS1B) are contacted with the biological sample under conditions that allow SAS1B polypeptide/antibody complexes (including, for example, a complex of an antibody or antigen-binding portion thereof and a cell expressing SAS1B on its surface) to form. The SAS1B polypeptide/antibody complexes are detected. The detection of the SAS1B polypeptide/antibody complexes is an indication that the mammal has a hyperproliferative disorder. The lack of detection of the polypeptide/antibody complexes is an indication that the mammal does not have a hyperproliferative disorder.

In one embodiment, the SAS1B polypeptide/antibody complex is detected when an indicator reagent, such as an enzyme conjugate, which is bound to the antibody, catalyzes a detectable reaction. Optionally, an indicator reagent comprising a signal generating compound can be applied to the polypeptide/antibody complex under conditions that allow formation of a polypeptide/antibody/indicator complex. The polypeptide/antibody/indicator complex is detected. Optionally, the polypeptide or antibody can be labeled with an indicator reagent prior to the formation of a polypeptide/antibody complex. The method can optionally comprise a positive or negative control.

In one embodiment, one or more antibodies are attached to a solid phase or substrate. A test sample potentially comprising a polypeptide of this disclosure is added to the substrate. One or more antibodies that specifically bind SAS1B are added. The antibodies can be the same antibodies used on the solid phase or can be from a different source or species and can be linked to an indicator reagent, such as an enzyme conjugate. Wash steps can be performed prior to each addition. A chromophore or enzyme substrate is added and color is allowed to develop. The color reaction is stopped and the color can be quantified using, for example, a spectrophotometer.

Assays include, but are not limited to those based on competition, direct reaction or sandwich-type assays, including, but not limited to enzyme linked

immunosorbent assay (ELISA), multiplex fluorescent immunoassay (MFI or MFIA) western blot, IFA, radioimmunoassay (RIA), western blot, hemagglutination (HA), fluorescence polarization immunoassay (FPIA), in situ hybridization, fluorescence-activated cell sorting (FACS), and microtiter plate assays (any assay done in one or more wells of a microtiter plate).

Assays can use solid phases or substrates or can be performed by immunoprecipitation or any other methods that do not utilize solid phases. Where a solid phase or substrate is used, one or more antibodies or antigen-binding portions thereof are directly or indirectly attached to a solid support or a substrate such as a microtiter well, magnetic bead, non-magnetic bead, column, matrix, membrane, fibrous mat composed of synthetic or natural fibers (*e.g.*, glass or cellulose-based materials or thermoplastic polymers, such as, polyethylene, polypropylene, or polyester), sintered structure composed of particulate materials (*e.g.*, glass or various thermoplastic polymers), or cast membrane film composed of nitrocellulose, nylon, polysulfone or the like (generally synthetic in nature). All of these substrate materials can be used in suitable shapes, such as films, sheets, or plates, or they may be coated onto or bonded or laminated to appropriate inert carriers, such as paper, glass, plastic films, or fabrics. Suitable methods for immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like.

In one type of assay format, one or more antibodies or antigen-binding portions thereof can be coated on a solid phase or substrate. A test sample suspected of containing SAS1B polypeptides or SAS1B-positive cells is incubated with an indicator reagent comprising a signal generating compound conjugated to an antibody or antigen-binding antibody fragment specific for SAS1B (indicator reagent composition) for a time and under conditions sufficient to form antigen/antibody complexes of either SAS1B polypeptides of the test sample to the antibodies or antigen-binding fragments thereof of the solid phase or the indicator reagent compound. The reduction in binding of the indicator reagent can be quantitatively measured. A measurable reduction in the signal compared to the signal generated from a confirmed negative SAS1B test sample indicates the presence of SAS1B in the test sample. This type of assay can quantitate the amount of SAS1B in a test sample.

The formation of a polypeptide/antibody complex or a polypeptide/antibody/indicator complex (including, for example, a complex of an

antibody or antigen-binding portion thereof and a cell expressing SAS1B on its surface or a complex of an antibody or antigen-binding portion thereof, an indicator reagent, and a cell expressing SAS1B on its surface) can be detected by e.g., radiometric, colorimetric, fluorometric, size-separation, or precipitation methods. Optionally, detection of a polypeptide/antibody complex is by the addition of a secondary antibody that is coupled to an indicator reagent comprising a signal generating compound. Indicator reagents comprising signal generating compounds (labels) associated with a polypeptide/antibody complex can be detected using the methods described above and include chromogenic agents, catalysts such as enzyme conjugates fluorescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as dioxetanes, acridiniums, phenanthridiniums, ruthenium, and luminol, radioactive elements, direct visual labels, as well as cofactors, inhibitors, magnetic particles, and the like. Examples of enzyme conjugates include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

Formation of the complex (including, for example, antibodies 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, 31H1/31K1, antigen-binding fragments thereof, and antibody molecules that compete with 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, 31H1/31K1 for binding to SAS1B) can be indicative of the presence of SAS1B-positive cells in a test sample. Therefore, the methods can be used to diagnose a hyperproliferative disease in a mammal.

The methods can also indicate the amount or quantity of SAS1B in a test sample. With many indicator reagents, such as enzyme conjugates, the amount of SAS1B present is proportional to the signal generated. Depending upon the type of test sample, it can be diluted with a suitable buffer reagent, concentrated, or contacted with a solid phase without any manipulation. For example, it usually is preferred to test samples that previously have been diluted, or concentrated specimens, in order to determine the presence and/or amount of SAS1B present.

Vectors and Host Cells

A polypeptide can be expressed in systems, *e.g.*, cultured cells, which result in substantially the same post-translational modifications present as when the

polypeptide is expressed in a native cell, or in systems that result in the alteration or omission of post-translational modifications, *e.g.*, glycosylation or cleavage, present when expressed in a native cell.

An expression vector can be, for example, a plasmid, such as pBR322, pUC, or ColE1, or an adenovirus vector, such as an adenovirus Type 2 vector or Type 5 vector. Vectors suitable for use include, for example, bacterial vectors, mammalian vectors, viral vectors (such as retroviral, adenoviral, adeno-associated viral, herpes virus, simian virus 40 (SV40)) and baculovirus-derived vectors for use in insect cells. Polynucleotides in such vectors can be operably linked to a promoter, which is selected based on, *e.g.*, the cell type in which expression is sought.

The expression vector can be transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody. An embodiment includes host cells containing polynucleotides encoding an antibody (*e.g.*, whole antibody, a heavy or light chain thereof, or portion thereof, or a single chain antibody, or a fragment or variant thereof), operably linked to a heterologous promoter. For the expression of entire antibody molecules, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule.

Host cells into which vectors, such as expression vectors, comprising polynucleotides can be introduced include, for example, prokaryotic cells (*e.g.*, bacterial cells) and eukaryotic cells (*e.g.*, yeast cells; fungal cells; plant cells; insect cells; and mammalian cells). Such host cells are available from a number of different sources that are known to those skilled in the art, *e.g.*, the American Type Culture Collection (ATCC), Manassas, VA. Host cells into which the polynucleotides have been introduced, as well as their progeny, even if not identical to the parental cells, due to mutations, are included herein. Host cells can be transformed with the expression vectors to express the antibodies or antigen-binding fragments thereof. Host cells expressing antibodies or antigen-binding fragments thereof include cells and hybridomas transformed with a polynucleotide described herein.

One embodiment provides methods of producing a recombinant cell that expresses an SAS1B antibody, antigen-binding fragment thereof or portion thereof, comprising transfecting a cell with a vector comprising a polynucleotide. An SAS1B antibody, or fragment, or portion thereof, can then be produced by expressing the polypeptide in the recombinant host cell.

Isolation and purification of polypeptides produced in the systems described above can be carried out using conventional methods, appropriate for the particular system. For example, preparative chromatography and immunological separations employing antibodies, such as monoclonal or polyclonal antibodies, can be used.

Antibody-Drug Conjugates

Antibodies and antigen-binding fragments thereof (e.g., antibodies 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, 31H1/31K1 , antigen-binding fragments thereof, and antibody molecules that compete 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, 31H1/31K1 for binding to SAS1B) that specifically bind SAS1B can be conjugated to a therapeutic agent or effector molecule to form an "antibody-drug conjugate". A therapeutic agent is an agent with a biological activity directed against a particular target molecule or a cell bearing a target molecule. Therapeutic agents can include, for example, various drugs such as vinblastine, daunomycin, cytotoxins such as maytansinoids and maytansinoid analogs, a prodrug, tomaymycin derivatives, taxoids, a leptomycin derivative, CC-1065 and CC-1065 analogs, encapsulating agents (such as liposomes) that contain pharmacological compositions, therapeutic agents, toxins (e.g., ricin, abrin, diphtheria toxin and subunits thereof, botulinum toxins A through F, variants of toxins (see, e.g., U.S. Pat. Nos. 5,079,163 and 4,689,401), *Pseudomonas exotoxin* (PE) (see e.g., U.S. Pat. No. 5,602,095) and variants thereof (see, e.g. U.S. Pat. Nos. 4,892,827; 5,512,658; 5,602,095; 5,608,039; 5,821,238; and 5,854,044; PCT Publication No. WO 99/51643; Pai *et al.,* Proc. Natl. Acad. Sci. USA 88:3358, 1991; Kondo *et al.,* J. Biol. Chem. 263:9470, 1988; Pastan *et al.,* Biochim. Biophys. Acta 1333:C1-C6, 1997)), radioactive agents such as ^{125}I, ^{32}P, ^{14}C, ^{3}H and ^{35}S and other labels, target moieties and ligands. An effector molecule is a small molecule that selectively binds to a protein and regulates its biological activity.

Effector or therapeutic molecules can be linked to an antibody using any number of means known to those of skill in the art, for example by covalent or noncovalent attachment. Therapeutic agents or effector molecules that are polypeptides will typically contain a variety of functional groups; such as carboxylic acid (COOH), free amine (--NH_2) or sulfhydryl (--SH) groups, which are available for reaction with a suitable functional group on an antibody to result in the binding of the effector molecule or therapeutic agent. Alternatively, the antibody is derivatized to

expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of known linker molecules. The linker can be any molecule used to join the antibody to the effector molecule. The linker is capable of forming covalent bonds to both the antibody and to the effector molecule or therapeutic agent. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the antibody and the effector molecule are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (such as through a disulfide linkage to cysteine) or to the alpha carbon amino and carboxyl groups of the terminal amino acids.

In some circumstances, it is desirable to free the effector molecule or therapeutic agent from the antibody when the antibody-drug conjugate has reached its target site. Therefore, antibody-drug conjugates can comprise linkages that are cleavable in the vicinity of the target site. Cleavage of the linker to release the effector molecule or therapeutic molecule from the antibody can be accomplished by, for example, enzymatic activity or conditions to which the antibody-drug conjugate is subjected either inside the target site or in the vicinity of the target site.

In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, labels (such as enzymes or fluorescent molecules) drugs, toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given therapeutic agent or effector molecule to an antibody or other polypeptide.

Antibodies can be labeled with a detectable moiety. Detectable moieties include, for example, fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors, bioluminescent markers (e.g., luciferase, green fluorescent protein (GFP), yellow fluorescent protein (YFP)), enzymes (e.g., horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase, glucose oxidase), a magnetic agent (e.g. gadolinium), lanthanides (e.g., europium and dysprosium), manganese, paramagnetic particles (e.g., superparamagnetic iron oxide), polypeptide epitopes recognized by a secondary reporter (such as leucine zipper pair sequences, radiolabeled amino acids, binding sites for secondary antibodies, metal binding domains, epitope tags). Detectable moieties can be attached to antibodies by spacer arms of various lengths to reduce potential steric

hindrance.

Radioimmunoconjugates

An antibody or binding fragment thereof can be conjugated to a radionuclide molecule for use as a radioimmunoconjugate for treatment for cancer or for imaging and detection of cancer cells. Radioimmunoconjugates allow radiation therapy to be delivered directly to the surface of tumor cells in relatively high doses while sparing normal tissues from the effects of radiation. Radioimmunoconjugates can inhibit tumor cell proliferation and *microvascularization* around *tumor cells* and can be used to eradicate the last microscopic clusters of tumor cells after treatment.

An antibody of the invention can be conjugated to an alpha emitter or a beta emitter, for example ^{177}Lu, ^{131}I, ^{90}Y, ^{188}Re, ^{186}Re, ^{67}Cu, ^{212}Bi, ^{213}Bi or ^{211}At, ^{149}Tb, ^{225}Ac, ^{212}Pb, ^{225}Ac, ^{227}Th, ^{199}Ah, ^{199}Au, ^{194}Ir, ^{166}Ho, ^{159}Gd, ^{153}Sm, ^{149}Pm, ^{142}Pr, ^{111}Ag, ^{109}Pd, and ^{77}As.

A radioimmunoconjugate can be comprised of an antibody or an antigen-binding fragment of an antibody that specifically binds SAS1B linked to radionuclide molecule via a linker. Any type of linker with sufficient complexing ability and a functional group allowing direct or indirect conjugation to a protein or a peptide can be used. For example, an antibody or binding fragment thereof can be linked to a radionuclide by a chelating agent. A chelating agent is attached to the antibody or binding fragment thereof and functions to chelate the radionuclide. The binding or linking of the radionuclide to the antibody can be done in any manner known in the art, as long as the targeting specificity of the antibody or binding fragment thereof is not decreased by a substantial amount. Chelating agents are known in the art and include, for example, DOTA, cDTPA, TETA and CHX-A-DTPA (cyclohexyldiethylenetriamine pentaacetic acid). A radionuclide can be attached to an antibody or binding fragment thereof by reacting a bifunctional chelating agent, e.g., p-SCN-bn-DOTA with the antibody, followed by purification to remove unconjugated chelator, and then reaction of the chelator antibody conjugate with the radionuclide, followed by purification to remove any unconjugated radionuclide. Alternatively, the chelation agent and the radionuclide can be combined and then subsequently conjugated to the antibody.

Examples of bifunctional cyclic chelating agents that can be used in the methods of the invention include p-SCN-bn-DOTA, and DOTA-NHS-ester. Bifunctional linear chelators like p-SCN-Bn-DTPA and CHX-A"-DTPA can also be

used. Cyclic, linear or branched chelating agents can be used, for example polyaminopolyacid chelating agents that comprise a linear, cyclic or branched polyazaalkane backbone with acidic (e.g. carboxyalkyl) groups attached at backbone nitrogens. Other examples of suitable chelating agents include DOTA derivatives such as p-isothiocyanatobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (p-SCN-Bz-DOTA) and DTPA derivatives such as p-isothiocyanatobenzyl-diethylenetriaminepentaacetic acid (p-SCN-Bz-DTPA).

An embodiment provides a pharmaceutical composition comprising a radioimmunoconjugate as described herein, and a pharmaceutically acceptable carrier and/or excipient. Acceptable pharmaceutical carriers include but are not limited to non-toxic buffers, fillers, isotonic solutions, and the like. The carrier can contain a radiolytic stabilizer, e.g., ascorbic acid, which can protect the integrity of the radioimmunoconjugate during storage and shipment.

A radioimmunoconjugate can be provided as a pharmaceutical composition comprising a radionuclide linked to an antibody or a fragment thereof dissolved in a buffer solution, which substantially maintains the chemical integrity of the radioimmunoconjugate. The pharmaceutical composition can be physiologically acceptable for infusion into patients.

A patient can be treated by administering one or more radioimmunoconjugates to a patient. The one or more radioimmunoconjugates can be administered to a patient in conjunction with one or more clearing agents or chelating agents to prevent unbound radioisotope from accumulating in and damaging organs and tissues in the body.

A pharmaceutical composition of the invention can by administered by, for example, intravenous infusion or intravenous injection. In an embodiment a pharmaceutical composition can be administered directly in a vein by a peripheral cannula connected to a drip chamber that prevents air embolism and allows an estimate of flow rate into the patient.

In an embodiment a pharmaceutical composition can be administered in a repeated fashion (e.g., 2, 3, 4, 5, 6, or more times over, e.g., 1, 2, 3, 4, 5, days or more, or 1, 2 3, 4, 5 weeks or more, or 1, 2, 3, 4, 5, weeks or more). In another embodiment a radioimmunoconjugate can be administered in a repeated fashion, but with different radionuclides, e.g., beta-radioimmunotherapy can be followed by alpha-radioimmunotherapy or vice versa.

A radioimmunoconjugate can be administered alone or in combination with or in addition to one or more additional therapies. An additional therapy can be, for example, pretreatment, chemotherapy, monoclonal antibody therapy, surgery, radioimmunotherapy, photodynamic therapy, bone marrow transplantation, and stem cell transplantation and/or therapy.

In an embodiment of the present invention the antibody dosing is about 1, 5, 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, or more mg per patient, and a radionuclide amounting to about 1, 2, 5, 10, 25, 50, 75, 100, 125, 150, 175, 200 or more MBq/kg of bodyweight.

An embodiment provides a kit for the production of a radioimmunoconjugate, which can comprise two or more vials, wherein one vial contains a conjugate comprising a linker such as a chelator linked to an antibody or antibody fragment of the disclosure and a second vial contains a radionuclide. Some procedures can be performed, e.g., radiolabeling and/or purification before infusion. The contents of one or more of the vials can be dried, lyophilized, or in a solution. In an embodiment the radioimmunoconjugate is produced by mixing the content of the two vials.

Methods of Treatment

Antibodies and antigen-binding fragments thereof (e.g., antibodies 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, 31H1/31K1 , antigen-binding fragments thereof, and antibody molecules that compete with 7H2, 6C1, 1H2/1K2, 1H3/1K3, 2H2/2K5, 6B1, 3F2, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, 31H1/31K1 for binding to SAS1B) can be conjugated to a cytotoxic agent, such as duocarmycin, maytansanoids, and auristatins, to form a drug having specific cytotoxicity towards SAS1B-expressing cells by targeting the drug to SAS1B. Cytotoxic conjugates comprising such antibodies and/or antigen-binding fragments thereof and a drug or cytotoxin can be used as a therapeutic for treatment of hyperproliferative disorders such as such as any SAS1B-positive cancer, including, but not limited to, uterine cancer, pancreatic cancer, and ovarian cancer.

