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(54) **Titre : ANTICORPS, COMPOSITIONS ET PROCEDES D'IMMUNOHISTOCHEMIE PERMETTANT LA DETECTION DE C4.4A**  
(54) **Title: ANTIBODIES, COMPOSITIONS, AND IMMUNOHISTOCHEMISTRY METHODS FOR DETECTING C4.4A**

(57) **Abrégé/Abstract:**

Antibodies, compositions, systems, and methods for detecting C4.4a, for example immunohistochemistry methods for detecting C4.4a using a C4.4a antibody. The antibody may be obtained by immunizing a host with a C4.4a protein such as a peptide downstream of the signal peptide. The antibodies may be adapted to detect the uPAR-like domain 1 and uPAR-like domain 2. Also featured are methods for diagnosing C4.4a-associated tumors using C4.4a antibodies disclosed herein.



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(54) Title: ANTIBODIES, COMPOSITIONS, AND IMMUNOHISTOCHEMISTRY METHODS FOR DETECTING C4.4A

(57) Abstract: Antibodies, compositions, systems, and methods for detecting C4.4a, for example immunohistochemistry methods for detecting C4.4a using a C4.4a antibody. The antibody may be obtained by immunizing a host with a C4.4a protein such as a peptide downstream of the signal peptide. The antibodies may be adapted to detect the uPAR-like domain 1 and uPAR-like domain 2. Also featured are methods for diagnosing C4.4a-associated tumors using C4.4a antibodies disclosed herein.



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**ANTIBODIES, COMPOSITIONS, AND IMMUNOHISTOCHEMISTRY  
METHODS FOR DETECTING C4.4A**

**5      FIELD OF THE INVENTION**

The present invention relates to immunohistochemistry assays and reagents, more particularly to antibodies directed to the tumor-associated antigen C4.4a and methods of use, for example for diagnostic use.

**BACKGROUND OF THE INVENTION**

10      C4.4a is a protein homologue of the urokinase receptor. It is a GPI (glycosyl-phosphatidylinoditol) binding protein that has a similar structure to urokinase receptor (uPAR) and belongs to Ly-6 family as uPAR. While most of Ly-6 family molecules consist of single domain, uPAR is of three cysteine-rich domains. C4.4A is a membrane protein that binds to cell membranes in GPI binding sites,  
15      and consists of two cysteine-rich domains and a cysteine-lacking third domain. C4.4A was isolated from metastatic rat pancreatic cancer cell lines in 1989, and its human homolog was isolated in 2001 (Hansen et al., 2005, Thrombosis and Haemostasis, 93(4):A33, XP009145645). C4.4a is also considered to be a tumor-associated antigen, e.g., in lung cancer, esophageal cancer, cervical cancer, skin  
20      cancer, colon cancer, urothelial cancer, etc.

Several polyclonal C4.4a antibodies have been previously produced (e.g., Catalog No. 28073 from Clonetech; Catalog No. NBP2-32598 from Novus; Catalog Nos. LS-A9857, A9856, and LS-C186776 from LifeSpan Biosciences). However, these antibodies do not perform well in immunohistochemistry (IHC) assays. As of  
25      the filing of this application, Inventors are not aware of any other group who has been able to produce a monoclonal C4.4a antibody, e.g., a monoclonal C4.4a antibody that is adapted for IHC assays and/or for diagnostic purposes. One common method of producing monoclonal antibodies is using mouse hybridoma techniques. However, it is not uncommon for hybridomas to lose productivity or  
30      for the hybridoma cells to change, resulting in changes in the antibody (and effectiveness of the antibody). And, high sequence similarity between human C4.4a and its rabbit counterpart adds to the difficulty of producing an anti-C4.4a antibody directed to the uPAR-like domain 1 or the uPAR-like domain 2.

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U.S. Pat. Application No. 2012/0321619 is directed to C4.4a antibodies. Without wishing to limit the present invention to any theory or mechanism, it appears that the C4.4a antibodies in U.S. Pat. Application No. 2012/0321619 were used for C4.4a detection purposes in cell lines and in other *in vitro* assays, and these antibodies would not work well in formalin-fixed paraffin-embedded (FFPE) tissue samples, e.g., for diagnostic purposes. Hansen et al. (Biochem J, 2004, 380:845-857) discloses C4.4a antibodies; however, these antibodies are polyclonal antibodies.

### **SUMMARY OF THE INVENTION**

As of the filing of this application, Inventors have not been able to find a monoclonal C4.4a antibody, e.g., a monoclonal C4.4a antibody adapted for IHC assays, e.g., IHC assays in FFPE tissue samples. The present invention features monoclonal C4.4a antibodies, methods of detecting C4.4a using the monoclonal antibodies disclosed herein, as well as methods for detecting C4.4a-associated cancers using the monoclonal C4.4a antibodies disclosed herein. The monoclonal C4.4a antibodies of the present invention can be used to detect C4.4a in FFPE tissue samples.