One embodiment provides methods of treating or preventing hyperproliferative disorders comprising administering an effective amount of an antibody or antigen-binding fragment thereof or an antibody-drug conjugate to a mammal in need thereof.

An "effective amount" is an amount sufficient to effect beneficial or desired results. For example, an effective amount is one that achieves the desired therapeutic effect. An effective amount can be administered in one or more administrations, applications or dosages. An effective amount of a pharmaceutical composition (i.e., an effective dosage) depends on the pharmaceutical composition selected. The compositions can be administered from one or more times per day to one or more times per week; including once every other day. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with an effective amount of the pharmaceutical compositions described herein can include a single treatment or a series of treatments.

"Treatment" means the administration of one or more pharmaceutical compositions to a subject. The term treatment also includes an adjustment (e.g., increase or decrease) in the dose or frequency of one or more pharmaceutical agents that a subject can be taking, the administration of one or more new pharmaceutical agents to the subject, or the removal of one or more pharmaceutical agents from the subject's treatment plan. Treatment also refers to any amelioration of one or more symptoms of a hyperproliferative disease, improvement in patient survival, or the reversal of the disease.

A subject can be an animal, for example, a mammal, a human, monkey, dog, cat, horse, cow, pig, goat, rabbit, or mouse.

A "pharmaceutical composition" is a sterile or aseptic composition of an antibody or antigen-binding fragment thereof or antibody-drug conjugate formulated with a pharmaceutically acceptable carrier, which can be safely administered to a subject. The pharmaceutical composition does not cause undesirable side effects when administered to a patient that outweigh the beneficial effects.

One embodiment provides a pharmaceutical composition for the treatment of a hyperproliferative disorder in a mammal, which comprises an effective amount of an antibody or antigen-binding portion thereof or an antibody-drug conjugate and a pharmaceutically acceptable carrier. The pharmaceutical composition can be used for the treatment of cancer, including (but not limited to) the following: carcinoma, including that of the bladder, breast, endometrium, colon, kidney, liver, lung, ovary,

pancreas, stomach, cervix, thyroid and skin; including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Burkitt's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; other tumors, including melanoma, seminoma, tetratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscarama, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer and teratocarcinoma, and other cancers yet to be determined in which SAS1B is expressed. An embodiment provides pharmaceutical compositions comprising: an effective amount of an antibody, antibody fragment or antibody-drug conjugate, and a pharmaceutically acceptable carrier, which may be inert or physiologically active.

"Pharmaceutically-acceptable carriers" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, and the like that are physiologically compatible. Examples of suitable carriers, diluents and/or excipients include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, and the like, as well as combination thereof. Isotonic agents, such as sugars, polyalcohols, or sodium chloride can be present in compositions. Examples of suitable carriers include, for example: Dulbecco's phosphate buffered saline, pH about 7.4, containing or not containing about 1 mg/ml to 25 mg/ml human serum albumin; 0.9% saline (0.9% w/v sodium chloride (NaCl)), and 5% (w/v) dextrose. Carriers can also contain an antioxidant such as tryptamine and a stabilizing agent such as TWEEN20® (polysorbate).

Administration can be by any method, including, for example parenteral (e.g. intravenous, intramuscular, intraperinoneal, subcutaneous, intra-articular, intrasynovial, intratumoral, peritumoral, intralesional, or perilesional). Compositions can be administered intravenously as a bolus or by continuous infusion over a period of time.

Sterile or aseptic compositions for parenteral administration can be prepared by incorporating the antibody, antigen-binding fragment or antibody-drug conjugate in the required amount in the appropriate solvent, followed by sterilization by

microfiltration. As solvent or vehicle include, for example, water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, and the like, as well as a combination thereof. Isotonic agents, such as sugars, polyalcohols, or sodium chloride can be present in compositions. These compositions may also contain adjuvants, in particular wetting, isotonizing, emulsifying, dispersing and stabilizing agents. Sterile compositions for parenteral administration can also be prepared in the form of sterile solid compositions, which can be dissolved at the time of use in sterile water or any other injectable sterile medium.

The antibodies, antigen-binding fragments thereof or antibody-drug conjugates can also be orally administered as a solid composition (tablets, pills, powders gelatin capsules, sachets) or granules) or liquid compositions (pharmaceutically acceptable solutions, suspensions, emulsions, syrups and elixirs containing inert diluents such as water, ethanol, glycerol, vegetable oils or paraffin oil). These compositions may comprise substances other than diluents, for example wetting, sweetening, thickening, flavoring or stabilizing products.

The doses depend on the desired effect, the duration of the treatment and the route of administration used; they are generally between 5 mg and 1000 mg per day orally for an adult with unit doses ranging from 1 mg to 250 mg of active substance.

Antibodies, antibody-binding portions thereof, or antibody-drug conjugates can be used for the treatment of a hyperproliferative disorder in a mammal. The antibodies, antibody-binding portions thereof, or antibody-drug conjugates can also be used to treat the neovascularization of a tumor.

Similarly, an embodiment provides a method for inhibiting the growth of selected cell populations comprising contacting target cells, or tissue containing target cells, with an effective amount of an antibody, antigen-binding fragment or antibody-drug conjugate, or an antibody, antigen-binding fragment or a therapeutic agent comprising a cytotoxic conjugate, either alone or in combination with other cytotoxic or therapeutic agents.

Methods for inhibiting the growth of selected cell populations expressing SAS1B can be practiced *in vitro, in vivo*, or *ex vivo*. "Inhibiting growth" means slowing the growth of a cell, decreasing cell viability, causing the death of a cell, lysing a cell and inducing cell death, over a short period of time (e.g., minutes to hours) or a long period of time (e.g., days to months).

Examples of *in vitro* uses include treatments of autologous bone marrow prior to their transplant into the same patient in order to kill diseased or malignant cells; treatments of bone marrow prior to its transplantation in order to kill competent T cells and prevent graft-versus-host-disease (GVHD); treatments of cell cultures in order to kill all cells except for desired variants that do not express the target antigen; or to kill variants that express undesired antigen.

Examples of clinical *ex vivo* use include the removal of tumor cells or lymphoid cells from bone marrow prior to autologous transplantation in cancer treatment or in treatment of autoimmune disease, or to remove T cells and other lymphoid cells from autologous or allogeneic bone marrow or tissue prior to transplant in order to prevent graft versus host disease (GVHD).

For clinical *in vivo* use, the antibody, the antigen-binding fragment, or the antibody-drug conjugate can be supplied as solutions that are sterile and contain appropriate levels of endotoxin. Examples of suitable protocols of antibody-drug conjugate administration are as follows. Antibodies, antigen-binding fragments thereof or antibody-drug conjugates can be given weekly for 4 weeks as an i.v. bolus each week. Bolus doses are given in 50 to 100 ml of normal saline to which 5 to 10 ml of human serum albumin can be added. Dosages can be 10 μg to 100 mg per administration, i.v. (range of 100 ng to 1 mg/kg per day). Dosages can range from 50 μg to 30 mg. Dosages can range from 1 mg to 20 mg. After four weeks of treatment, the patient can continue to receive treatment on a weekly basis. Specific clinical protocols with regard to route of administration, excipients, diluents, dosages, times, etc., can be determined by one of ordinary skill in the art as the clinical situation warrants.

The antibodies or antigen-binding fragments can also be used to detect SAS1B in a biological sample *in vitro* or *in vivo*. Antibodies or fragments thereof can be used to determine the level of SAS1B in a tissue or in cells derived from the tissue. The tissue can be diseased tissue, a tumor or a biopsy of a tumor. The tissue or biopsy thereof can be frozen, fixed, permeabilized or non-permeabilized.

The above-described method can be used to diagnose a cancer in a subject known to or suspected to have a cancer, wherein the level of SAS1B measured in said patient's tissues, blood or serum is compared with that of a normal reference subject or standard. The method can then be used to determine whether a tumor or cells of tissue of the patient expresses SAS1B, which may suggest that the tumor or

patient will respond well to treatment with the antibodies, antigen-binding fragments or antibody-drug conjugates described herein.

A method for diagnosis is also provided in which labeled antibodies, antigen-binding fragments thereof, or antibody-drug conjugates are administered to a subject suspected of having a cancer, and the distribution of the label within the body of the subject is measured or monitored.

All patents, patent applications, and other scientific or technical writings referred to anywhere herein are incorporated by reference herein in their entirety. The embodiments illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms, while retaining their ordinary meanings. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope claimed. Thus, it should be understood that although the present invention has been specifically disclosed by embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this disclosure as defined by the description and the appended claims.

In addition, where features or aspects are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that embodiments also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

The following are provided for exemplification purposes only and are not intended to limit the scope described in broad terms above.

Examples

Example 1 SAS1B in Human Lung Tumors

The SAS1B protein is encoded by the *Astl* gene (astacin-like metalloendopeptidase). ASTL expression is upregulated in lung adenocarcinomas. ASTL has a gain of copy number ranking of 1389, placing ASTL in the top 8% of

genes upregulated in lung adenocarcinomas. This data in primary tumors is supported by upregulation of ASTL in lung adenosquamous and adenocarcinoma cell lines.

ASTL expression in several squamous and adenocarcinoma lung cancer cell lines has been confirmed. PCR amplification of the human SAS1B catalytic domain of 579 bp occurred in lung cancer cell lines NCI-H226, A549, HEK-293, human ovary, MMMT539, while the control water was negative. Western blotting of protein extracts from lung cancer H226 and A549 cell lines using a guinea pig polyclonal anti-human SAS1B antibody showed expression of full length (46 kD) and truncated SAS1B proteins in both lung cancer cell lines H226 and A549. SAS1B can be localized to the surfaces of both H226 and A549 cells. Immunofluorescent staining of unpermeabilized A549 cells and cell surface staining of H226 cells was observed. Polyclonal antibodies to SAS1B in the presence of complement lyse (kill) tumor cells that express SAS1B, while inactivated complement has no effect.

Example 2 Production and Characterization of SAS1B and Antibodies

The human SAS1B protein is encoded by the *Astl* gene (astacin-like metalloendopeptidase). ASTL expression is upregulated in lung adenocarcinomas. ASTL has a gain of copy number ranking of 1389, placing ASTL in the top 8% of genes upregulated in lung adenocarcinomas. This data in primary tumors is supported by upregulation of ASTL in lung adenosquamous and adenocarcinoma cell lines. ASTL expression was confirmed in several squamous and adenocarcinoma lung cancer cell lines. PCR amplification of the human SAS1B catalytic domain of 579 bp occurred in lung cancer cell lines NCI-H226, A549, HEK-293, human ovary, MMMT539, while the control water was negative. Western blotting of protein extracts from lung cancer H226 and A549 cell lines using a guinea pig polyclonal anti-human SAS1B antibody showed expression of full length (46 kD) and truncated SAS1B proteins in both lung cancer cell lines H226 and A549. Most importantly, SAS1B can be localized to the surfaces of both H226 and A549 cells. Immunofluorescent staining of unpermeabilized A549 cells and cell surface staining of H226 cells was observed. Polyclonal antibodies to SAS1B in the presence of complement lyse (kill) tumor cells that express SAS1B, while inactivated complement has no effect.

A diagram of the human SAS1B polypeptide is shown in Figure 1. SAS1B has been divided into domains. These include a classic signal peptide,

propetide, putative transmembrane, catalytic region embedded in overall larger protease domain and C-terminus domain. SAS1B shows protein microheterogeneity in both mice and humans.

SAS1B has several splice variant forms: SV-A, SV-B, SV-C, SV-D, SV-E, and SV-F. Splice variants A and C were PCR amplified using variant specific forward and reverse primers. SV-A and SV-C were amplified from the following RNA sources: 1) SNU539 cell line (malignant mixed Mullerian tumor); 2) pancreatic ductal adenocarcinoma (PDAC) mouse xenograft tumor samples (human primary patient tumors were affixed into pancreas of nude mice and then propagated; tumor RNA interrogated here from approximately F5 generation); 3) human primary patient head and neck squamous cell carcinoma (HNSCC) samples. PCR products were subcloned and sequenced and sequence identity was used to confirm SV-A, SV-C, or SV-D splice variants.

Splice variant B was identified by sequencing data generated by subcloning PCR products from SV-A primer set. DNA sequences were identified from all three RNA sources listed above confirming presence of SV-B transcript in different tumor types.

Splice variant D was PCR amplified using variant specific primers from SNU539 cell line RNA. Identification was confirmed by subcloning and sequencing PCR amplicon.

These results show identification of splice variants A, B, C, and D transcripts present in various cancer types.

Amplification of each of the splice variants reveals that SV-B lacks 18 amino acids from Exon 8, SV-C has a novel N-terminal transmembrane domain, SV-D has a novel transmembrane domain and an ORF stop after Exon 7. SV-E and SV-F have a novel transmembrane domain and an N-terminal ORF stop after Exon 4. SV-E and SV-F have a C-terminal ORF that starts from extended Exon 5.

Amino acids 55-289 of splice variant A of SAS1B were expressed in in *E. coli*, and used to generate mouse antibodies. The immunogen was denatured prior to generation of antibodies. Monoclonal antibodies 6B1 and 3F2 were generated.

Amino acids 280-430 of splice variant A of human SAS1B ("recombinant

SAS1B-CT") were expressed in *E. coli* and used to generate mouse antibodies. Monoclonal antibodies 6C1 and 7H2 were generated. Clones 6C1 and 7H2 recognized recombinant SAS1B-CT in a western blot demonstrating that they are positive clones.

Amino acids 280-430 ("recombinant SAS1B-CT") of splice variant A of human SAS1B were expressed in *E. coli* and used to generate rabbit antibodies. The recombinant SAS1B-CT was a 17 kDa protein according to SDS-PAGE analysis. Monoclonal antibodies 1H2/1K2, 1H3/1K2, 2H2/2K5, 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5, and 31H1/31K1 were generated.

Example 3 Antibody Screening

Monoclonal antibodies 6C1, 7H2, and 3A7 (a negative control) were screened by western blotting using SAS1B-SVA transfected HEK293 T cell extracts. HEK293 cells were transfected with SAS1B-SVA plasmids to express the SAS1B protein. The cell lysate from the untransfected and transfected cells were prepared, electrophoresed, and blotted. Blots were then probed with the anti-SAS1B-CT monoclonal antibodies (6C1 and 7H2), the negative control 3A7 antibody, SB3, which is a mouse monoclonal antibody known to react with SAS1B-transfected cells, and an anti-His antibody. SAS1B-CT monoclonal antibodies 6C1 and 7H3 reacted with the 52 kDa SAS1B protein. 3A7 did not react with the SAS1B protein.

Culture supernatants of cells transfected with SAS1B-CT were tested by western blot using the mammalian cell-expressed full-length SAS1B-SVA (mol weight ~52kDa). Where cell lysates from untransfected cells were used, the antibodies did not recognize any protein. The antibodies did, however, recognize the 52 kDa SAS1B protein from the transfected cells, which is the only additional protein present in the transfected cells.

Secondary screens of the SAS1B-CT rabbit clones were completed with western blotting. HEK293-cells transfected with SAS1B-SVA (~52kDa) lysates, untransfected HEK-cell lysates, and recombinant SAS1B-CT were tested against rabbit antibodies 1H2/1K2, 1H3/1K2 and 2H2/2K5. All three clones tested reacted with the full-length recombinant SAS1B SVA protein as well as the truncated SAS1B-CT protein which was used as the immunogen to generate the monoclonal antibodies.

A western blot analysis of the SAS1B-anti-mouse (6B1 and 3F2) and anti-rabbit (1H2/1K2, 1H3/1K2 and 2H2/2K5) monoclonal antibodies on endogenous SAS1B using an uterine cancer derived cell line SNU 539 cell lysate was performed. SNU 539 cell lysates were made in RIPA or Celis extraction buffer. The experiment was done to determine pattern of reactivity of the antibodies and also to determine if they specifically bind endogenous SAS1B. Extraction with RIPA buffer gave a better result that the Celis buffer. Rabbit 1H2/1K2 and 1H3/1K2 monoclonal antibodies showed an identical pattern recognizing a 120kDa band. Mouse monoclonal antibodies 6B1 and 3F2 recognized 75 and 120 kDa bands respectively.

A western blot analysis of the SAS1B-anti-mouse and anti-rabbit monoclonal antibodies on endogenous SAS1B using a colon cancer cell line SW480 cell lysate was performed. The SW480 cell lysate was made in Celis extraction buffer. A rabbit polyclonal antibody recognized a 65 kDa band and a higher molecular weight band of about ~120 kDa which was also recognized by rabbit 1H3/1K2 and mouse monoclonal 6B1. Mouse monoclonal antibody 3F2 recognized a strong band at ~100 kDa and 75 kDa and lower molecular weight bands at 37 kDa and 30 kDa.

2-D gel electrophoresis and western blotting using 17 cm IPG (immobilized pH gradient) strips was performed. SNU 539 cell lysate in Celis (8 M urea, 2 M thiourea, 2% NP-40 and 100mM DTT and 0.2% ampholines) was prepared. 400mg of the protein per IPG strip was loaded as follows: -pH 4-7 NL, 17cm. The proteins resolved by IEF (isoelectric focusing) were further separated by SDS-PAGE using 12% gels. After transblotting the proteins, the membranes were probed with polyclonal and monoclonal antibodies with appropriate controls. An SAS1B rabbit polyclonal antibody recognized mainly a ~65kDa band, a ~50 kDa band (pI 4.5) and a ~37kDa (pI 7) band on a 2D Western blot in which the lysate was prepared in Celis. The pattern shown by the rabbit monoclonal antibody 1H3/1K2 is similar to the one displayed on 1D Western, showing multiple charge variants at around 120 kDa and pI 6.5. 6B1 recognized a strong band at around 75 kDa at a pI~ 5.5. Mouse monoclonal 3F2 recognized a band little higher, very close to the spots recognized by rabbit monoclonal 1H3 /1K2, with slight acidic pI. Mouse monoclonal 3A7 did not recognize the recombinant SAS1B but recognized the *E. coli* protein

contaminant in the immunogen. Therefore, it was used as a negative control. 3A7 did not recognize any major bands in a SNU539 cell lysate.