For example, the present invention features an isolated antibody (e.g., a monoclonal antibody) specific for C4.4a, wherein the antibody binds specifically to a particular sequence or region of C4.4a, e.g., a C4.4a epitope as described herein (e.g., an epitope within the uPAR-like domain 1, an epitope within the uPAR-like domain 2, etc.). In some embodiments, the antibody comprises clone S42H9L5 or clone S20H1L1 as disclosed herein. The present invention also features a monoclonal C4.4a antibody or C4.4a binding fragment, wherein the antibody or binding fragment has the same epitopic specificity as an antibody selected from the group consisting of clone S42H9L5 and clone S20H1L1.

In some embodiments, the C4.4a antibody of the present invention comprises SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, a fragment thereof, a peptide that is at least 60% identical to SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, a peptide that is at least 60% identical to a fragment of one of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, or a combination thereof.

The present invention also features a host cell expression system expressing a C4.4a antibody according to the present invention. The present invention also

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features a labeled tissue sample (a labeled FFPE tissue sample), wherein the tissue sample is labeled with a C4.4a antibody according to the present invention.

5 The present invention also features a kit comprising a C4.4a antibody according to the present invention. The C4.4a antibody may be adapted for immunohistochemistry. In some embodiments, the kit further comprises a detection system (e.g., a chromogenic system, a fluorescence system, any other appropriate system) for making the C4.4a antibody visible. The kit may further comprise any other appropriate reagents, e.g., a secondary antibody directed to the C4.4a antibody, buffers, etc.

10 The present invention also features a method (e.g., automated method, manual method) of detecting C4.4a. In some embodiments, the method comprises providing a sample, contacting the sample with a C4.4a antibody according to the present invention, and making the antibody visible (e.g., via a detection system such as a chromogenic system, a fluorescence system, any other appropriate system).  
15 Detecting the antibody may be indicative of the presence of C4.4a.

The present invention also features methods of producing a C4.4a antibody (e.g., a C4.4a antibody according to the present invention). In some embodiments, the method comprises immunizing a host animal with the antibody using an immunogen as described herein.

20 The present invention also features a method of diagnosing a C4.4a-associated tumor (e.g., lung tumor, cervical tumor, skin carcinoma, esophageal tumor, and tumors with squamous differentiation), said method comprising detecting C4.4a according to a method of the present invention. Detection of C4.4a may be indicative of the C4.4a-associated tumor.

25 The present invention also features a labeled tissue sample (e.g., FFPE sample) labeled with a C4.4a antibody according to the present invention. The tissue sample may be obtained by a method according to the present invention.

The present invention also features a closed system for detecting C4.4a, e.g., a closed automated system adapted to perform a method according to the present  
30 invention, e.g., an automated immunohistochemistry assay.

The present invention also features a stainer machine programmed to perform a method according to the present invention.

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Any feature or combination of features described herein are included within the scope of the present invention provided that the features included in any such combination are not mutually inconsistent as will be apparent from the context, this specification, and the knowledge of one of ordinary skill in the art. Additional advantages and aspects of the present invention are apparent in the following detailed description and claims.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

**FIG. 1A** shows the results of C4.4a immunohistochemistry on mouse xenograft tissues (SCC-T9, H293-T3, and MCF-7 models) using clone S42H9L5. DISCOVERY XT platform protocol: STD CC1, anti-human C4.4a antibody (at 1:400) for 16 min at room temperature (RT), standard ChromoMap DAB detection. Expression of C4.4A in mouse xenograft: SCC-T9 - strong (+++); H293-T3 - moderate (++); MCF-7 -weak (+); PC3-negative (-). When clone S42H9L5 is used for IHC test, the staining intensity of mouse xenografts (SCC-T9, H293-T3, MCF-7, and PC3) matches the expression level of C4.4A.

**FIG. 1B** shows the results of C4.4a immunohistochemistry on mouse xenograft tissues (SCC-T9, H293-T3, and MCF-7 models) like in FIG. 1A but with clone S20H1L1. Clone S20H1L1 showed weaker binding to C4.4a than clone S42H9L5 (the staining intensity is weaker in mouse xenografts).

**FIG. 2A** shows the results of C4.4a immunohistochemistry on various human tissues (squamous cell carcinoma, skin, cervix, and esophagus) using clone S42H9L5. BenchMark XT platform protocol: STD CC1, anti-human C4.4a antibody (at 1:400) at RT, ultraView Dab Detection. Strong membrane staining is noticed in human skin squamous cell carcinoma, skin, cervix, and esophagus when clone S42H9L5 is used.

**FIG. 2B** shows the results of C4.4a immunohistochemistry on various human tissues (squamous cell carcinoma, skin, cervix, and esophagus) as in FIG. 2A but using clone S20H1L1. Strong membrane staining is noticed in human skin squamous cell carcinoma, skin, cervix, and esophagus when clone S20H1L1 is used.

### **TERMS**

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

a disclosed invention belongs. The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. “Comprising” means “including.” Hence “comprising A or B” means “including A” or “including B” or “including A and B.”

Suitable methods and materials for the practice and/or testing of embodiments of the disclosure are described below. Such methods and materials are illustrative only and are not intended to be limiting. Other methods and materials similar or equivalent to those described herein can be used. For example, conventional methods well known in the art to which the disclosure pertains are described in various general and more specific references, including, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, 1989; Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 3d ed., Cold Spring Harbor Press, 2001; Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Associates, 1992 (and Supplements to 2000); Ausubel *et al.*, *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, 4th ed., Wiley & Sons, 1999; Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1990; and Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1999.

In case of conflict, the present specification, including explanations of terms, will control.

Although methods and materials similar or equivalent to those described herein can be used to practice or test the disclosed technology, suitable methods and materials are described below. The materials, methods, and examples are illustrative only and not intended to be limiting.

In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

**Antibody:** A polypeptide that includes at least a light chain or heavy chain immunoglobulin variable region and specifically binds an epitope of an antigen (such as HER2 protein or ER protein). Antibodies include monoclonal antibodies, polyclonal antibodies, or fragments of antibodies. An antibody can be conjugated

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or otherwise labeled with a detectable label, such as an enzyme, hapten, or fluorophore.

**Buffers:** Buffer solutions are commonly used to maintain correct pH levels for biological and chemical systems. Many of the exemplary embodiments disclosed herein include using a buffer solution. Representative buffering agents or salts that may be present in the buffer include, but are not limited to Tris, Tricine, HEPES, MOPS, TAPS, Bicine, TAPSO, TES, PIPES, Cacodylate, SSC, MES, KCl, NaCl, potassium acetate, NH<sub>4</sub>-acetate, potassium glutamate, NH<sub>4</sub>Cl, ammonium sulphate, MgCl<sub>2</sub>, magnesium acetate and the like. One commonly used buffer solution is phosphate buffered saline (PBS). Another commonly used buffer solution is biotin ligase reaction buffer (0.1 M KCl, 5.5 mM MgCl<sub>2</sub>, 50 mM Tris·HCl (pH = 8.0), 0.05% Brij-35, 0.1 mM dithiothreitol (DTT), 3 mM ATP, and 60 μM biotin). The amount of buffering agent may range from about 5 to 150 mM, e.g., from about 10 to 100 mM, e.g., from about 20 to 50 mM, etc., however the buffering agent is not limited to those ranges. In some embodiments, the buffering agent helps provide a pH ranging from about 5.0 to about 9.5, e.g., 6.0 to 8.0, e.g., 6.5 to 7.5, etc. (e.g., at room temperature). Other agents that may be present in the buffer medium include chelating agents, such as EDTA, EGTA and the like.

**Contacting:** placement that allows association between two or more moieties, particularly direct physical association, for example both in solid form and/or in liquid form (for example, the placement of a biological sample, such as a biological sample affixed to a slide, in contact with a composition, such as a solution containing the probes disclosed herein).

**Detectable label:** A molecule or material that can produce a signal (such as a visual, electrical, or other signal) that indicates the presence and/or amount of a target (such as a protein or nucleic acid) in a sample. When conjugated to a specific binding molecule (for example, an antibody or nucleic acid probe), the detectable label can be used to locate and/or quantify the target to which the specific binding molecule is directed. A detectable label can be detected directly or indirectly, and several different detectable labels can be used in combination to detect one or more targets. For example, a first detectable label, such as a hapten conjugated to an antibody specific to a target, can be detected indirectly by using a second detectable label that is conjugated to a molecule that specifically binds the first detectable label. In addition, multiple detectable labels that can be separately detected can be conjugated to different specific binding molecules that specifically bind different



targets to provide a multiplex assay that can provide detection of the multiple targets in a single sample.