Immunoprecipitation-specific binding of SAS1B-CT monoclonal antibodies to recombinant full-length SAS1B-SVA was tested in a transfection model. This immunoprecipitation experiment was done to determine if the monoclonal antibodies 6C1 and 7H2 capture the recombinantly-produced SAS1B in a transfection model. Lysates of HEK 293 cells transfected with recombinant full-length SAS1B-SVA were incubated with the antibody, and then mixed with Protein G coupled to DYNABEADS® magnetic beads. The DYNABEADS® magnetic beads were washed several times and the bound antigen was eluted with the elution buffer. Both the eluted protein and the bead pellet was checked by western blotting for the presence of the SAS1B protein using an anti-His antibody. Immmunoprecipitation from both 6C1 and 7H2 showed that both antibodies are capable of binding and capturing the recombinant SAS1B.

Immunohistochemical experiments were performed with mouse ovarian tissue sections. Culture supernatants from 6B1 and 3F2 showed oocyte staining in Bouins fixed mouse ovarian section. The antibodies also showed immunoreactivity in oocytes within secondary antral follicles, while immunoreactivity was not seen in oocytes contained within primordial or primary follicles. Control mouse IgG showed no staining to any cell type.

Immunohistochemical experiments were also performed with monkey ovarian tissue sections. Bouins fixed monkey ovary sectioned stained with control mouse IgG showed no staining to any cell type. Protein A purified monoclonal antibodies 3F2 and 6B1 used on Bouin's fixed monkey ovary tissue sections revealed intense immunoreactivity to oocytes housed in secondary follicles. There was staining in the ooplasm within a follicle at the primary-to-secondary transition, where the persistent unilaminar granulosa cell layer and incomplete bilaminar layers are noted. Oocytes in primordial and primary follicles were SAS1B negative.

Protein A purified monoclonal antibody 3F2 was used on either zinc formalin or neutral buffered formalin fixed monkey ovary section. Intense immunoreactivity to oocytes housed in secondary follicles was revealed. Oocytes in primordial and primary follicles were SAS1B negative. Control

mouse IgG did not stain any cell type.

Protein A purified monoclonal antibody 3F2 was used on neutral buffered formalin-fixed monkey normal breast/mammary glands, uterus, pancreas and kidney sections. The results revealed no immunoreactivity to any cell types. Control mouse IgG (Ms IgG) did not stain any cell type. SNU539 uterine cancer cells were injected subcutaneously into nude mice. The cells formed solid tumors. Excised tumors were fixed in Bouin's fixative followed by SAS1B immunohistochemical staining. Monoclonal antibody culture supernatants 6B1 and 3F2 were used to stain xenograft tissue sections. Both antibodies immunostained the tumor section. Control mouse IgG (Ms IgG) did not stain any cells.

Monoclonal antibody culture supernatants 6B1 and 3F2 were used to stain live SNU539 uterine cancer cells. The antibodies did not immunoreact to membrane structures indicating that the epitope for these antibodies could be buried within the cell. However, the same antibodies when used to probe a western blot of the same cell line total protein extract, showed immunoreactivity to several protein bands in the native extract and also reacted to the recombinant HS4 antigen at ~35 kDa. Control mouse IgG showed no immunostaining to the cells or extracts.

Zinc formalin-fixed clear cell papillary renal cell carcinoma cells were immunostained with monoclonal antibody 3F2. The antibody immunostained the tumor section. Control mouse IgG (Ms IgG) did not stain any cells.

Example 4 Fluorescence-activated cell sorting (FACS) analysis

FACS analysis was performed using SAS1B positive cell lines and rabbit monoclonal antibodies. The following samples were used: un-stained cells, secondary antibody alone, positive Integrin α4 Rb mAb, mAb 34 mock control, mAb 1, mAb 2, mAb 3 (1H2/1K2), mAb 5 (1H3/1K2), and mAb 7 (2H2/2K5). 2 million cells/tube of SNU539 cells were allowed to recover for 2-3 hours at 37°C in media. Cells were blocked with warm 5% NGS media and chilled in 0.1% azide containing media on ice. The cells were then incubated with the rabbit monoclonal antibody supernatants mAb (8ug/ml) or controls in blocking media for 2 hours on ice. The cells were washed with azide buffer. The secondary antibody was goat anti-rabbit R-PE (R-phycoerythrin). The cells were fixed

with 4% PFA (paraformaldehyde) for 15 mins at room temperature, washed and analyzed on the flow cytometer (Calibur).

The integrin α4 monoclonal antibody was used as a positive control for the flow cytometry assay to determine expression of surface integrin protein on the cancer cells. The mock control is the media in which the monoclonal antibodies were obtained. These were SAS1B negative. Monoclonal antibodies 1, 3 and 5 showed good surface staining by flow cytometry.

Example 5 Double immunofluorescence ("IF") assays

Experiments were conducted to determine the specificity of anti-human SAS1B monoclonal antibodies 3F2 and 6B1 against the human SAS1B protein with a C-terminal V5 tag expressed by HEK293T cells by double immunofluorescence from two different SAS1B epitopes. HEK293T cells were seeded at ~100,000 per well of 24 well plate containing a lysine-coated 12 mm coverslip. After 24 hours the cells were transfected with 1 ug of SV-A plasmid with 2 ul of TURBOFECT® transfection reagent followed by no change of media. 48 h later the cells were PFA fixed and methanol permeabilized for an IF study as follows.

The cells were removed from cell culture media and fixed in 4% PFA in PBS, 300 ul for 15 min. The cells were washed in 1 ml PBS in wells for 3 times and permeabilized in methanol (100%) for 15 minutes in 0.5 ml at room temperature. The cells were washed in PBS (1 ml) 3 times and blocked in complete culture media (DMEM) with 5% normal goat serum (NGS) for 30 min (i.e., BB, blocking buffer). The first antibody in BB was added for 1 h at 10 ug/ml for monoclonal antibodies 3F2 and 6B1 and 5 ug/ml for anti-V5 DYLIGHT® 488 rabbit (a rabbit polyclonal antibody to V5 tag). The cells were washed in PBS 3 times. The secondary antibody in BB (goat anti-mouse ALEXA FLUOR® 594) was added for 1 h at 5 ug/ml (1:200 dilution). The cells were washed in PBS 3 times. The cells were mounted in SLOWFADE® antifade mountant with DAPI nuclear stain ((3ul) and stored at 4°C before confocal imaging.

The mock transfected HEK293T cells showed no V5 signal or SAS1B signal when probed with anti-V5 antibody or mouse IgG control antibody or secondary antibody alone. The anti-V5 antibody confirmed expression of

human SAS1B with C-terminal V5 tag in fixed and permeabilized HEK293T cells. The V5 tag antibody confirmed the expression of SAS1B SV-A epitopes in transfected HEK293T cells. The V5 tag antibody confirmed the expression of SAS1B SV-A epitopes in transfected HEK293T cells. SAS1B mAbs 3F2 and 6B1 IF colocalizes (merge) with the V5-tag epitope signal confirming the specificity of 3F2 and 6B1 monoclonal antibodies for the SAS1B epitope.

Therefore, double epitope probing of HEK293T cells transfected with human SAS1B SV-A containing a V5 epitope revealed colocalization of the IF pattern from 3F2 and 6B1 mAbs with the V5 tag antibody IF pattern confirmed the specificity of these mouse mAbs towards human SAS1B protein in fixed and permeabilized cells.

Example 6 Live Immunofluorescence

Anti-Human SAS1B mouse monoclonal antibodies reactive to a SAS1B epitope on live cancer cell surfaces were identified by indirect immuno-fluorescence (IF) for the development of therapeutic antibodies. M1 (renal cancer cell line) and MMMT539 (uterine cancer cell line) cells were seeded at 20,000 cells in 1ml per well on collagen coated coverslips in 24 well plates. The media was replaced 22 h post seeding. For antibody probing, cells were kept on ice for ~8 to 18 min following 47 h post seeding. The first antibody was added at 50 ug or 20 ug or 10 ug/ml in complete culture media with 15 mM NaN3 (pre-chilled) incubated on ice for 60 min, and the cells washed in complete culture media with 15 mM NaN3 in cold 1ml, x3. The second antibody, goat-antimouse-AF488 (pre-chilled), was added to the wells at 5ug/ml in complete culture media with 15 mM NaN3 for 60 min on ice. The cells were washed in complete media with 15 mM NaN3, 1 ml, x3 on ice. The cells were fixed in 4% PFA in PBS for 20min on ice, then washed in distilled water at RT, 1ml, x1, and mounted in 8 ul of SLOWFADE® Diamond Antifade Mountant with DAPI nuclear stain. An anti-EGFR monoclonal antibody was used as a positive control for live M1 cells. Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein and member of protein kinase superfamily. Both fluorescence and confocal microscopy confirmed the EGFR expression on the cell surface of the live M1 renal carcinoma cell line. Most of the cells expressed the receptor and validated the live cell surface IF staining methodology for the screening of anti-human SAS1B mouse monoclonal

antibodies. The receptor molecule was found on the cell surfaces. Anti-human CABYR monoclonal antibody 3A4 was used as a negative control for live MMMT539 cells. CABYR is a calcium binding cytoskeleton sperm protein with no transmembrane domain. Anti-CABYR mAb 3A4 showed no fluorescence signal on the live MMMT539 cancer cell line by fluorescence or confocal microscopy.

Anti-SAS1B C-terminal monoclonal antibodies 6C1 and 7H2 showed no fluorescence signal on the surface of live uterine tumor cells like the anti-CABYR monoclonal antibody 3A4, suggesting a lack of SAS1B C-terminal epitope between residues 280 to 431 being exposed to the cell surface.

Live MPanc96 cells (pancreatic ductal adenocarcinoma cell line) were incubated with different anti-SAS1B monoclonal antibodies (as well as DAPI stain for nucleus localization). Cells were fixed and permeabilized after primary and secondary antibody incubation thus fluorescence staining observed represents surface localized SAS1B. Monoclonal antibodies 1H3/1K2 and 2H2/2K5 were used, as well as normal rabbit or mouse IgG's as a negative control. All primary antibodies used at a concentration of 20 ug/mL. The results indicated that SAS1B rabbit monoclonal antibodies 1H3/1K2 and 2H2/2K5 immunoreact with cell surface localized SAS1B in pancreatic cancer cells. Similar results were obtained for an additional PDAC cell line known as 366. These live immunofluorescent data further validate and qualify rabbit monoclonal antibodies 1H3/1K2 and 2H2/2K5 as potential candidates for future therapeutic uses as they recognize cell surface SAS1B in pancreatic cancer cell lines.

SAS1B monoclonal antibodies directed to the N-terminus of SAS1B and monoclonal antibodies directed to the C-terminus of SAS1B show differential cell surface staining patterns in MPanc96 cells. Monoclonal antibodies that recognize the N-terminus of SAS1B showed punctate SAS1B surface expression while monoclonal antibodies directed against the C-terminus of SAS1B showed homogenous SAS1B expression. One hypothesis is that both N- and C- termini are exposed extracellularly where the N-terminus has a short peptide exposed compared to a more bulky C-terminus of the protein which causes staining to appear more homogenous.

SAS1B-CT rabbit monoclonal antibodies were used in live

immunofluorescent staining of SAS1B in SNU 539 cancer cell lines. Acutase split cells were plated on plain coverslips at 7000 cells/well. The cells were blocked with 5% NGS media and chilled in 0.1% azide-containing media. The cells were incubated with undiluted rabbit monoclonal antibody supernatants and Rb2 IM serum at 1:50 dilution for 2 hours on ice and then washed with azide buffer. The secondary antibody (goat anti-rabbit Alexa 488, 1:500 dilution) was added in blocking buffer and incubated 1 hour on ice in the dark. The cells were washed with azide buffer and fixed with 4% PFA for 15 mins at room temperature. The cells were mounted in antifade reagent with DAPI. When used at a concentration of 20ug/ml both rabbit monoclonal antibodies 1H2/1K2 and 1H3/1K2 showed surface staining on SNU 539 cells.

Example 7 Immunoprecipitation Assays

HEK293T cells were transfected with human SAS1B SV-A full length cDNA for 66 h. Immunoprecipitation ("IP") of SAS1B was completed with 7H2 monoclonal antibodies. Detection of the SAS1B immunoprecipitation product was also completed with HRP peroxidase conjugated mouse anti-His mAb at 1:3000 or 1:5000 dilution. HEK293T cells were seeded at 250,000. The cells were transfected with 20 mg of SAS1B-SVA plasmid into each of the two T-75 flasks (at ~90% confluency) for ~66 h at 37°C and 5% CO_2. Following transfection, two saline washes were completed with 1X HBSS. Lysed cells were incubated with RIPA+protease inhibitor for one hour on ice with mixing to ensure homogenous lysate and supernatant was used for IP (RIPA: 25mM Tris-HCl (pH 7.6)), 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS). For the immunoprecipitation, 3 mg magnetic bead-coupled protein G was suspended and incubated with 20 mg (in 200mL PBST) SAS1B monoclonal antibody for 10 minutes. The magnetic bead/antibody complex was washed with PBST once, followed by incubation with 300 mL antigen extract for 20 min. The post immunoprecipitation sample was saved for comparison. Three 200 mL washes with washing buffer+protease inhibitor. The fourth wash used only 100 mL of wash buffer+protease inhibitor. The magnetic beads were transferred to a new micro-centrifuge tube. 1X Lamelli buffer was added to bead/antibody/antigen complex for subsequent analysis. The sample was boiled at 98°C for 10 min in 1X Lamelli buffer and the

supernatant transferred to a new tube. The immunoprecipitation sample was analyzed by western blot and Coomassie blue staining. The SDS-PAGE samples (pre-IP, IP, and post IP extracts) were resolved with 12% TGX Criterion gels at 30 milliamps for 1 hr. Coomassie stain was added to determine the profile of IP samples. Following SDS-PAGE resolution, bands were transferred to nitrocellulose membrane at 1 Amp for 1h at 5°C. The membrane was blocked with 5% dry milk/PBST, and incubated with mouse anti-His monoclonal antibody conjugated with HRP for 1 hr at RT at 1:3000 or 1:5000 dilution. The membranes were imaged with TMB solution.

The western blot showed the SAS1B antigen was pulled down by monoclonal antibody 7H2. The 3A7 mAb and protein G bead did not pull down rSAS1B. The remaining SAS1B in the post IP ("PIP") sample was compared with starting material ("SM"). 7H2 pulled down recombinant SAS1B from HEK293T extract. The negative control mAb 3A7 did not pull down the antigen. 3A7 showed no reduction of SAS1B in post IP samples.

The IP elutes of the 7H2, 3A7, and blank beads (BB) samples were analyzed. The 3A7 mAb and the blank beads were used as negative controls (SM, starting material). The heavy chain was stained at about 50kD and the light chain stained at about 25 kD. The 7H2 IP reaction pulled down unique 35 kD and 12 kD proteins. The 7H2 and 3A5 mAbs showed no cell killing or inhibition of cell proliferation.

Example 8 Hybridoma Sequencing of Hybridoma Cell Lines SAS1B 6C1 & 7H2

Parental line clone hybridoma cell lines SAS1B 6C1, SAS1B 7H2 (heavy IgG1; light-kappa) were sequenced. RT-PCR was carried out using 5' RACE and gene specific reverse primers which amplify mouse immunoglobulin heavy chain (IgG1) and light chain (kappa) variable region sequences. The specific bands were excised and cloned into pCR-Blunt II-TOPO® vector for sequencing, and the constructs were transformed into *E. coli.* 16-24 colonies of each chain were picked and PCR screened for the presence of amplified regions prior to sequencing; additional colonies were picked as necessary (see Table 5). PCR positive clones were selected (8 heavy chain and 8 kappa chain) and sequenced. DNA sequences were analyzed by BLAST and SnapGene to confirm homology to mouse antibody

sequences.

Table 5: Summary of PCR Testing and DNA Sequencing of SAS1B Clones

Sample	Colonies Picked	PCR Screening Positive	Number of PCR Positives Sequenced	Number of Sequences with Antibody ORF
SAS1B 6C1 heavy	16	16(100%)	8	4 (50%)
SAS1B 6C1 kappa	24	20(83.3%)	8	2 (25%)
SAS1B 7H2 heavy	16	16(100%)	8	8 (100%)
SAS1B 7H2 kappa	16	14(87.5%)	8	4 (50%)

The DNA sequences were determined for eight each of SAS1B 6C1 and 7H2 IgG1 heavy chain, five SAS1B 6C1 kappa chain and seven SAS1B 7H2 kappa chain variable regions.