5 Detectable labels include chromogenic, fluorescent, phosphorescent and/or luminescent molecules, catalysts (such as enzymes) that convert one substance into another substance to provide a detectable signal (such as by converting a colorless substance into a colored substance or vice versa, or by producing a precipitate or increasing sample turbidity), haptens that can be detected through antibody-hapten binding interactions using additional detectably labeled antibody conjugates, and paramagnetic and magnetic molecules or materials. Particular examples of  
10 detectable labels include: enzymes, such as horseradish peroxidase, alkaline phosphatase, acid phosphatase, glucose oxidase,  $\beta$ -galactosidase or  $\beta$ -glucuronidase; fluorophores, such as fluoresceins, luminophores, coumarins, BODIPY dyes, resorufins, and rhodamines (many additional examples of fluorescent molecules can be found in The Handbook — A Guide to Fluorescent  
15 Probes and Labeling Technologies, Molecular Probes, Eugene, OR); nanoparticles, such as quantum dots (U.S. Patent Nos. 6,815,064, 6,682,596 and 6,649,138);

metal  
chelates, such as DOTA and DPTA chelates of radioactive or paramagnetic metal ions like  $Gd^{3+}$ ; and liposomes, for example, liposomes containing trapped  
20 fluorescent molecules. Where the detectable label includes an enzyme, a detectable substrate such as a chromogen, a fluorogenic compound, or a luminogenic compound is used in combination with the enzyme to generate a detectable signal (a wide variety of such compounds are commercially available, for example, from Life Technologies, Carlsbad, CA).

25 Alternatively, an enzyme can be used in a metallographic detection scheme. In some examples, metallographic detection methods include using an enzyme, such as alkaline phosphatase, in combination with a water-soluble metal ion and a redox-inactive substrate of the enzyme. The substrate is converted to a redox-active agent by the enzyme, and the redox-active agent reduces the metal ion, causing it to  
30 form a detectable precipitate (see, for example, U.S. Pat. Nos. 7,642,064; 7,632,652).

In other examples, metallographic detection methods include using an oxido-reductase enzyme (such as horseradish peroxidase) along with a water soluble metal ion, an oxidizing agent and a reducing agent, again to form a  
35 detectable precipitate (see, for example, U.S. Patent No. 6,670,113).

Haptens are small

5 molecules that can be bound by antibodies. Exemplary haptens include dinitrophenyl (DNP), biotin, digoxigenin (DIG), and fluorescein. Additional haptens include oxazole, pyrazole, thiazole, nitroaryl, benzofuran, triperpene, urea, thiourea, rotenoid, coumarin and cyclolignan haptens, such as those disclosed in U.S. Pat. No. 7,695,929.

10 **Hapten:** A hapten is a molecule, typically a small molecule that can combine specifically with an antibody, but typically is substantially incapable of being immunogenic except in combination with a carrier molecule. Many haptens are known and frequently used for analytical procedures, such as di-nitrophenyl, biotin, digoxigenin, fluorescein, rhodamine, or combinations thereof. Plural different haptens may be coupled to a polymeric carrier. Moreover, compounds, such as haptens, can be coupled to another molecule using a linker, such as an NHS-PEG linker.

15 **Immune complex:** The binding of antibody to a soluble antigen forms an immune complex. The formation of an immune complex can be detected through conventional methods known to the person of ordinary skill in the art, for instance immunohistochemistry, immunoprecipitation, flow cytometry, immunofluorescence microscopy, ELISA, immunoblotting (e.g., Western blot),  
20 magnetic resonance imaging, CT scans, X-ray and affinity chromatography. Immunological binding properties of selected antibodies may be quantified using methods well known in the art.

25 **Immunohistochemistry (IHC):** A method of determining the presence or distribution of an antigen in a sample by detecting interaction of the antigen with a specific binding agent, such as an antibody. A sample is contacted with an antibody under conditions permitting antibody-antigen binding. Antibody-antigen binding can be detected by means of a detectable label conjugated to the antibody (direct detection) or by means of a detectable label conjugated to a secondary antibody, which binds specifically to the primary antibody (e.g., indirect detection).

30 **Multiplex, -ed, -ing:** Embodiments of the present invention allow multiple targets in a sample to be detected substantially simultaneously, or sequentially, as desired, using plural different conjugates. Multiplexing can include identifying and/or quantifying nucleic acids generally, DNA, RNA, peptides, proteins, both individually and in any and all combinations. Multiplexing also can include

detecting two or more of a gene, a messenger and a protein in a cell in its anatomic context.

5       **Probe:** An isolated nucleic acid (such as an isolated synthetic oligonucleotide), attached to a detectable label or reporter molecule. Typical labels include  
10       radioactive isotopes, enzyme substrates, co-factors, ligands, chemiluminescent or fluorescent agents, haptens (including, but not limited to, DNP), and enzymes. Methods for labeling and guidance in the choice of labels appropriate for various purposes are discussed, *e.g.*, in Sambrook et al. (In *Molecular Cloning: A Laboratory Manual*, CSHL, New York, 1989) and Ausubel et al. (In *Current Protocols in Molecular Biology*, Greene Publ. Assoc. and Wiley-Intersciences, 1992).

15       Probes can be selected to provide a desired specificity, and may comprise at least 15, 20, 25, 30, 35, 40, 45, 50 or more nucleotides of a target nucleic acid. In particular examples, probes can include at least 100, 250, 500, 600, 1000, or more nucleotides of a target nucleic acid. In some examples, the probe includes segments of nucleotides that are from non-contiguous portions of a target nucleic acid, such as a HER2 genomic nucleic acid.

20       **Sample:** The term "sample" refers to any liquid, semi-solid or solid substance (or material) in or on which a target can be present. In particular, a sample can be a biological sample or a sample obtained from a biological material. Exemplary biological samples include tissue samples and/or cytology samples, for example, obtained from an animal subject, such as a human subject. In other examples, a biological sample can be a biological fluid obtained from, for example, blood,  
25       plasma, serum, urine, bile, ascites, saliva, cerebrospinal fluid, aqueous or vitreous humor, or any bodily secretion, a transudate, an exudate (for example, fluid obtained from an abscess or any other site of infection or inflammation), or fluid obtained from a joint (for example, a normal joint or a joint affected by disease). A biological sample can also be a sample obtained from any organ or tissue  
30       (including a biopsy or autopsy specimen, such as a tumor biopsy) or can include a cell (whether a primary cell or cultured cell) or medium conditioned by any cell, tissue or organ.

35       **Specific binding:** A term that refers to the binding of agent that preferentially binds to a defined target (such as an antibody to a specific protein or antigen or a nucleic acid probe to a specific nucleic acid sequence). With respect to a target

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protein, “specifically binds” refers to the preferential association of an antibody or other ligand, in whole or part, with a specific polypeptide. “Specifically binds” refers to the preferential association of a nucleic acid probe, in whole or part, with a specific nucleic acid, when referring to a target nucleic acid.

5 A specific binding agent binds substantially only to a particular target. A minor amount of non-specific interaction may occur between a specific binding agent and a non-target protein or nucleic acid. Antibody to antigen specific binding typically results in greater than 2-fold, such as greater than 5-fold, greater than 10-fold, or greater than 100-fold increase in amount of bound antibody or other ligand (per  
10 unit time) to a target protein, as compared to a non-target protein. Immunoassay formats can be used to select antibodies that specifically react with a particular protein (such as antibodies that specifically bind HER2 protein or ER protein). See Harlow & Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York (1988), for a description of immunoassay formats and  
15 conditions.

Specific binding of a nucleic acid probe to a target nucleic acid molecule typically results in greater than 2-fold, such as greater than 5-fold, greater than 10-fold, or greater than 100-fold increase in amount of bound nucleic acid probe to a target nucleic acid as compared to a non-target nucleic acid. A variety of ISH conditions  
20 are appropriate for selecting nucleic acid probes that bind specifically with a particular nucleic acid sequence (such as a HER2-specific probe or a chromosome 17 centromere probe).

**Subject:** Any multi-cellular vertebrate organism, such as human or non-human mammals (*e.g.*, veterinary subjects).

## 25 **DETAILED DESCRIPTION OF THE INVENTION**

Referring now to FIG. 1-2, the present invention features antibodies directed to C4.4a, methods (*e.g.*, immunohistochemistry) of detecting C4.4a using the antibodies disclosed herein, as well as methods for detecting C4.4a-associated cancers using the C4.4a antibodies disclosed herein. The present invention also  
30 includes any cDNA sequence encoding any peptide disclosed herein.