Of the 8 PCR positive SAS1B 6C1 IgG1 heavy chain clones sent for sequencing, 4 of the resulting sequences had open reading frames (ORFs) that came back as positive matches for a mouse IgG1 chain in a BLAST search. All four of these sequences contained the exact same nucleotide sequence for the variable region, from the putative ATG start codon to the beginning of the consensus IgG1 constant region. This consensus DNA sequence for SAS1B 6C1 IgG1 heavy chain variable region provided the longest ORF found in the total sequenced area and is shown below:

```
1       ATGGGCAGGCTTACTTCTTCATTCCTGCTACTGATTGTCCCTGCATATGTCCTGTCCCAG
61      GTTACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGCCCTCCCAGACCCTCAGTCTGACT
121     TGTTCTTTCTCTGGGTTTTCACTGAGCACTTCTACTATGGGTGTAGGCTGGATTCGTCAG
181     CCATCAGGGAAGGGTCTGGAGTGGCTGGCACACATTTGGTGGGATGATGTCAAGCGCTAT
241     AACCCAGCCCTGAAGAGCCGACTGACTATCTCCAAGGATACCTCCAGCAGCCAGGTATTC
301     CTCAAGATCGCCAGTGTGGACACTGCAGACTCTGCCACATACTACTGTGCTCGAATACCT
361     AATGATGGTTACTGTGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA
SEQ ID NO:62.
```

The translation of the consensus SAS1B 6C1 IgG1 heavy variable chain DNA sequence is shown below:

```
1       MGRLTSSFLLLIVPAYVLSQVTLKESGPGILQPSQTLSLTCSFSGFSLSTSTMGVGWIRQ
61      PSGKGLEWLAHIWWDDVKRYNPALKSRLTISKDTSSSQVFLKIASVDTADSATYYCARIP
121     NDGYCAMDYWGQGTSVTVSS
SEQ ID NO:77.
```

Of the 8 PCR positive kappa chain clones sent for sequencing, 5 of the resulting sequences had ORFs which came back as positive matches for a murine

kappa chain in a BLAST search. Of these 5, two (K4 & K14) contained the exact same nucleotide sequence for the variable region, from the putative ATG start codon to the beginning of the consensus kappa chain constant region. A third sequence (K8), while containing all the same nucleotides as the consensus sequence, also contained a 155 nucleotide insert which moved the sequence out of frame, and resulting in a truncated ORF. A final pair of sequences (K7 & K20) contains a 4 base pair deletion, which shifts the reading frame so that the "RADA" amino acid sequence which identifies the beginning of the kappa constant region is not translated. Therefore, it was determined that the sequence given by sequences K4 & K14 is the consensus sequence. This consensus DNA sequence for the SAS1B 6C1 kappa chain variable region is shown below:

```
1       ATGGAGACAGACACACTCCTGTTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGT
61      GACATTGTGCTGACACAGTCTCCTGCTTCCTTAGCTGTATCTCTGGGGCAGAGGGCCACC
121     ATCTCATGCAGGGCCAGCAAAAGTGTCAGTACATCTGGCTATAGTTTTATGCACTGGTAC
181     CAACAGAAACCAGGACAGCCACCCAAACTCCTCATCTATCTTGCATCCAACCTAGAATCT
241     GGGGTCCCTGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACCCTCAACATCCAT
301     CCTGTGGAGGAGGAGGATGCTGCAACCTATTACTGTCAGCACAGTAGGGAGCTTCCGTAC
361     ACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA
SEQ ID NO:63.
```

The translation of the consensus SAS1B 6C1 kappa chain DNA sequence is shown below.

```
1       METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSFMHWY
61      QQKPGQPPKLLIYLASNLESGVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHSRELPY
121     TFGGGTKLEIK
SEQ ID NO:78.
```

Of the 8 PCR positive SAS1B 7H2 IgG1 heavy chain clones sent for sequencing, all 8 of the resulting sequences had ORFs which came back as positive matches for a murine IgG1 heavy chain in a BLAST search. Of these 8, all 8 contained the exact same nucleotide sequence for the variable region, from the putative ATG start codon to the beginning of the consensus IgG1 constant region. This consensus sequence provided the longest ORF found in the total sequenced area. The consensus DNA sequence for SAS1B 7H2 IgG1 heavy chain variable region is shown below:

```
1       ATGGGCAGGCTTACTTCTTCATTCCTGCTACTGATTGTCCCTGCATATGTCCTGTCCCAG
61      GTTACTCTGAAAGAGTCTGGCCCTGGGATATTGCAGCCCTCCCAGACCCTCAGTCTGACT
121     TGTTCTTTCTCTGGGTTTTCACTGAGCACTTCTGGTATGGGTGTAGGCTGGATTCGTCAG
181     CCATCAGGGAAGGGTCTGGAGTGGCTGGCACACATTTGGTGGGATGATGTCAAGCGCTAT
```

241 AATCCAGCCCTGAAGAGCCGACTGACTATCTCCAAGGATACCTCCAGCAGCCAGGTATTC
301 CTCAAGATCGCCAGTGGGGACACTATAGATACTGCCACATACTACTGTGCTCGAATACCT
361 ACTGATGATTACTATGCTTTGGACCACTGGGGTCAAGGAGCCTCAGTCACCGTCTCCTCA
SEQ ID NO:60.

The translation of consensus SAS1B 7H2 IgG1 heavy variable chain DNA sequence is shown below:

1 MGRLTSSFLLLIVPAYVLSQVTLKESGPGILQPSQTLSLTCSFSGFSLSTSGMGVGWIRQ
61 PSGKGLEWLAHIWWDDVKRYNPALKSRLTISKDTSSSQVFLKIASGDTIDTATYYCARIP
121 TDDYYALDHWGQGASVTVSS
SEQ ID NO:75.

Of the 8 PCR positive SAS1B 7H2 kappa chain clones sent for sequencing, 7 of the resulting sequences had ORFs which came back as positive matches for a murine kappa chain in a BLAST search. Of these 7, two (K5 & K10) contained the exact same nucleotide sequence for the variable region, from the putative ATG start codon to the beginning of the consensus kappa chain constant region. A third sequence (K2) was the same as the consensus sequence except for 1 nucleotide difference, which changed a "T" to a "C" at position 96. This nucleotide change would not change the amino acid sequence, as codons "GCT" and "GCC" both code for the amino acid alanine. A fourth sequence (K4) contains a single "T" to "A" mutation at position 329, which would change a phenylalanine to a tyrosine in the amino acid sequence. The remaining 3 sequences (K9, K1 & K6) contain a four amino acid deletion which would shift the reading frame, so that the "RADA" sequence which identifies the beginning of the kappa constant region is not translated. The consensus DNA sequence for SAS1B 7H2 kappa chain variable region is shown below (the bolded “T” at position 96 can optionally be a “C”; The bolded T at position 329 can optionally be an “A”):

1 ATGGAGACAGACACACTCCTGTTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGT
61 GACATAGTGCTGACACAGTCTCCTGCTTCCTTAGC**T**GTATCTCTGGGGCAGAGGGCCACC
121 ATCTCATGCAGGGCCAGCAAAAGTGTCAGTACATCTGGTTATAGTTTTATGCACTGGTAC
181 CAACAGAAACCAGGACAGCCACCCAAACTCCTCATCTATCTTGCATCCAACCTAGAATCT
241 GGGGTCCCTGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACCCTCAACATCCAT
301 CCTGTGGAGGAGGAGGATGCTGCAACCT**T**TTACTGTCAGCACAGTAGGGAGCTTCCGTAC
361 ACGTTCGGAGGGGGGACCAAGCTGGAAATAAAA
SEQ ID NO:61.

The translation of consensus SAS1B 7H2 kappa variable chain DNA sequence is shown below (the bold underlined “F” amino acid can optionally be “Y”)

1 METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSFMHWY
61 QQKPGQPPKLLIYLASNLESGVPARFSGSGSGTDFTLNIHPVEEEDAAT**F**YCQHSRELPY

121 TFGGGTKLEIK
SEQ ID NO:76.

The CDR sequences were determined as shown below:

Heavy Chain CDR sequences for mouse anti-SAS1B mAbs

CDR1:	7H2	GFSLSTSG (SEQ ID NO:1)
	6C1	GFSLSTST (SEQ ID NO:7)
CDR2:	7H2	AHIWWDDV (SEQ ID NO:2)
	6C1	AHIWWDDV (SEQ ID NO:2)
CDR3:	7H2	IPTDDYYALDH (SEQ ID NO:3)
	6C1	IPNDGYCAMDY (SEQ ID NO:8)

Light Chain CDR sequences for mouse anti-SAS1B mAbs

CDR1:	7H2	KSVSTSGYSF (SEQ ID NO:4)
	6C1	KSVSTSGYSF (SEQ ID NO:4)
CDR2:	7H2	LASNL (SEQ ID NO:5)
	6C1	LASNL (SEQ ID NO:5)
CDR3:	7H2	QHSRELPYT (SEQ ID NO:6)
	6C1	QHSRELPYT (SEQ ID NO:6)

Example 9 Sequencing of Monoclonal Antibody 2H2/2K5

Antibody 2H2/2K5 was sequenced. The consensus DNA sequence of 2H2 rabbit IgG variable heavy chain is shown below.

```
1       ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAG
61      TCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGC
121     ACAGTCTCTGGATTCTCCCTCAGTAGCTATGCAATGGTCTGGGTCCGCCAGGCTCCAGGG
181     GAGGGGCTGGAATGGATCGGAGCCATTAATACTGGTGGTGTCACATTCTACGCGAGCTGG
241     GCGAAAGGCCGATTCACCATCTCCAAAACCTCGACCACAGTGGATCTGAAAATCACCAGT
301     CCGACAACCGAGGACACGG
SEQ ID NO:67.
```

The consensus DNA sequence of 2H2 rabbit IgG constant heavy chain is shown below.

```
1       CCACCTATTTCTGTGCCAGAGCCTTTGAATTCTGGGGCCAGGGGACCCTGGTCACCGTCT
61      CGAGCGGACAGCCGAAAGCCCCGTCGGTGTTTCCACTGGCGCCCTGCTGTGGCGATACGC
121     CTTCGTCCACCGTGACCCTTGGCTGTCTTGTGAAGGGTTACCTTCCCGAGCCGGTCACTG
181     TAACATGGAATTCAGGGACACTCACGAACGGGGTCAGGACTTTCCCATCAGTCAGACAGT
241     CATCCGGTCTGTATTCACTTAGCTCGGTAGTGTCCGTAACTTCCTCCAGCCAGCCGGTAA
301     CATGTAACGTAGCGCACCCCGCCACCAATACCAAGGTGGACAAGACCGTGGCCCCCTCAA
361     CATGCTCGAAACCTACGTGCCCTCCGCCTGAACTTCTCGGGGGTCCCAGCGTCTTTATCT
421     TCCCTCCTAAGCCCAAAGATACGCTGATGATCTCGCGGACCCCGGAGGTGACTTGTGTCG
481     TGGTCGATGTCTCCCAAGACGATCCCGAAGTACAGTTCACCTGGTACATTAACAACGAGC
541     AAGTCCGAACGGCCAGGCCACCGTTGCGCGAGCAGCAATTCAATTCGACGATCCGGGTGG
601     TATCAACGTTGCCGATCACTCATCAGGACTGGTTGCGAGGGAAGGAATTCAAATGCAAGG
661     TCCACAACAAGGCCCTTCCGGCACCAATCGAGAAAACGATCAGCAAGGCGAGGGGGCAGC
```

```
721     CCCTGGAACCCAAGGTCTATACAATGGGACCACCCAGGGAAGAGTTGTCATCCCGGTCCG
781     TATCGCTTACATGCATGATTAACGGTTTCTATCCTTCAGACATTTCAGTAGAGTGGGAGA
841     AGAATGGAAAAGCCGAAGATAACTACAAAACAACCCCCGCAGTACTTGACTCCGACGGAT
901     CGTACTTCTTGTACAACAAGCTCTCGGTGCCCACGTCAGAATGGCAACGAGGGGATGTCT
961     TTACATGCTCGGTGATGCATGAGGCACTCCACAATCATTACACGCAGAAAAGCATCTCCC
1021    GCTCGCCGGGAAAGTGA
SEQ ID NO:73.
```

The translation of the consensus DNA sequence for 2H2 rabbit IgG heavy chain variable region is shown below.

```
1       METGLRWLLLVAVLKGVQCQSVEESGGRLVTPGTPLTLTCTVSGFSLSSYAMVWVRQAPG
61      EGLEWIGAINTGGVTFYASWAKGRFTISKTSTTVDLKITSPTTEDTATYFCARAFEFWGQ
121     GTLVTVSS
SEQ ID NO:81.
```

The translation of the consensus DNA sequence for 2H2 rabbit IgG heavy chain constant region is shown below.

```
1       GQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSS
61      GLYSLSSVVSVTSSSQPVTCNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIFP
121     PKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVVS
181     TLPITHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVS
241     LTCMINGFYPSDISVEWEKNGKAEDNYKTTPAVLDSDGSYFLYNKLSVPTSEWQRGDVFT
301     CSVMHEALHNHYTQKSISRSPGK
SEQ ID NO:87.
```

The consensus DNA sequence of 2K5 rabbit kappa chain variable region is shown below.

```
1       ATGGACACGAGGGCCCCCATTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCC
61      ACATTTGCCCAAGTGCTGACCCAGACTGCATCCTCCGTGTCTGCAGCTGTGGGAGGCACA
121     GTCACCATCAGTTGCCAGTCCAGTCAGAGTGTTTATGACAACTGGTTAGCCTGGTATCAG
181     CAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGATGCATCCGATCTGGCATCTGGG
241     GTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGAC
301     CTGGAGTGTGACGATGCTGCCACTTACTACTGTCTAGGCGGTTATAGTGGTAATATTTAT
361     ACTTTCGGCGGAGGGACCGAGGTGGTGGTCAAACGTAC
SEQ ID NO:68.
```

The consensus DNA sequence of 2K5 rabbit kappa chain constant region is shown below.

```
1       GCCCGTGGCACCCACTGTACTCCTGTTTCCGCCTTCCTCGGATGAGGTGGCGACGGGCAC
61      GGTCACAATCGTCTGCGTGGCGAATAAGTACTTTCCGGATGTCACAGTGACGTGGGAGGT
121     GGACGGGACAACACAGACCACAGGTATTGAAAACAGCAAAACACCGCAGAATTCGGCTGA
181     CTGTACGTATAACTTGTCCTCCACTCTTACGTTGACATCAACACAGTACAATTCGCACAA
241     GGAGTATACGTGCAAGGTAACCCAGGGTACGACAAGCGTAGTCCAGTCCTTCAGCAGGAA
301     GAACTGCTGA
SEQ ID NO:74.
```

The translation of the 2K5 consensus variable region of the rabbit kappa chain is shown below:

```
1       MDTRAPIQLLGLLLLWLPGATFAQVLTQTASSVSAAVGGTVTISCQSSQSVYDNWLAWYQ
61      QKPGQPPKLLIYDASDLASGVPSRFKGSGSGTQFTLTISDLECDDAATYYCLGGYSGNIY
121     TFGGG
SEQ ID NO:82.
```

The translation of the 2K5 consensus constant region of the rabbit kappa chain is shown below:

```
1       TEVVVKRTPVAPTVLLFPPSSDEVATGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENS
61      KTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQSFSRKNC
SEQ ID NO:66.
```

Example 10 Sequencing of Monoclonal Antibody 1H2/1K2

The consensus DNA sequence of 1H2 rabbit IgG heavy variable region is shown below:

```
1       ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAG
61      TCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGC
121     ACAGTCTCTGGATTCTCCCTCAGTAGCAACGACATGAAGTGGGTCCGCCAGGCTCCAGGG
181     AAGGGGCTGGAATACATCGGATACATTTTCAGTAGTGGTAGTATATACTACGCGAGCTGG
241     GCGAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGGTGGATCTGAAAATCACCAGC
301     CCGACAACCGAGGACACGGCCACCTATTTCTGTAGCAGAGGAGGCTTGTGGGGCCCGGGC
361     ACCCTGGTCACCGTCTCGAG
SEQ ID NO: 64.
```

The consensus DNA sequence of 1H2 rabbit IgG heavy constant region is shown below:

```
1       CGGACAGCCGAAAGCCCCGTCGGTGTTTCCACTGGCGCCCTGCTGTGGCGATACGCCTTC
61      GTCCACCGTGACCCTTGGCTGTCTTGTGAAGGGTTACCTTCCCGAGCCGGTCACTGTAAC
121     ATGGAATTCAGGGACACTCACGAACGGGGTCAGGACTTTCCCATCAGTCAGACAGTCATC
181     CGGTCTGTATTCACTTAGCTCGGTAGTGTCCGTAACTTCCTCCAGCCAGCCGGTAACATG
241     TAACGTAGCGCACCCCGCCACCAATACCAAGGTGGACAAGACCGTGGCCCCCTCAACATG
301     CTCGAAACCTACGTGCCCTCCGCCTGAACTTCTCGGGGGTCCCAGCGTCTTTATCTTCCC
361     TCCTAAGCCCAAAGATACGCTGATGATCTCGCGGACCCCGGAGGTGACTTGTGTCGTGGT
421     CGATGTCTCCCAAGACGATCCCGAAGTACAGTTCACCTGGTACATTAACAACGAGCAAGT
481     CCGAACGGCCAGGCCACCGTTGCGCGAGCAGCAATTCAATTCGACGATCCGGGTGGTATC
541     AACGTTGCCGATCACTCATCAGGACTGGTTGCGAGGGAAGGAATTCAAATGCAAGGTCCA
601     CAACAAGGCCCTTCCGGCACCAATCGAGAAAACGATCAGCAAGGCGAGGGGGCAGCCCCT
661     GGAACCCAAGGTCTATACAATGGGACCACCCAGGGAAGAGTTGTCATCCCGGTCCGTATC
721     GCTTACATGCATGATTAACGGTTTCTATCCTTCAGACATTTCAGTAGAGTGGGAGAAGAA
781     TGGAAAAGCCGAAGATAACTACAAAACAACCCCCGCAGTACTTGACTCCGACGGATCGTA
841     CTTCTTGTACAACAAGCTCTCGGTGCCCACGTCAGAATGGCAACGAGGGGATGTCTTTAC
901     ATGCTCGGTGATGCATGAGGCACTCCACAATCATTACACGCAGAAAAGCATCTCCCGCTC
961     GCCGGGAAAGTGA
SEQ ID NO:71.
```

The translation of consensus DNA sequence for 1H2 rabbit IgG heavy variable chain is shown below.

```
1       METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTPLTLTCTVSGFSLSSNDMKWVRQAPG
61      KGLEYIGYIFSSGSIYYASWAKGRFTISKTSTTVDLKITSPTTEDTATYFCSRGGLWGPG
121     TLVTVSS
SEQ ID NO:79.
```

The translation of consensus DNA sequence for 1H2 rabbit IgG heavy constant chain is shown below.

```
1       GQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSS
61      GLYSLSSVVSVTSSSQPVTCNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIFP
121     PKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVVS
181     TLPITHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVS
241     LTCMINGFYPSDISVEWEKNGKAEDNYKTTPAVLDSDGSYFLYNKLSVPTSEWQRGDVFT
301     CSVMHEALHNHYTQKSISRSPGK
SEQ ID NO:85.
```