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For reference, the sequence of C4.4a is shown below (antigen UniProt number O95274) (SEQ ID NO: 1):

```

      10      20      30      40      50
MDPARKAGAQ AMIWTAGWLL LLLLRRGGAQA LECYSCVQKA DDGCSPNKMK
      60      70      80      90     100
TVKCAPGVDV CTEAVGAVET IHGQFSLAVR GCGSGLPGKN DRGLDLHGLL
     110     120     130     140     150
AFIQLQQCAQ DRCNAKLNLT SRALDPAGNE SAYPPNGVEC YSCVGLSREA
     160     170     180     190     200
CQGTSPPVVS CYNASDHVYK GCFDGNVTLT AANVTVSLPV RGCYQDEFCT
     210     220     230     240     250
RDGVTGPGFT LSGSCCQGSR CNSDLRNKTY FSPRIPLVR LPPPEPTTVA
     260     270     280     290     300
STTSVTTSTS APVRPTSTTK PMPAPTSQTP RQGEVEHEASR DEEPRLTGGA
     310     320     330     340
AGHQDRSNSG QYPAKGGPQQ PHNKGCVAPT AGLAALLLAV AAGVLL

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### **Immunogens**

- 5 New Zealand White rabbits were immunized with recombinant protein (see epitope sequences and descriptions below) emulsified with complete Freund's adjuvant followed by a series of booster doses of immunogen emulsified with incomplete Freund's adjuvant.

	Sequence	Description
S42H9L5	Amino acids 31-308 as immunogen (SEQ ID NO: 2, see below)	Domain after signal peptide (Signal peptide is amino acids 1-30) was used as immunogen. ELISA testing using recombinant proteins for uPAR-Like domain 1 (amino acids 31-116) (SEQ ID NO: 3, see below) and uPAR-like domain 2 (amino acids 139-224) (SEQ ID NO: 4, see below) showed this clone binds to uPAR-like domain 2 (SEQ ID NO: 4, see below).

	Sequence	Description
S20H1L1	Amino acids 31-308 as immunogen (SEQ ID NO: 2)	Domain after signal peptide (amino acids 1-30). ELISA testing using recombinant proteins for uPAR-Like domain 1 (amino acids 31-116) (SEQ ID NO: 3, see below) and uPAR-like domain 2 (amino acids 139-224) (SEQ ID NO: 4, see below) showed this clone binds to uPAR-like domain 1 (SEQ ID NO: 3, see below).

The immunogen used as described above corresponds to amino acids 31-308 of C4.4a:

5 LECYSCVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIHGQFSLAV  
 RGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRCNAKLNLTSRALDPAGN  
 ESAYPPNGVECYSCVGLSREACQGTSPPVVSCYNASDHVYKGCDFDGNVTL  
 TAANVTVSLPVRGCVQDEFCTRDGVTGPGFTLSGSCCQGSRCNSDLRNKT  
 YFSPRIPLVRLPPPEPTTVASTTSVTTSTAPVRPTSTTKPMPAPTSQTPRQG  
 VEHEASRDEEPRLTGGAAGHQDRSN (SEQ ID NO: 2).

10 For reference, the uPAR-like domain 1 corresponds to amino acids 31-116:  
 LECYSCVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIHGQFSLAV  
 RGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRCNAK (SEQ ID NO: 3).

For reference, the uPAR-like domain 2 corresponds to amino acids 139-224:  
 15 ECYSCVGLSREACQGTSPPVVSCYNASDHVYKGCDFDGNVTLTAANVTVSL  
 PVRGCVQDEFCTRDGVTGPGFTLSGSCCQGSRCNSD (SEQ ID NO: 4).

The present invention is not limited to the immunogen sequences disclosed herein. For example, in some embodiments, the immunogen comprises a fragment of SEQ ID NO: 2, e.g., all or a portion of the uPAR-like domain 1 (SEQ ID NO: 3), all or a portion of the uPAR-like domain 2 (SEQ ID NO: 4), etc. Fragments may be any  
 20 appropriate length, e.g., between 270-277 amino acids, between 260-277 amino acids, between 250-277 amino acids, between 240-277 amino acids, between 230-277 amino acids, between 220-277 amino acids, between 210-277 amino acids, between 200-277 amino acids, between 190-277 amino acids, between 180-277 amino acids, between 170-277 amino acids, between 160-277 amino acids,  
 25 between 150-277 amino acids, between 140-277 amino acids, between 130-277