The consensus DNA sequence of 1K2 rabbit kappa variable chain is shown below.

```
1       ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCC
61      ACATTTGCCCAAGTGCTGACCCAGACTGCATCGCCCGTGTCTGCAGCTGTGGGAGGCACA
121     GTCACCATCAATTGCCAGGCCAGTCAGAGTGTTTATAATAATAACCGCTTATCCTGGTAT
181     CAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGATGCATCCAATCTAGATTCT
241     GGGGTCTCATCGCGGTTCAGCGGCAGTGGATCTGGGACAGAGTTCACTCTCACCATCAGC
301     GACGTGCAGTGTGACGATGCTGCCACTTACTACTGTCTAGGCACTTATGATTGTAATAGT
361     GCTGATTGCCATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAAACGTAC
SEQ ID NO:65.
```

The consensus DNA sequence of 1K2 rabbit kappa constant chain is shown below.

```
1       GCCCGTGGCACCCACTGTACTCCTGTTTCCGCCTTCCTCGGATGAGGTGGCGACGGGCAC
61      GGTCACAATCGTCTGCGTGGCGAATAAGTACTTTCCGGATGTCACAGTGACGTGGGAGGT
121     GGACGGGACAACACAGACCACAGGTATTGAAAACAGCAAAACACCGCAGAATTCGGCTGA
181     CTGTACGTATAACTTGTCCTCCACTCTTACGTTGACATCAACACAGTACAATTCGCACAA
241     GGAGTATACGTGCAAGGTAACCCAGGGTACGACAAGCGTAGTCCAGTCCTTCAGCAGGAA
301     GAACTGCTGA
SEQ ID NO:72.
```

The translation of consensus DNA sequence for 1K2 kappa variable chain is shown below.

```
1       MDTRAPTQLLGLLLLWLPGATFAQVLTQTASPVSAAVGGTVTINCQASQSVYNNNRLSWY
61      QQKPGQPPKLLIYDASNLDSGVSSRFSGSGSGTEFTLTISDVQCDDAATYYCLGTYDCNS
121     ADCHAFGGG
SEQ ID NO:80.
```

The translation of consensus DNA sequence for 1K2 kappa constant chain is shown below.

```
1       TEVVVKRTPVAPTVLLFPPSSDEVATGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENS
61      KTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQSFSRKNC
SEQ ID NO:86.
```

Example 11 Sequencing of Monoclonal Antibody 1H3/1K2

The consensus DNA sequence of 1H3 rabbit IgG heavy variable chain is shown below.

```
1       ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAG
61      TCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGC
```

```
121     ACAGTCTCTGGATTCTCCCTCAGTAGCAACGACATGAAGTGGGTCCGCCAGGCTCCAGGG
181     AAGGGGCTGGAATACATCGGATACATTTTCAGTAGTGGTAGTATATACTACGCGAGCTGG
241     GCGAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGGTGGATCTGAAAATCACCAGC
301     CCGACAACCGAGGACACGGCCACCTATTTCTGTAGCAGAGGAGGCTTGTGGGGCCCGGGC
361     ACCCTGGTCACCGTCTCGAG
SEQ ID NO: 64.
```

The consensus DNA sequence of 1H3 rabbit IgG heavy constant chain is shown below.

```
1       CGGACAGCCGAAAGCCCCGTCGGTGTTTCCACTGGCGCCCTGCTGTGGCGATACGCCTTC
61      GTCCACCGTGACCCTTGGCTGTCTTGTGAAGGGTTACCTTCCCGAGCCGGTCACTGTAAC
121     ATGGAATTCAGGGACACTCACGAACGGGGTCAGGACTTTCCCATCAGTCAGACAGTCATC
181     CGGTCTGTATTCACTTAGCTCGGTAGTGTCCGTAACTTCCTCCAGCCAGCCGGTAACATG
241     TAACGTAGCGCACCCCGCCACCAATACCAAGGTGGACAAGACCGTGGCCCCCTCAACATG
301     CTCGAAACCTACGTGCCCTCCGCCTGAACTTCTCGGGGGTCCCAGCGTCTTTATCTTCCC
361     TCCTAAGCCCAAAGATACGCTGATGATCTCGCGGACCCCGGAGGTGACTTGTGTCGTGGT
421     CGATGTCTCCCAAGACGATCCCGAAGTACAGTTCACCTGGTACATTAACAACGAGCAAGT
481     CCGAACGGCCAGGCCACCGTTGCGCGAGCAGCAATTCAATTCGACGATCCGGGTGGTATC
541     AACGTTGCCGATCACTCATCAGGACTGGTTGCGAGGGAAGGAATTCAAATGCAAGGTCCA
601     CAACAAGGCCCTTCCGGCACCAATCGAGAAAACGATCAGCAAGGCGAGGGGGCAGCCCCT
661     GGAACCCAAGGTCTATACAATGGGACCACCCAGGGAAGAGTTGTCATCCCGGTCCGTATC
721     GCTTACATGCATGATTAACGGTTTCTATCCTTCAGACATTTCAGTAGAGTGGGAGAAGAA
781     TGGAAAAGCCGAAGATAACTACAAAACAACCCCCGCAGTACTTGACTCCGACGGATCGTA
841     CTTCTTGTACAACAAGCTCTCGGTGCCCACGTCAGAATGGCAACGAGGGGATGTCTTTAC
901     ATGCTCGGTGATGCATGAGGCACTCCACAATCATTACACGCAGAAAAGCATCTCCCGCTC
961     GCCGGGAAAGTGA
SEQ ID NO:71.
```

The translation of consensus DNA sequence for 1H3 rabbit IgG heavy variable chain is shown below.

```
1       METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTPLTLTCTVSGFSLSSNDMKWVRQAPG
61      KGLEYIGYIFSSGSIYYASWAKGRFTISKTSTTVDLKITSPTTEDTATYFCSRGGLWGPG
121     TLVTVSS
SEQ ID NO:79.
```

The translation of consensus DNA sequence for 1H3 rabbit IgG heavy constant chain is shown below.

```
1       GQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSS
61      GLYSLSSVVSVTSSSQPVTCNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIFP
121     PKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVVS
181     TLPITHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVS
241     LTCMINGFYPSDISVEWEKNGKAEDNYKTTPAVLDSDGSYFLYNKLSVPTSEWQRGDVFT
301     CSVMHEALHNHYTQKSISRSPGK
SEQ ID NO:85.
```

The consensus DNA sequence of 1K2 rabbit kappa variable chain is shown below.

```
1       ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCC
61      ACATTTGCCCAAGTGCTGACCCAGACTGCATCGCCCGTGTCTGCAGCTGTGGGAGGCACA
121     GTCACCATCAATTGCCAGGCCAGTCAGAGTGTTTATAATAATAACCGCTTATCCTGGTAT
181     CAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGATGCATCCAATCTAGATTCT
241     GGGGTCTCATCGCGGTTCAGCGGCAGTGGATCTGGGACAGAGTTCACTCTCACCATCAGC
301     GACGTGCAGTGTGACGATGCTGCCACTTACTACTGTCTAGGCACTTATGATTGTAATAGT
```

361 GCTGATTGCCATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAAACGTAC
SEQ ID NO:65.

The consensus DNA sequence of 1K2 rabbit kappa constant chain is shown below.

1 GCCCGTGGCACCCACTGTACTCCTGTTTCCGCCTTCCTCGGATGAGGTGGCGACGGGCAC
61 GGTCACAATCGTCTGCGTGGCGAATAAGTACTTTCCGGATGTCACAGTGACGTGGGAGGT
121 GGACGGGACAACACAGACCACAGGTATTGAAAACAGCAAAACACCGCAGAATTCGGCTGA
181 CTGTACGTATAACTTGTCCTCCACTCTTACGTTGACATCAACACAGTACAATTCGCACAA
241 GGAGTATACGTGCAAGGTAACCCAGGGTACGACAAGCGTAGTCCAGTCCTTCAGCAGGAA
301 GAACTGCTGA
SEQ ID NO:72.

The translation of consensus DNA sequence for 1K2 kappa variable chain is shown below.

1 MDTRAPTQLLGLLLLWLPGATFAQVLTQTASPVSAAVGGTVTINCQASQSVYNNNRLSWY
61 QQKPGQPPKLLIYDASNLDSGVSSRFSGSGSGTEFTLTISDVQCDDAATYYCLGTYDCNS
121 ADCHAFGGG
SEQ ID NO:80.

The translation of consensus DNA sequence for 1K2 kappa constant chain is shown below.

1 TEVVVKRTPVAPTVLLFPPSSDEVATGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENS
61 KTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQSFSRKNC
SEQ ID NO:86.

Example 12 ELISA Analysis

An indirect ELISA analysis of purified rabbit monoclonal antibodies 1H2/1K2, 1H3/1K2, and 2H2/2K5 was performed. Antibody 1H2/1K2 was used at a concentration of 1.8 mg/ml, antibody 1H3/1K2 was used at a concentration of 2.05 mg/ml, and antibody 2H2/2K5 was used at a concentration of 1.92 mg/ml. Tartrate resistant acid phosphatase (TRAP) ELISA plates were coated with 1:10,000 rabbit TRAP in carbonate coating buffer (pH 9.6) overnight at 4°C (100µl/well). Plates were coated with 0.1µg/well/100µl SAS1B-CT antigen in CCB and incubated overnight at 4°C. All plates were blocked with 3% skim milk powder in PBST for 1 hour at room temperature. Monoclonal antibodies 1H2/1K2, 1H3/1K, and 2H2/2K5 were all diluted to a concentration of 1.0 µg/ml. 25 µl of PBSt were added to each well, then 25 µl of each monoclonal antibody were added to the plates and titrated across the plates at 25 µl increments. The plates were incubated for 1 hour at 37°C with shaking. Secondary antibody goat anti-rabbit IgG-HRP (1/10,000) was added at 10 µl/well and incubated for 1 hour at 37°C with shaking. TMB substrate was added

at 50 µl/well for 2 minutes at room temperature in the dark. The reaction was stopped with 1M HCl 50 µl/well and read at 450 nm.

Table 6.

	SAS1B –CT												
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1H2/1K2	**A**	2.554	2.266	2.908	2.628	2.263	1.826	1.127	0.741	0.466	0.29	0.154	0.136
1H3/1K2	**B**	2.811	2.833	2.869	2.705	2.396	1.927	1.398	0.896	0.542	0.305	0.196	0.147
2H2/2K5	**C**	2.606	2.358	2.62	2.43	2.003	1.423	0.943	0.576	0.355	0.204	0.142	0.115
pos. control	**D**	2.358	2.498	2.494	2.567	2.266	2.255	2.383	2.337	2.205	2.1	2.046	1.859
PBST	**E**	0.113	0.097	0.069	0.058	0.055	0.054	0.055	0.052	0.054	0.054	0.054	0.059
1H2/1K2	**F**	2.607	2.967	2.914	2.561	2.151	1.736	1.183	0.732	0.406	0.233	0.151	0.109
1H3/1K2	**G**	2.932	2.857	2.869	2.638	2.333	1.854	1.294	0.785	0.433	0.241	0.148	0.106
2H2/2K5	**H**	2.705	2.824	2.714	2.501	1.976	1.45	0.929	0.562	0.302	0.184	0.125	0.091
	TRAP												
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1H2/1K2	**A**	2.383	2.263	2.266	2.073	1.693	1.26	0.747	0.558	0.336	0.203	0.181	0.144
1H2/1K2	**B**	2.32	2.356	2.274	2.017	1.627	1.17	0.741	0.461	0.307	0.198	0.165	0.148
1H3/1K2	**C**	2.453	2.319	2.305	2.136	1.803	1.33	0.841	0.514	0.307	0.195	0.162	0.116
1H3/1K2	**D**	2.419	2.344	2.266	2.11	1.772	1.288	0.817	0.519	0.306	0.193	0.198	0.122
2H2/2K5	**E**	2.47	2.327	2.092	1.744	1.317	0.842	0.532	0.323	0.187	0.128	0.098	0.102
2H2/2K5	**F**	2.499	2.409	2.168	1.766	1.322	0.904	0.561	0.351	0.213	0.137	0.105	0.109
Std	**G**	2.518	2.38	2.32	2.081	1.708	1.282	0.923	0.578	0.348	0.218	0.157	0.135
Std	**H**	2.48	2.304	2.325	2.107	1.757	1.287	0.821	0.509	0.296	0.191	0.129	0.126
	con(ng/ml)	**500**	250	125	62.5	31.3	15.6	7.81	3.9	1.95	0.98	0.49	0.24
	Dilut. Factor	**2**	4	8	16	32	64	128	256	512	1024	2048	4096

Example 13 3F2 and 6B1 Sequencing

Total RNA was extracted from 3F2 and 6B1 hybridoma cells. RACE (Rapid Amplification of cDNA Ends) was performed to amplify DNA for V_H and V_L. Positive clones were identified by gel electrophoresis. The positive DNA was cloned and sequenced and the DNA and amino acid sequences for V_H, V_L and CDRs were identified using VBASE2.

The amino acid sequence of the heavy chain variable region of 3F2 is as follows:

```
1       EVQLQQSGAELARPGASVKMSCKASGFTFINYWMHWVKQRPGQGLEWIGAIYPGKSDISY
61      SQKFNGKAKLTAVTSANTAYMELSSLTSEDSAVYYCTRGGAMDYWGQGTSVTVSS
SEQ ID NO:83.
```

The nucleotide sequence of the heavy chain variable region of 3F2 is as follows:

```
1       GAGGTTCAGCTCCAGCAGTCTGGGGCTGAGCTGGCAAGACCTGGGGCTTCAGTGAAGATG
61      TCCTGCAAGGCTTCTGGCTTCACCTTTATCAACTACTGGATGCACTGGGTAAAACAGAGG
121     CCTGGACAGGGTCTGGAATGGATTGGCGCTATTTATCCTGGAAAGAGTGATATTAGTTAT
181     AGTCAGAAGTTCAACGGCAAGGCCAAACTGACTGCAGTCACTTCCGCCAACACTGCCTAC
241     ATGGAGCTCAGCAGCCTAACAAGTGAGGACTCTGCGGTCTATTATTGTACAAGAGGAGGG
301     GCTATGGACTATTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA
SEQ ID NO:69.
```

CDR1 of the 3F2 heavy chain variable region is GFTFINYW (SEQ ID NO:20).

CDR2 of the 3F2 heavy chain variable region is IYPGKSDI (SEQ ID NO:21). CDR 3 of the 3F2 heavy chain variable region is TRGGAMDY (SEQ ID NO:22).

The amino acid sequence of the light chain variable region of 3F2 is as follows:

```
1       DIVMTQSPSSLSMSVGQKVTMNCKSSQSLLYSSDQKNYLAWYQQKPGQSPKLLVYFASIR
61      ESGVPDRFIGSGSGTDFTLTISNVQAEDLAVYFCQQHYNTPLTFGAGTKLELK
SEQ ID NO:84.
```

The nucleotide sequence of the light chain variable region of 3F2 is as follows:

```
1       GACATTGTGATGACACAGTCTCCATCGTCCCTGTCTATGTCAGTGGGACAGAAGGTCACT
61      ATGAACTGCAAGTCCAGTCAGAGCCTTTTATACAGTAGTGATCAGAAGAACTATTTGGCC
121     TGGTATCAGCAGAAACCAGGACAGTCTCCTAAACTTCTGGTATACTTTGCATCCATTAGG
181     GAATCTGGGGTCCCTGATCGCTTCATAGGCAGTGGATCTGGGACAGATTTCACTCTTACC
241     ATCAGCAATGTGCAGGCTGAAGACCTGGCAGTTTACTTCTGTCAGCAACACTATAACACT
301     CCTCTCACGTTCGGTGCTGGGACCAAACTGGAGCTGAAAC
SEQ ID NO:70.
```

CDR1 of the light chain variable region of 3F2 is QSLLYSSDQKNY (SEQ ID NO:23). CDR2 of the light chain variable region is FAS. CDR3 of the light chain variable region is QQHYNTPLT(SEQ ID NO:24).

The amino acid sequence of the 6B1 heavy chain variable region is shown below:

```
1       EVQLQQSGAELARPGASVKMSCKASGFTFINYWMHWVKQRPGQGLEWIGAIYPGKSDISY
61      SQKFNGKAKLTAVTSANTAYMELSSLTSEDSAVYYCTRGGAMDYWGQGTSVTVSS
SEQ ID NO:83.
```

The nucleotide sequence of the 6B1 heavy chain variable region is shown below:

```
1       GAGGTTCAGCTCCAGCAGTCTGGGGCTGAGCTGGCAAGACCTGGGGCTTCAGTGAAGATG
61      TCCTGCAAGGCTTCTGGCTTCACCTTTATCAACTACTGGATGCACTGGGTAAAACAGAGG
121     CCTGGACAGGGTCTGGAATGGATTGGCGCTATTTATCCTGGAAAGAGTGATATTAGTTAT
181     AGTCAGAAGTTCAACGGCAAGGCCAAACTGACTGCAGTCACTTCCGCCAACACTGCCTAC
241     ATGGAGCTCAGCAGCCTAACAAGTGAGGACTCTGCGGTCTATTATTGTACAAGAGGAGGG
301     GCTATGGACTATTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA
SEQ ID NO:69.
```

CDR1 of the 6B1 heavy chain variable region is GFTFINYW (SEQ ID NO:20). The CDR2 of the 6B1 heavy chain variable region is IYPGKSDI (SEQ ID NO:21). The CDR3 of the 6B1 heavy chain region is TRGGAMDY (SEQ ID NO:22).

The amino acid sequence of the 6B1 light chain variable region is shown below:

```
1       DIVMTQSPSSLSMSVGQKVTMNCKSSQSLLYSSDQKNYLAWYQQKPGQSPKLLVYFASIR
61      ESGVPDRFIGSGSGTDFTLTISNVQAEDLAVYFCQQHYNTPLTFGAGTKLELK
SEQ ID NO:84.
```

The nucleotide sequence of the 6B1 light chain variable region is shown below:

```
1       GACATTGTGATGACACAGTCTCCATCGTCCCTGTCTATGTCAGTGGGACAGAAGGTCACT
61      ATGAACTGCAAGTCCAGTCAGAGCCTTTTATACAGTAGTGATCAGAAGAACTATTTGGCC
```

```
121     TGGTATCAGCAGAAACCAGGACAGTCTCCTAAACTTCTGGTATACTTTGCATCCATTAGG
181     GAATCTGGGGTCCCTGATCGCTTCATAGGCAGTGGATCTGGGACAGATTTCACTCTTACC
241     ATCAGCAATGTGCAGGCTGAAGACCTGGCAGTTTACTTCTGTCAGCAACACTATAACACT
301     CCTCTCACGTTCGGTGCTGGGACCAAACTGGAGCTGAAAC
SEQ ID NO:70.
```

CDR1 of 6B1 light chain variable region is QSLLYSSDQKNY (SEQ ID NO:23). The CDR2 of the 6B1 light chain variable region is FAS. The CDR3 of the 6B1 light chain variable region is QQHYNTPLT (SEQ ID NO:24).

Example 14 Sequencing of Rabbit Monoclonal Antibodies

Recombinant rabbit clones 3H4/3K5, 5H2/5K1, 6H2/6K1, 7H1/7K3, 9H1/9K5 and 31H1/31K1 (generated against a C-terminal fragment of recombinant human SAS1B protein, aa 280-431) were sequenced and analyzed. Variable region alignments of both DNA sequences and translated DNA sequences show that all six clones are different in sequence. It is likely that the six recombinant rabbit clones recognize SAS1B via different epitopes.

The nucleotide sequences of the variable region of the six heavy chains were aligned, from the putative ATG start codon to the beginning of the consensus constant region. The translated variable sequences of the heavy chains were also aligned, from the initial methionine amino acid to the beginning of the constant region.

The consensus sequence of variable region of 3H4 rabbit IgG heavy chain is shown below.

```
1       ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAG
61      TCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGC
121     ACAGCCTCTGGATTCTCCCTCAGTACCTACTACATGAGCTGGGTCCGCCAGGCTCCAGGG
181     AAGGGGCTGGAATGGATCGGAATCGTTGTTAGTACTGGTGACACATACTACGCGAGCTGG
241     GCGAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGGTGGATCTGAAAATCACCAGT
301     CCGACAACCGAGGACACGGCCACCTATTTCTGTGCCGGAAATTATGATGGTAGTACTTAT
361     TATTTAGACTTGTGGGGCCCGGGGACCCTGGTCACCGTCTCGAGC
SEQ ID NO:88.
```

The consensus sequence of the variable region of the 3H4 rabbit IgG heavy chain was translated into the amino acid sequence shown below.

```
1       METGLRWLLLVAVLKGVQCQSVEESGGRLVTPGTPLTLTCTASGFSLSTYYMSWVRQAPG
61      KGLEWIGIVVSTGDTYYASWAKGRFTISKTSTTVDLKITSPTTEDTATYFCAGNYDGSTY
121     YLDLWGPGTLVTVSS
SEQ ID NO:100.
```

The consensus sequence of variable region of 5H2 rabbit IgG heavy chain was translated into the amino acid sequence shown below:

```
1       ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAG
```

```
61      TCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGC
121     ACAGTGTCTGGATTCTCCCTCAGTAGCTATGCAATGAGCTGGGTCCGCCAGGCTCCAGGG
181     AAGGGGCTGGAATGGATCGGAATAATTGCTATTAGTGGTAACACATACTACGCGAGCTGG
241     GCGAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGGTGGATCTGAAAATCACCAGT
301     CCGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGAGATCCCTACGATGACTATGGT
361     GATTGGCTGTGGGGCCCGGGGACCCTCGTCACCGTCTCGAGC
SEQ ID NO:90.
```

The translation of the consensus 5H2 variable heavy chain DNA sequence is shown below.

```
1       METGLRWLLLVAVLKGVQCQSVEESGGRLVTPGTPLTLTCTVSGFSLSSYAMSWVRQAPG
61      KGLEWIGIIAISGNTYYASWAKGRFTISKTSTTVDLKITSPTTEDTATYFCARDPYDDYG
121     DWLWGPGTLVTV
SEQ ID NO:102.
```

The consensus sequence of variable region of 6H2 rabbit IgG heavy chain is shown below.

```
1       ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAG
61      TCGCTGGAGGAGTCCGGGGGTCGCCTGGTCAAGCCTGACGAAACCCTGACAATCACCTGC
121     ACAGTCTCTGGAATCGACCTCAGTAGCTATGTAATGGGCTGGGTCCGCCAGGCTCCAGGG
181     AAGGGGCTGGAATGGATCGGATTCATTTATTCTGGTGGTAGCGCGTACTACGCGAGCTGG
241     GCGAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGGTGGATCTGAAAATGACCAGT
301     CTGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGACGATATGATTTGTGGGGCCCG
361     GGGACCCTGGTCACCGTCTCGAGC
SEQ ID NO:92.
```

The translation of consensus 6H2 variable heavy chain DNA sequence is shown below.

```
1       METGLRWLLLVAVLKGVQCQSLEESGGRLVKPDETLTITCTVSGIDLSSYVMGWVRQAPG
61      KGLEWIGFIYSGGSAYYASWAKGRFTISKTSTTVDLKMTSLTTEDTATYFCARRYDLWGP
121     GTLVTVSS
SEQ ID NO:104.
```

The consensus sequence of variable region of 7H1 rabbit IgG heavy chain is shown below:

```
1       ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAG
61      TCAGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGC
121     ACAGTCTCTGGATTCTCCCTCAGTAGCTTTGCAATGAGCTGGGTCCGCCAGGCTCCAGGG
181     AAGGGGCTGGAATGGATCGGAATCATTGCTACTAGTAGCACATACTTCGCGACCTGGGCG
241     AAAGGCCGATTCACCATCTCCAAAACCTCGTCGACCACGGTGGATCTGAAAATCACCAGT
301     CCGGCAATCGAGGACACGGCCTCCTATTTCTGTGCCAGAGTTGGTGGTGATCCTGCACAT
361     ACTTATATTACGGCTTTTGATCCCTGGGGCCCGGGCACCCTGGTCACCGTCTCGAGC
SEQ ID NO:94.
```

The translation of consensus 7H1 DNA sequence of the heavy chain variable region is shown below.

```
1       METGLRWLLLVAVLKGVQCQSVEESGGRLVTPGTPLTLTCTVSGFSLSSFAMSWVRQAPG
61      KGLEWIGIIATSSTYFATWAKGRFTISKTSSTTVDLKITSPAIEDTASYFCARVGGDPAH
121     TYITAFDPWGPGTLVTVSS
```

SEQ ID NO:106.

The consensus sequence of the variable region of 9H1 rabbit IgG heavy chain is shown below:

```
1       ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAG
61      TCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGC
121     ACAGCCTCTGGATTCTCCCTCAGTAGCTACTACATGAGCTGGGTCCGCCAGGCTCCAGGG
181     AAGGGGCTGGAATGGATCGGAATCGTTGTTAGTACTGCTGACACATACTACGCGAGCTGG
241     GCGAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGGTGGATCTGAAAATCACCAGT
301     CCGACAACCGAGGACACGGCCACCTATTTCTGTGCCGGAAATTATGATGGTAGTACTTAT
361     TATTTAGACTTGTGGGGCCCGGGGACCCTCGTCACCGTCTCGAGC
```

SEQ ID NO:96.

The translation of consensus 9H1 variable heavy DNA sequence is shown below.

```
1       METGLRWLLLVAVLKGVQCQSVEESGGRLVTPGTPLTLTCTASGFSLSSYYMSWVRQAPG
61      KGLEWIGIVVSTADTYYASWAKGRFTISKTSTTVDLKITSPTTEDTATYFCAGNYDGSTY
121     YLDLWGPGTLVTVSS
```

SEQ ID NO:108.

The consensus sequence of variable region of 31H1 rabbit IgG heavy is shown below:

```
1       ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTGTCCAGTGTCAG
61      TCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCCTGACACTCACCTGC
121     ACAGTCTCTGGATTCTCCCTCAGTAACTATGCAATGGTCTGGGTCCGCCAGGCTCCAGGG
181     GAGGGGCTGGAATGGATCGGAGCCATTAATACTGGTGGTGTCACATTCTACGCGAGCTGG
241     GCGAAAGGCCGATTCACCATCTCCAAAACCTCGACCACAGTGGATCTGAAAATCACCAGT
301     CCGACAACCGAGGACACGGCCACCTATTTCTGTGCCAGAGCCTTTGAATTGTGGGGCCCG
361     GGGACCCTCGTCACCGTCTCGAGC
```

SEQ ID NO:98.

The translation of consensus 31H1 variable heavy region DNA sequence is shown below.

```
1       METGLRWLLLVAVLKGVQCQSVEESGGRLVTPGTPLTLTCTVSGFSLSNYAMVWVRQAPG
61      EGLEWIGAINTGGVTFYASWAKGRFTISKTSTTVDLKITSPTTEDTATYFCARAFELWGP
121     GTLVTVSS
```

SEQ ID NO:110.

The nucleotide sequences of the variable region of the six kappa chains were aligned, from the putative ATG start codon to the beginning of the consensus constant region. The translated variable sequences of the heavy chains were also aligned, from the initial methionine amino acid to the beginning of the constant region.

The consensus sequence of variable region of 3K5 rabbit kappa chain is shown below.

```
1       ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCC
61      ACATTTGCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAGCTGTGGGAGGCACA
121     GTCAGCATCAGTTGCCAGTCCAGTCAGAGTGTTTATAGCAACAACTGGTTATCCTGGTAT
181     CAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCACTCTGGCATCT
241     GGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGC
301     GACGTGCAGTGTGACGATGCTGCCACTTACTACTGTCTAGGCACTTATAGTAGTACTAGT
361     GATATGTATGTTTTCGGCGGAGGG
SEQ ID NO:89.
```

The translation of consensus 3K5 variable kappa chain DNA sequence is shown below.

```
1       MDTRAPTQLLGLLLLWLPGATFAAVLTQTPSPVSAAVGGTVSISCQSSQSVYSNNWLSWY
61      QQKPGQPPKLLIYGASTLASGVPSRFKGSGSGTQFTLTISDVQCDDAATYYCLGTYSSTS
121     DMYVFGGG
SEQ ID NO:101.
```

The consensus sequence of variable region of 5K1 rabbit kappa chain is shown below:

```
 1      ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCC
61      ACATTTGCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAGCTGTGGGAGGCACA
121     GTCAGCATCAGTTGCCAGTCCAGTAAGAGTGTTTATAATAATAACTGGTTATCCTGGTTT
181     CAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCACTCTGGCATCT
241     GGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGC
301     GACGTGCAGTGTGACGATGCTGCCACTTACTACTGTGCGGGCGGTTATAGTAGTGTTAGT
361     GATTTTTTTGCTTTCGGCGGAGGG
SEQ ID NO:91.
```

The translation of consensus 5K1 variable kappa chain DNA sequence is shown below.

```
1       MDTRAPTQLLGLLLLWLPGATFAAVLTQTPSPVSAAVGGTVSISCQSSKSVYNNNWLSWF
61      QQKPGQPPKLLIYGASTLASGVPSRFKGSGSGTQFTLTISDVQCDDAATYYCAGGYSSVS
121     DFFAFGGG
SEQ ID NO:103.
```

The consensus sequence of variable region of 6K1 rabbit kappa chain is shown below:

```
1       ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCC
61      ACATTTGCACAAGTGCTGACCCAGACTCCAGCCTCCGTGTCTGCAGCTGTGGGAGGCACA
121     GTCACCATCAACTGCCAGTCCAGTCAGAGTGTTTATGATAACAACTGGTTAGCCTGGTAT
181     CAGCAGAAACCAGGGCAGCCTCCCAAACTCCTGATCTATTATGCATCCACTCTGTATTCT
241     GGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGC
301     GGCGTGCAGTGTGACGATGCTGCCACTTACTACTGTGAAGGCGAATTTAGTTGTAGTAAT
361     GGTGATTGTGTTGTTTTCGGCGGAGGG
SEQ ID NO:93.
```

The translation of consensus 6K1 variable kappa chain DNA sequence is shown below.

```
1       MDTRAPTQLLGLLLLWLPGATFAQVLTQTPASVSAAVGGTVTINCQSSQSVYDNNWLAWY
61      QQKPGQPPKLLIYYASTLYSGVSSRFKGSGSGTQFTLTISGVQCDDAATYYCEGEFSCSN
121     GDCVVFGGG
SEQ ID NO:105.
```

The consensus sequence of variable region of 7K3 rabbit kappa chain is shown below.

```
1       ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCC
61      ACATTTGCCCAAGTGCTGACCCAGACTCCATCTTCCACGTCTGCAGCTGTGGGAGGCACA
121     GTCACCATCAATTGCCAGTCCAGTCAGAGTGTTTATGATAACAACGAATTATCCTGGTAT
181     CAACAAAAACCAGGGCAGCCTCCCAAGCTTCTGATCTATTCTGCATCCAAACTGACATCT
241     GGGGTCTCATCGCGTTTCAGCGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGC
301     GGCGTGCAGTGTGACGATGTTGCCACTTACTACTGTCTAGGCGGTTATTATGATAGTGGT
361     TGGTACTTTGCTTTCGGCGGAGGG
SEQ ID NO:95.
```

The translation of consensus 7K3 variable kappa chain DNA sequence is shown below.

```
1       MDTRAPTQLLGLLLLWLPGATFAQVLTQTPSSTSAAVGGTVTINCQSSQSVYDNNELSWY
61      QQKPGQPPKLLIYSASKLTSGVSSRFSGSGSGTQFTLTISGVQCDDVATYYCLGGYYDSG
121     WYFAFGGG
SEQ ID NO:107.
```

The consensus sequence of variable region of 9K5 rabbit kappa chain is shown below:

```
1       ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCC
61      ACATTTGCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAGCTGTGGGAGGCACA
121     GTCAGCATCAGTTGCCAGTCCAGTCAGAGTGTTTTTGATAACAACTGGTTATCCTGCTAT
181     CAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCACTCTGGCATCT
241     GGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGC
301     GACGTGCAGTGTGACGATGCTGCCACTTACTACTGTCTAGGCACTTATACTAGTACTAGT
361     GATATGTATGTTTTCGGCGGAGGG
SEQ ID NO:97.
```

The translation of the consensus 9K5 rabbit kappa DNA sequence is shown below.

```
1       MDTRAPTQLLGLLLLWLPGATFAAVLTQTPSPVSAAVGGTVSISCQSSQSVFDNNWLSCY
61      QQKPGQPPKLLIYGASTLASGVPSRFKGSGSGTQFTLTISDVQCDDAATYYCLGTYTSTS
121     DMYVFGGG
SEQ ID NO:109.
```

The consensus sequence of variable region of 31K1 rabbit kappa chain is shown below:

```
1       ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTGCC
61      ACATTTGCCCAAGTGCTGACCCAGACTGCATCCTCCGTGTCTGCAGCTGTGGGAGGCACA
121     GTCACCATCAGTTGCCAGTCCAGTCAGAGTGTTTATGACAACTGGTTAGCCTGGTATCAG
181     CAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGATGCATCCGATCTGGCATCTGGG
241     GTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCACCGAC
```

```
301     CTGGAGTGTGACGATGCTGCCACTTACTACTGTCTAGGCGGTTATAGTGGTAATATTTAT
361     ACTTTCGGCGGAGGG
```

SEQ ID NO:99.