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amino acids, between 120-277 amino acids, between 110-277 amino acids, between 100-277 amino acids, between 90-277 amino acids, between 80-277 amino acids, between 70-277 amino acids, between 60-277 amino acids, between 50-277 amino acids, between 40-277 amino acids, etc. In some embodiments, the immunogen comprises a sequence that is at least 60% identical (e.g., at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, etc.) to SEQ ID NO: 2. Shown below are non-limiting examples of other immunogen peptides, e.g., fragments of SEQ ID NO: 2:

SEQ ID NO:	Amino Acid Length	Immunogen Sequence
5	260	LECYSCVQKADDGCSPNKMKTVCAPGVDVCTEAVGAVETIH GQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDR NAKLNLTSRALDPAGNESAYPPNGVECYSCVGLSREACQGTS PPVWSCYNASDHVYKGCFDGNVTLTAANVTVSLPVRGCVQDE FCTRDGVTGPGFTLSGSCCQGSRCNSDLRNKTYFSPRIPLV RLPPPEPTTVASTTSVTTSTSAPVRPTSTTKPMPAPTSQTPRQ GVEHEASR
6	250	CVQKADDGCSPNKMKTVCAPGVDVCTEAVGAVETIHGQFSL AVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRCAKLNLT SRALDPAGNESAYPPNGVECYSCVGLSREACQGTSPPVVS CYNASDHVYKGCFDGNVTLTAANVTVSLPVRGCVQDEFCTR DGVGTGPGFTLSGSCCQGSRCNSDLRNKTYFSPRIPLVRLPP PEPTTVASTTSVTTSTSAPVRPTSTTKPMPAPTSQTPRQGVE

SEQ ID NO:	Amino Acid Length	Immunogen Sequence
7	245	LECYSCVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIH GQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRC NAKLNLTSRALDPAGNESAYPPNGVECYSCVGLSREACQGTS PPVVSCYNASDHVYKGCFDGNVTLTAANVTVSLPVRGCVQDE FCTRDGVTGPGFTLSGSCCQGSRCNSDLRNKTYFSPRIPLV RLPPPEPTTVASTTSVTTSTAPVRPTSTTKPMPAP
8	200	LECYSCVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIH GQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRC NAKLNLTSRALDPAGNESAYPPNGVECYSCVGLSREACQGTS PPVVSCYNASDHVYKGCFDGNVTLTAANVTVSLPVRGCVQDE FCTRDGVTGPGFTLSGSCCQGSRCNSDLRNKTY
9	190	CVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIHGQFSL AVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRCNAKLNLT SRALDPAGNESAYPPNGVECYSCVGLSREACQGTSPPVVS CYNASDHVYKGCFDGNVTLTAANVTVSLPVRGCVQDEFCTR DGVGTGPGFTLSGSCCQGSRCNSDL
10	180	TVKCAPGVDVCTEAVGAVETIHGQFSLAVRGCGSGLPGKNDR GLDLHGLLAFIQLQQCAQDRCNAKLNLTSRALDPAGNESAYP PNGVECYSCVGLSREACQGTSPPVVSCYNASDHVYKGCFDG NVTLTAANVTVSLPVRGCVQDEFCTRDGVTGPGFTLSGSCCQ GSRCNSDLRNKTY
11	165	TVKCAPGVDVCTEAVGAVETIHGQFSLAVRGCGSGLPGKNDR GLDLHGLLAFIQLQQCAQDRCNAKLNLTSRALDPAGNESAYP PNGVECYSCVGLSREACQGTSPPVVSCYNASDHVYKGCFDG NVTLTAANVTVSLPVRGCVQDEFCTRDGVTGPGFTLSGSC



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SEQ ID NO:	Amino Acid Length	Immunogen Sequence
12	140	LECYSCVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIH GQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRC NAKLNLTSRALDPAGNESAYPPNGVECYSCVGLSREACQGTS PPVVSCYNASDHVYK
13	125	PNKMKTVKCAPGVDVCTEAVGAVETIHGQFSLAVRGCGSGLP GKNDRGLDLHGLLAFIQLQQCAQDRCNAKLNLTSRALDPAGN ESAYPPNGVECYSCVGLSREACQGTSPVVSCYNASDHVYK
14	120	LECYSCVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIH GQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRC NAKLNLTSRALDPAGNESAYPPNGVECYSCVGLSREA
15	110	CTEAVGAVETIHGQFSLAVRGCGSGLPGKNDRGLDLHGLLAFI QLQQCAQDRCNAKLNLTSRALDPAGNESAYPPNGVECYSCV GLSREACQGTSPVVSCYNASDHVYK
16	100	CVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIHGQFSL AVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRCNAKLNLT SRALDPAGNESAYPP

The present invention is not limited to immunogens comprising SEQ ID NO: 2, fragments thereof, or sequences that have at least 60% identity with SEQ ID NO: 2. For example, in some embodiments, the immunogen comprises a fragment of full length C4.4a (SEQ ID NO: 1), e.g., amino acids 1-330, amino acids 1-310, amino acids 1-308, amino acids 1-300, amino acids 1-280, amino acids 1-250, amino acids 10-330, amino acids 10-310, amino acids 10-308, amino acids 10-300, amino acids 10-280, amino acids 10-250, amino acids 20-330, amino acids 20-310, amino acids 20-308, amino acids 20-300, amino acids 20-280, amino acids 20-250, amino acids 30-330, amino acids 30-310, amino acids 30-308, amino acids 30-300, amino