The translation of consensus 31K1 DNA sequence is shown below.

```
1       MDTRAPTQLLGLLLLWLPGATFAQVLTQTASSVSAAVGGTVTISCQSSQSVYDNWLAWYQ
61      QKPGQPPKLLIYDASDLASGVPSRFKGSGSGTQFTLTITDLECDDAATYYCLGGYSGNIY
121     TFGGG
SEQ ID NO:111.
```

Heavy Chain CDR sequences for rabbit anti-SAS1B mAbs

CDR1:	3H4	SYAMS (SEQ ID NO:25)
	5H2	TYYMS (SEQ ID NO:31)
	6H2	SYVMG (SEQ ID NO:37)
	7H1	LSSFAMS (SEQ ID NO:43)
	9H1	LSSYYMS (SEQ ID NO:48)
	31H1	LSNYAMV (SEQ ID NO:54)
CDR2:	3H4	IVVSTGDTYYASWAKG (SEQ ID NO:26)
	5H2	IIAISGNTYYASWAKG (SEQ ID NO:32)
	6H2	FIYSGGSAYYASWKG (SEQ ID NO:38)
	7H1	IIATSSTYFATWAKG (SEQ ID NO:44)
	9H1	IWSTADTYYASWAKG (SEQ ID NO:49)
	31A1	AINTGGVTFYASWAKG (SEQ ID NO:55)
CDR3:	3H4	NYDGSTYYLDL (SEQ ID NO:27)
	5H2	DPYDDYGDWL (SEQ ID NO:33)
	6H2	RYDL (SEQ ID NO:39)
	7H1	VGGDPAHTYITAFDP (SEQ ID NO:45)
	9H1	NYDGSTYYLDL (SEQ ID NO:50)
	31H1	AFEL (SEQ ID NO:56)

Light Chain CDR sequences for rabbit anti-SAS1B mAbs

CDR1:	3K5	QSSQSVYSN (SEQ ID NO:28)
	5K1	QSSKSVYNNN (SEQ ID NO:34)
	6K3	QSSQSVYDNN (SEQ ID NO:40)
	7K3	QSSQSVYDNNELS (SEQ ID NO:46)
	9K5	QSSQSVFDNN (SEQ ID NO:51)
	31K1	QSSQSVYDN (SEQ ID NO:57)
CDR2:	3K5	GASTLA (SEQ ID NO:29)
	5K1	GASTLAS (SEQ ID NO:35)
	6K3	YASTLYS (SEQ ID NO:41)
	7K3	SASKLTS (SEQ ID NO:47)

	9K5	GASTLAS (SEQ ID NO:35)
	31K1	DASDLAS (SEQ ID NO:58)
CDR3:	3K5	LGTYSSTSDMYV (SEQ ID NO:30)
	5K7	AGGYSSVSDFFA (SEQ ID NO:36)
	6K3	EGEFSCSNGDCVV (SEQ ID NO:42)
	7K3	EGEFSCSNGDCVV (SEQ ID NO:42)
	9K5	LGTYTSTSDMYV (SEQ ID NO:53)
	31K1	LGGYSGNIYT (SEQ ID NO:59)

Example 15 Pancreatic Cell Surface Staining by SAS1B Monoclonal antibodies

Experiments were conducted to determine if SAS1B localizes to the cell surface of pancreatic cell lines (MPanc96 & 366), to identify which pancreatic cell line displays the most robust SAS1B cell surface staining, and to identify anti-SAS1B antibodies that show good cell surface staining. The live indirect immunofluorescence protocol is as follows. The cells were dissociated with Acutase and plated on fibronectin coated coverslips. MPanc96 cells were allowed to grow 2-3 days and 366 cells were allowed to grow for 3-4 days. The cells were blocked (blocking 1 solution) with 5% NGS in media for 30 min at RT. Then the cells were blocked with 5% NGS in chilled media + 0.1% sodium azide (blocking 2 solution) for 30 min on ice. The primary antibody (1H3/1K2, 2H2/2K5 or control) was added at 20ug/mL in blocking 2 solution for 2 hours on ice at 4°C. The cells were washed 3x for 5 minutes each on ice. The secondary antibody (goat anti-Ms/Rb Alexa488 (1:500)) was added in blocking 2 solution for 1 hour in dark on ice at 4°C. The cells were washed 3x for 5 min each on ice. The cells were fixed with 4% PFA-PBS for 15 min in the dark at RT. The cells were washed 2x for 5 min each at RT. The cells were stained with DAPI stain (1:1000) for 10 min at RT. The cells were washed 2x for 5 min each at RT. The cells were treated with PROLONG® gold antifade. 2H2/2K5 showed about 60-70% cell staining for MPanc96 cells, but only about 5% cell staining for 366 cells. About 10-25% of the 1H3/1K2 treated MPanc96 and 366 cells stained for SASB1.

Example 16 Cell Surface Density of SAS1B

The cell surface density of SAS1B was examined in SNU cells (endometrial tumor cells), 3T3(T) cells (positive control cells transfected with SAS1B), and K562 cells (myelogenous leukemia) with antibody 2H2/2K5 (also called RCT-7 antibody). The results are shown in Figure 2. The SNU cells have about 8,000 copies of

SAS1B on their surface while the 3T3(T) cells and K562 cells have significantly fewer copies of SAS1B.

Example 17 Cell Screening

Several cells were screened for SAS1B expression with a control antibody (Novus propeptide antibody) and the 2H2/2K5 antibody (also called RCT-7 antibody). The cells screened included 3T3(UT) (a negative control), 3T3(T) (a positive control), M539 cells (tumor cells), A549 cells (tumor cells), H522 cells (tumor cells), H23 cells (tumor cells), M1X3 cells (tumor cells), HRE cells (normal cells), and HUVEC cells (normal cells). The results are shown in Figure 3. The RCT-7 antibody had similar results to the control antibody.

Example 18 Fluorescence-Activated Cell Sorting (FACS) Analysis

FACS analysis was performed using several different cell lines, antibody RCT-7 and a control antibody (Novus propeptide antibody). The cell lines were 3T3(T) (a positive control), M1X3 cells (tumor cells), H522 cells (tumor cells), H23 cells (tumor cells), SNU-539 cells (tumor cells), HRE cells (normal cells) and HUVEC cells (normal cells). Antibody RCT-7 results were similar to and tracked the control antibody.

CLAIMS:

We claim:

1. An isolated antibody or antigen-binding portion thereof comprising: (a) a VH CDR1 of SEQ ID NO:1, 7, 9, 14, 25, 31, 37, 43, 48, or 54; a VH CDR2 of SEQ ID NO:2, 10, 15, 26, 32, 38, 44, 49, or 55; a VH CDR3 of SEQ ID NO:3, 8, 16, 27, 33, 39, 45, 50, 56, or GGL; a VL CDR1 of SEQ ID NO:4, 11, 17, 28, 34, 40, 46, 51, or 57; a VL CDR2 of SEQ ID NO:5, 12, 18, 29, 35, 41, 47, 35, or 58; and a VL CDR3 of SEQ ID NO:6, 13, 19, 30, 36, 42, 53, or 59; or (b) a VH CDR1 of SEQ ID NO:20, a VH CDR2 of SEQ ID NO:21, a VH CDR3 of SEQ ID NO:22, a VL CDR1 of SEQ ID NO:23, a VL CDR2 of FAS, a VL CDR3 of SEQ ID NO:24 or 95% identity thereto.

2. An isolated antibody or antigen-binding portion thereof of claim 1, wherein the antibody comprises:

(a) a VH CDR1 of SEQ ID NO:1, a VH CDR2 of SEQ ID NO:2, a VH CDR3 of SEQ ID NO:3, a VL CDR1 of SEQ ID NO:4, a VL CDR2 of SEQ ID NO:5, a VL CDR3 of SEQ ID NO:6;

(b) a VH CDR1 of SEQ ID NO:7, a VH CDR2 of SEQ ID NO:2, a VH CDR3 of SEQ ID NO:8, a VL CDR1 of SEQ ID NO:4, a VL CDR2 of SEQ ID NO:5, a VL CDR3 of SEQ ID NO:6; or

(c) a VH CDR1 of SEQ ID NO:9, a VH CDR2 of SEQ ID NO:10, a VH CDR3 of GLL, a VL CDR1 of SEQ ID NO:11, a VL CDR2 of SEQ ID NO:12, a VL CDR3 of SEQ ID NO:13;

(d) a VH CDR1 of SEQ ID NO:14, a VH CDR2 of SEQ ID NO:15, a VH CDR3 of SEQ ID NO:16, a VL CDR1 of SEQ ID NO:17, a VL CDR2 of SEQ ID NO:18, a VL CDR3 of SEQ ID NO:19;

(e) a VH CDR1 of SEQ ID NO:25, a VH CDR2 of SEQ ID NO:26, a VH CDR3 of SEQ ID NO:27, a VL CDR1 of SEQ ID NO:28, a VL CDR2 of SEQ ID NO:29, a VL CDR3 of SEQ ID NO:30;

(f) a VH CDR1 of SEQ ID NO:31, a VH CDR2 of SEQ ID NO:32, a VH CDR3 of SEQ ID NO:33, a VL CDR1 of SEQ ID NO:34, a VL CDR2 of SEQ ID NO:35, a VL CDR3 of SEQ ID NO:36;

(g) a VH CDR1 of SEQ ID NO:37, a VH CDR2 of SEQ ID NO:38, a VH CDR3

of SEQ ID NO:39, a VL CDR1 of SEQ ID NO:40, a VL CDR2 of SEQ ID NO:41, a VL CDR3 of SEQ ID NO:42;

(h) a VH CDR1 of SEQ ID NO:43, a VH CDR2 of SEQ ID NO:44, a VH CDR3 of SEQ ID NO:45, a VL CDR1 of SEQ ID NO:46, a VL CDR2 of SEQ ID NO:47, a VL CDR3 of SEQ ID NO:42;

(i) a VH CDR1 of SEQ ID NO:48, a VH CDR2 of SEQ ID NO:49, a VH CDR3 of SEQ ID NO:50, a VL CDR1 of SEQ ID NO:51, a VL CDR2 of SEQ ID NO:35, a VL CDR3 of SEQ ID NO:53; or

(j) a VH CDR1 of SEQ ID NO:54, a VH CDR2 of SEQ ID NO:55, a VH CDR3 of SEQ ID NO:56, a VL CDR1 of SEQ ID NO:57, a VL CDR2 of SEQ ID NO:58, a VL CDR3 of SEQ ID NO:59.

3.. The isolated antibody or antigen-binding portion thereof of claim 1, wherein the antibody or antigen-binding portion thereof is a monoclonal antibody, a chimeric antibody, a humanized antibody, a synthetic antibody, a single chain antibody, a diabody, or a CDR-grafted antibody.

4. An isolated antibody or antigen-binding portion thereof that specifically binds human SAS1B, wherein said antibody binds the same human SAS1B epitope recognized by the monoclonal antibody of claim 3.

5. An isolated antibody or antigen-binding portion thereof, wherein said antibody or antigen-binding portion specifically binds human SAS1B, wherein the antibody or antigen-binding portion thereof competes for binding with the antibody or antigen-binding portion thereof of claim 1.

6. An isolated antibody or antigen-binding portion thereof, wherein said antibody or antigen-binding portion thereof inhibits the binding of the isolated antibody or antigen-binding portion thereof of claim 1 to human SAS1B.

7. The isolated antibody or antigen-binding portion thereof of claims 4 or 5, wherein said antibody or antigen-binding portion thereof specifically binds to a polypeptide consisting of amino acids 55-289 or 280-430 of SEQ ID NO:112.

8. The isolated antibody or antigen-binding portion thereof of claim 1, wherein the antibody or antigen-binding portion thereof comprises a VL amino acid sequence of SEQ ID NOs:76, 78, 80, 82, 84, 101,103, 105, 107, 109, or 111.

9. The isolated antibody or antigen-binding portion thereof of claim 1, wherein said antibody or antigen-binding portion thereof comprises the VH amino acid sequence of SEQ ID NOs:75, 77, 79, 81, 83, 100, 102, 104, 106, 108, or 110.

10. A composition comprising:

(a) the antibody or antigen-binding portion thereof of claim 1 and a pharmaceutically acceptable carrier; or

(b) the antibody or antigen-binding portion thereof of claim 1, wherein the antibody or antigen-binding portion thereof is conjugated to a therapeutic agent, and a pharmaceutically acceptable carrier.

11. An antibody-drug conjugate (ADC) comprising the antibody or antigen-binding portion thereof of claim 1(b), wherein the antibody or antigen-binding portion is conjugated to a therapeutic agent.

12. The isolated antibody or antigen-binding portion thereof of claim 1, wherein the antibody or antigen-binding portion thereof specifically binds human SAS1B with an affinity (K_d) of at least about 10^{-6} M.

13. The isolated antibody or antigen-binding portion thereof of claim 1(b), wherein said antibody or antigen-binding portion thereof binds to cancer cells.

14. A radioimmunoconjugate comprising an antibody of claim 1 linked to a radionuclide

15. An isolated polypeptide consisting of one of SEQ ID NO: 1-51, 53-59, 75-84, or 100-111.

16. An isolated polynucleotide encoding the polypeptide of claim 15.

17. An isolated polynucleotide encoding an anti-human SAS1B antibody or antigen-binding portion thereof, wherein said isolated polynucleotide encodes a heavy chain and a light chain, wherein: (a) the immunoglobulin heavy chain complementarity determining region (CDR) CDR1 comprises SEQ ID NO:1, 7, 9, 14, 25, 31, 37, 43, 48, or 54, CDR2 comprises SEQ ID NO:2, 10, 15, 26, 32, 38, 44, 49, or 55, and CDR3 comprises SEQ ID NO:3, 8, 16, 27, 33, 39, 45, 50, 56, or GGL, and wherein the immunoglobulin light chain CDR1 comprises SEQ ID NO:4, 11, 17, 28, 34, 40, 46, 51, or 57, CDR2 comprises SEQ ID NO:5, 12, 18, 29, 35, 41, 47, 52, or 58, and CDR3 comprises SEQ ID NO:6, 13, 19, 30, 36, 42, 53, or 59; or (b) the immunoglobulin heavy chain complementarity determining region (CDR) CDR1 comprises SEQ ID NO:20, CDR2 comprises SEQ ID NO:21, and CDR3 comprises SEQ ID NO:22, and wherein the immunoglobulin light chain CDR1 comprises SEQ ID NO:23, CDR2 comprises FAS, and CDR3 comprises SEQ ID NO:24.

18. A vector comprising one or more polynucleotides of claim 17.

19. A host cell comprising the vector of claim 18.

20. A method for producing a human SAS1B antibody or antigen-binding portion thereof, comprising culturing the isolated host cell of claim 19 and recovering said antibody.

21. An isolated antibody or antigen-binding portion thereof of claim 1, wherein the antibody is a chimeric antibody comprising VL and VH domains obtained from a mouse antibody, wherein said VL and VH domains comprise sequences capable of binding to human SAS1B, and the VL and VH domains are fused to human CL and CH domains, respectively.

22. A method of treating a hyperproliferative disorder comprising administering the composition of claim 10 to a mammal in need thereof.

23. A method of detecting a SASB1 polypeptide in a sample comprising:

(a) contacting one or more antibodies of claim 1 with a test sample under conditions that allow polypeptide/antibody complexes to form; and

(b) detecting polypeptide/antibody complexes;

wherein the detection of polypeptide/antibody complexes is an indication that the human SAS1B polypeptide is present in the sample.

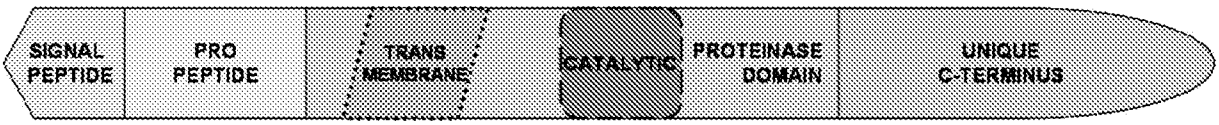
24. A method of detecting SAS1B-positive cells in a test sample comprising:

(a) contacting one or more antibodies of claim 1 with the test sample under conditions that allow SAS1B-positive cell/antibody complexes to form; and

(b) detecting SAS1B positive cell/antibody complexes;

wherein the detection of SAS1B positive cell/antibody complexes is an indication that SAS1B cells are present in the test sample.

25. The method of claim 24, wherein the sample is lymph node or tissue aspirate, serum, cellular suspension, lymphocytes, whole blood, plasma, circulating tumor cells, tumor cells or tissue, ascites fluid, urine, of ovarian cyst fluid, pap smear, or fluid effusion.

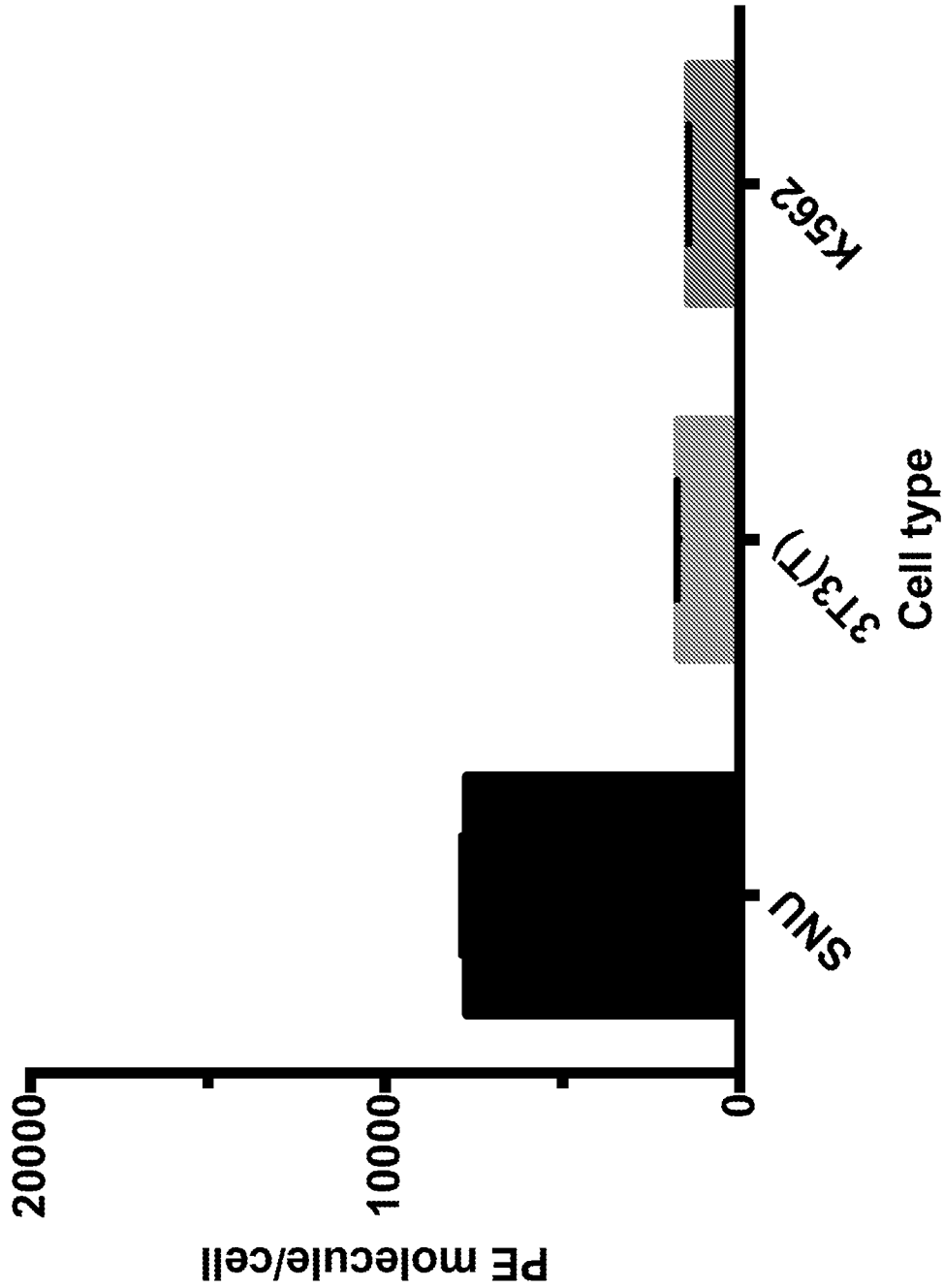



```
        10          20          30          40          50
MEGVGGLWPW VLGLLSLPGV ILGAPLASSC AGACGTSFPD GLTPEGTQAS
        60          70          80          90         100
GDKDIPAINQ GLILEETPES SFLIEGDIIR PSPFRLLSAT SNKWPMGGSG
       110         120         130         140         150
VVEVPFLLSS KYDEPSRQVI LEALAEFERS TCIRFVTYQD QRDFISIIPM
       160         170         180         190         200
YGCFSSVGRS GGMQVVSLAP TCLQKGRGIV LHELMHVLGF WHEHTRADRD
       210         220         230         240         250
RYIRVNWNEI LPGFEINFIK SQSSNMLTPY DYSSVMHYGR LAFSRRGLPT
       260         270         280         290         300
ITPLWAPSVH IGQRWNLSAS DITRVLKLYG CSPSGPRPRG RGSHAHSTGR
       310         320         330         340         350
SPAPASLSLQ RLLEALSAES RSPDPSGSSA GGQPVPAGPG ESPHGWESPA
       360         370         380         390         400
LKKLSAEASA RQPQTLASSP RSRPGAGAPG VAQEQSWLAG VSTKPTVPSS
       410         420         430
EAGIQPVPVQ GSPALPGGCV PRNHFKGMSE D
```

SAS1B amino acid sequence (SV-A splice form; reference sequence)

Figure 1

Fig. 2
Cell surface density of SAS1B



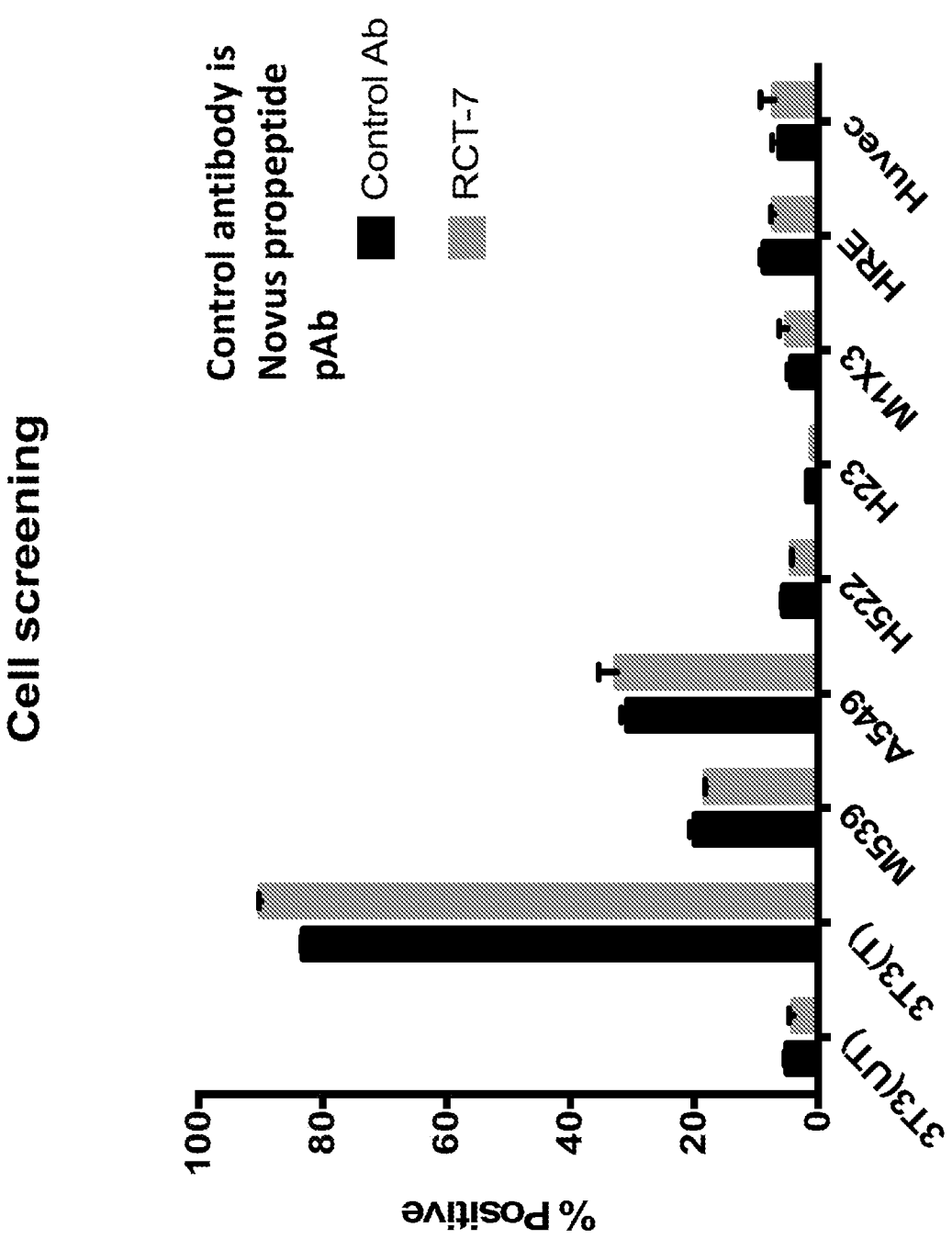

Cell screening
Control antibody is Novus propeptide pAb
Control Ab
RCT-7
% Positive
100
80
60
40
20
0
3T3(UT)
3T3(T)
M539
A549
H522
H23
M1X3
HRE
Huvec
Cell type

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/19052

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 47/48; C07K 16/30; G01N 33/574 (2017.01)
CPC - A61K 47/48, 47/48469, 47/48561, 47/48569; C07K 16/30; G01N 33/574, 33/57492

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/0191542 A1 (INNATE PHARMA, S.A.) July 9, 2015; paragraphs [0039], [0145], [0163]	15-16
A	US 2015/0125445 A1 (UNIVERSITY OF VIRGINIA PATENT FOUNDATION, D/B/A UNIVERSITY OF VIRGINIA LICENSING & VENTURES GROUP) May 7, 2015; paragraph [0145]	1-6, 7/4-5, 8-10, 12, 14, 18-25
A	US 2013/0058947 A1 (STULL, RA et al.) March 7, 2013; claim 99	1-6, 7/4-5, 8-10, 12, 14, 18-25
A	US 2010/0092457 A1 (ABURATANI, H et al.) April 15, 2010; claim 5	1-6, 7/4-5, 8-10, 12, 14, 18-25
A	US 2006/0233794 A1 (LAW, CL et al.) October 19, 2006; claim 3	1-6, 7/4-5, 8-10, 12, 14, 18-25
A	US 2006/0269550 A1 (HEIMAN, M) November 30, 2006; paragraph [0011]	1-6, 7/4-5, 8-10, 12, 14, 18-25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2017 (14.06.2017)	10 JUL 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/19052

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, 7/4-5, 8-10, 12, 14-25; SEQ ID NOs.: 1-6, 75-76

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US17/19052

-***-Continued from Box No. III Observations where unity of invention is lacking: -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-25; SEQ ID NO: 1 (HCDR1); SEQ ID NO: 2 (HCDR2); SEQ ID NO: 3 (HCDR3); SEQ ID NO: 4 (LCDR1); SEQ ID NO: 5 (LCDR2); SEQ ID NO: 6 (LCDR3); SEQ ID NO: 75 (VH); SEQ ID NO: 76 (VL) are directed toward anti-SAS1B antibodies, antigen-binding fragments thereof, and antibody-drug conjugates and methods of their use.

The SAS1B antibody will be searched to the extent that it encompasses SEQ ID NO: 1 (first exemplary HCDR1); SEQ ID NO: 2 (first exemplary HCDR2); SEQ ID NO: 3 (first exemplary HCDR3); SEQ ID NO: 4 (first exemplary LCDR1); SEQ ID NO: 5 (first exemplary LCDR2); SEQ ID NO: 6 (first exemplary LCDR3); SEQ ID NO: 75 (first exemplary VH); SEQ ID NO: 76 (first exemplary VL). Applicant is invited to elect additional pair(s) of SAS1B antibody light and heavy chain variable region sequence(s), and associated CDR region sequence(s), with specified SEQ ID NO: for each, to be searched. Additional pair(s) of SAS1B antibody light and heavy chain variable region sequence(s) and associated CDR region sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 3 (in-part), 4 (in-part), 5 (in-part), 6 (in-part), 7 (in-part), 8 (in-part), 9 (in-part), 10 (in-part), 12 (in-part), 14 (in-part), 15 (in-part), 16 (in-part), 17 (in-part), 18 (in-part), 19 (in-part), 20 (in-part), 21 (in-part), 22 (in-part), 23, (in-part), 24 (in-part), and 25 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (HCDR1); SEQ ID NO: 2 (HCDR2); SEQ ID NO: 3 (HCDR3); SEQ ID NO: 4 (LCDR1); SEQ ID NO: 5 (LCDR2); SEQ ID NO: 6 (LCDR3); SEQ ID NO: 75 (VH); SEQ ID NO: 76 (VL). Applicants must specify the claims that encompass any additionally elected pair(s) of SAS1B antibody light and heavy chain variable region sequence(s), and associated CDR region sequence (s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a SAS1B antibody encompassing SEQ ID NO: 7 (first exemplary elected HCDR1); SEQ ID NO: 10 (first exemplary elected HCDR2); SEQ ID NO: 8 (first exemplary elected HCDR3); SEQ ID NO: 11 (first exemplary elected LCDR1); SEQ ID NO: 12 (first exemplary elected LCDR2); SEQ ID NO: 13 (first exemplary elected LCDR3); SEQ ID NO: 77 (first exemplary elected VH); SEQ ID NO: 78 (first exemplary elected VL).

No technical features are shared between the antibody sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: an isolated antibody or antigen-binding portion thereof comprising: a VH CDR1; a VH CDR2; a VH CDR3; a VL CDR1; a VL CDR2; and a VL CDR3; an isolated antibody or antigen-binding portion thereof that specifically binds human SAS1 B, wherein said antibody binds the same human SAS1B epitope recognized by the monoclonal antibody; an isolated antibody or antigen-binding portion thereof, wherein said antibody or antigen-binding portion specifically binds human SAS1B, wherein the antibody or antigen-binding portion thereof competes for binding with the antibody or antigen-binding portion thereof; an isolated antibody or antigen-binding portion thereof, wherein said antibody or antigen-binding portion thereof inhibits the binding of the isolated antibody or antigen-binding portion thereof to human SAS1B; a composition comprising: (a) the antibody or antigen-binding portion thereof and a pharmaceutically acceptable carrier; or (b) the antibody or antigen-binding portion thereof, wherein the antibody or antigen-binding portion thereof is conjugated to a therapeutic agent, and a pharmaceutically acceptable carrier; an antibody-drug conjugate (ADC) comprising the antibody or antigen-binding portion thereof, wherein the antibody or antigen-binding portion is conjugated to a therapeutic agent; a radioimmunoconjugate comprising an antibody linked to a radionuclide; an isolated polypeptide; an isolated polynucleotide encoding the polypeptide; an isolated polynucleotide encoding an anti-human SAS1B antibody or antigen-binding portion thereof, wherein said isolated polynucleotide encodes a heavy chain and a light chain, comprising a CDR1, CDR2, and CDR3; a vector comprising one or more polynucleotides; a host cell comprising the vector; a method for producing a human SAS1B antibody or antigen-binding portion thereof, comprising culturing the isolated host cell and recovering said antibody; a method of treating a hyperproliferative disorder comprising administering the composition to a mammal in need thereof; a method of detecting a SASB1 polypeptide in a sample comprising: (a) contacting one or more antibodies with a test sample under conditions that allow polypeptide/antibody complexes to form; and (b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that the human SAS1B polypeptide is present in the sample; a method of detecting SAS1 B-positive cells in a test sample comprising: (a) contacting one or more antibodies with the test sample under conditions that allow SAS1B-positive cell/antibody complexes to form; and (b) detecting SAS1 B positive cell/antibody complexes; wherein the detection of SAS1 B positive cell/antibody complexes is an indication that SAS1B cells are present in the test sample.

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Previous Supplemental Box-***-

However, these shared technical features are previously shared by US 2015/0125445 A1 (University of Virginia Patent Foundation, d/b/a University of Virginia Licensing & Ventures Group) (hereinafter 'Virginia').

Virginia discloses an isolated antibody or antigen-binding portion thereof comprising: a VH CDR1; a VH CDR2; a VH CDR3; a VL CDR1; a VL CDR2; and a VL CDR3 (an entire immunoglobulin or antibody including heavy and light chain variable region CDRs; paragraph [0145]); an isolated antibody or antigen-binding portion thereof that specifically binds human SAS1B (an isolated antibody or antigen-binding portion thereof that specifically binds human SAS1B; paragraphs [0017], [0028]), wherein said antibody binds the same human SAS1B epitope recognized by a monoclonal antibody (the antibody is a monoclonal antibody (binds the same human SAS1B epitope recognized by a monoclonal antibody); paragraph [0020]); an isolated antibody or antigen-binding portion thereof, wherein said antibody or antigen-binding portion specifically binds human SAS1B (an isolated antibody or antigen-binding portion thereof that specifically binds human SAS1B; paragraphs [0017], [0028]), wherein the antibody or antigen-binding portion thereof competes for binding with an antibody or antigen-binding portion thereof (wherein the antibody or antigen-binding portion thereof competes for binding with an antibody or antigen-binding portion thereof; paragraph [0170]); an isolated antibody or antigen-binding portion thereof (an isolated antibody or antigen-binding portion thereof that specifically binds human SAS1B; paragraphs [0017], [0028]), wherein said antibody or antigen-binding portion thereof inhibits the binding of an isolated antibody or an antigen-binding portion thereof to human SAS1B (competes with another peptide for its cognate binding site (said antibody or antigen-binding portion thereof inhibits the binding of an isolated antibody or an antigen-binding portion thereof to human SAS1B); paragraph [0170]); a composition comprising: (a) the antibody and a pharmaceutically acceptable carrier (the antibody and a pharmaceutically acceptable carrier; paragraphs [0017]-[0018], [0241]); or (b) the antibody, wherein the antibody is conjugated to a therapeutic agent (the antibody, wherein the antibody is conjugated to a therapeutic agent; paragraphs [0017]-[0018], [0241]), and a pharmaceutically acceptable carrier (paragraph [0241]); an antibody-drug conjugate (ADC) comprising the antibody or antigen-binding portion thereof (an antibody-drug conjugate (ADC) comprising the antibody or antigen-binding portion thereof; paragraph [0024]), wherein the antibody or antigen-binding portion is conjugated to a therapeutic agent (wherein the antibody or antigen-binding portion is conjugated to a therapeutic agent; paragraph [0024]); a radioimmunoconjugate comprising an antibody linked to a Radionuclide (a radioimmunoconjugate comprising an antibody linked to a Radionuclide; paragraph [0497]); an isolated polypeptide (antibody (isolated polypeptide); paragraph [0153]); an isolated polynucleotide encoding the polypeptide (DNA molecule encoding the antibody; paragraphs [0153], [0198]); an isolated polynucleotide encoding an anti-human SAS1B antibody or antigen-binding portion thereof (an isolated polynucleotide encoding an anti-human SAS1B antibody or antigen-binding portion thereof; paragraphs [0017], [0153], [0198], [0301]), wherein said isolated polynucleotide encodes a heavy chain and a light chain (wherein said isolated polynucleotide encodes a heavy chain and a light chain; paragraph [0301]) comprising a CDR1, CDR2, and CDR3 (an entire immunoglobulin or antibody including heavy and light chain variable region CDRs; paragraph [0145]);; a vector comprising one or more polynucleotides (a vector comprising one or more polynucleotides; paragraphs [0297]-[0298]); a host cell comprising the vector (paragraph [0265]); a method for producing a human SAS1B antibody or antigen-binding portion thereof (a method for producing a human SAS1B antibody or antigen-binding portion thereof; paragraph [0267]), comprising culturing the isolated host cell of and recovering said antibody (production of antibodies by continuous cell lines in culture (culturing the isolated host cell of and recovering said antibody); paragraph [0318]); a method of treating a hyperproliferative disorder (a method of treating cancer (a hyperproliferative disorder); paragraphs [0016], [0019]) comprising administering the composition to a mammal in need thereof (paragraph [0019]); a method of detecting a SASB1 polypeptide in a sample (a method of detecting a SASB1 polypeptide in a sample; paragraphs [0016], [0022]) comprising: (a) contacting one or more antibodies with a test sample under conditions that allow polypeptide/antibody complexes to form (contacting one or more antibodies with a test sample under conditions that allow polypeptide/antibody complexes to form; paragraphs [0022], [0023]); and (b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that the human SAS1B polypeptide is present in the sample ((b) detecting polypeptide/antibody complexes; wherein the detection of polypeptide/antibody complexes is an indication that the human SAS1 B polypeptide is present in the sample; paragraphs [0022]-[0023]); and a method of detecting SAS1B-positive cells in a test sample (a method of detecting SAS1B-positive cells in a test sample; figure 10; paragraphs [0022], [0023]) comprising: (a) contacting one or more antibodies with the test sample under conditions that allow SAS1B-positive cell/antibody complexes to form ((a) contacting one or more antibodies with the test sample under conditions that allow SAS1B-positive cell/antibody complexes to form; paragraphs [0022]-[0023]); and (b) detecting SAS1B positive cell/antibody complexes; wherein the detection of SAS1B positive cell/antibody complexes is an indication that SAS1B cells are present in the test sample ((b) detecting SAS1 B positive cell/antibody complexes; wherein the detection of SAS1B positive cell/antibody complexes is an indication that SAS1B cells are present in the test sample; paragraphs [0022]-[0023]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Virginia reference, unity of invention is lacking.