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acids 30-280, amino acids 30-250, etc. Fragments of SEQ ID NO: 1 may be any appropriate length, e.g., between 330-345 amino acids, between 300-345 amino acids, between 280-345 amino acids, between 250-345 amino acids, between 200-345 amino acids, between 150-345 amino acids, between 100-345 amino acids, between 50-345 amino acids, between 30-345 amino acids, etc. In some embodiments, the immunogen comprises a sequence that is at least 60% identical, 65% identical, 70% identical, 75% identical, 80% identical, 85% identical, 90% identical, 95% identical, 98% identical, 99% identical, etc., to SEQ ID NO: 1.

The C4.4a antibodies of the present invention may be derived according to methods described herein (e.g., see above), however the C4.4a antibody is not limited to such methods and may be made by any other appropriate means.

#### **C4.4a Antibody Sequences and Configurations**

As previously discussed, the present invention features antibodies directed to C4.4a, e.g., monoclonal C4.4a antibodies. Shown below are non-limiting examples variable region sequences of two C4.4 antibody clones: S42H9L5 and S20H1L1 (hereinafter also referred to as “S42” and “S20,” respectively). In some embodiments, a C4.4a antibody of the present invention comprises one or more of the below sequences (SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20).

Clone	Chain	Sequence
S42H9L5	Heavy (SEQ ID NO: 17)	METGLRWLLLVAVLKGVCQSLEESGGRLVKPDETLTL TCTVSGFSLNTVAISWVRQAPGKGLEWIGFIHPTVNTYY ARWAKGRFTISRASSTTVDLKVTSLTFEDAATYFCVRGN AHYDIWGPGLTVTVSLGQPKAPSVFPLAPCCGDTSPST VTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPS
	Light (SEQ ID NO: 18)	MDTRAPTQLLGLLLLWLPGARCAFELTQTPSLVSAAVGG TVTISCQSSQSVYSDNYLAWYQQKPGQRPKLLIYKASDL ASGVPSRFKSGSGTEFTLTISDLECADAATYYCQSYYG VSSDSNAFGGGTEVVVKGDPVAPTVLIFPPSADLVATGT VTIVCVANKYFPDVTVTWEV

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Clone	Chain	Sequence
<b>S20H1L1</b>	Heavy  (SEQ ID NO: 19)	METGLRWLLLVAVLKGVQCQEQLEESGGGLVKPGGTLT LTCTASGFSLISTYYICWVRQAPGKGLEWIGCIPLSHSVS WYANWVNGRFSISKTSTTVTLKMASLTDADTATYFCG RGSSGWGVDSKLWGPGLTVTVSSGQPKAPSVFPLAPC CGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRT FPS
	Light  (SEQ ID NO: 20)	MDTRAPTQLLGLLLLWLPGAPFAAVLTQTPSPVSASVGG TVTINCQSSPSVASGYLSWFQQKPGQPPKLLIYRASTLV SGVPSRFKGS GSGTHFTLTISDVQCDDAATYYCAGAYS SRSDTTFGGGTEVVVKGDPVAPTVLIFPPAADQVATGTV TIVCVANKYFPDVTVTWEV

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For reference, the CDR and FR regions of the variable heavy and light chain sequences are highlighted below:

**Clone S42 Heavy Chain**

Chain	Sequence
Signal peptide (SEQ ID NO: 21)	METGLRWLLLVAVLKGVQC
FR1 (SEQ ID NO: 22)	QSLEESGGRLVKPDETLTLTCTVSGFSLN
CDR1 (SEQ ID NO: 23)	TVAIS
FR2 (SEQ ID NO: 24)	WVRQAPGKGGLEWIG
CDR2 (SEQ ID NO: 25)	FIHPTVNTYYARWAKG
FR3 (SEQ ID NO: 26)	RFTISRASSTTVDLKVTSLTFEDAATYFCVR
CDR3 (SEQ ID NO: 27)	GNAHYDI
FR4 (SEQ ID NO: 28)	WPGTLVTVSL

**Clone S42 Light Chain**

Chain	Sequence
Signal peptide (SEQ ID NO: 29)	MDTRAPTQLLGLLLLWLPGARC
FR1 (SEQ ID NO: 30)	AFELTQTPSLVSAAVGGTVTISC
CDR1 (SEQ ID NO: 31)	QSSQSVYSDNYLA
FR2 (SEQ ID NO: 32)	WYQQKPGQRPKLLIY
CDR2 (SEQ ID NO: 33)	KASDLAS
FR3 (SEQ ID NO: 34)	GVPSRFKGS GSGTEFTLTISDLECADAATYYC
CDR3 (SEQ ID NO: 35)	QSY YGVSSDSNA
FR4 (SEQ ID NO: 36)	FGGGTEV VVK

**Clone S20 Heavy Chain**

Chain	Sequence
Signal peptide (SEQ ID NO: 37)	METGLRWLLLVAVLKGVQC
FR1 (SEQ ID NO: 38)	QEQLLEESGGGLVKPGGTLTLTCTASGFSLI
CDR1 (SEQ ID NO: 39)	STYYIC
FR2 (SEQ ID NO: 40)	WVRQAPGKGGLEWIG
CDR2 (SEQ ID NO: 41)	CIPLSHSVSWYANWVNG
FR3 (SEQ ID NO: 42)	RFSISKTSSTTVTLKMASLTDADTATYFCGR
CDR3 (SEQ ID NO: 43)	GSSGWGVDSKL
FR4 (SEQ ID NO: 44)	WGPGTLVTVSS

**Clone S20 Light Chain**

Chain	Sequence
Signal peptide (SEQ ID NO: 45)	MDTRAPTQLLGLLLLWLPGAPFA
FR1 (SEQ ID NO: 46)	AVLTQTPSPVSASVGGTVTINC
CDR1 (SEQ ID NO: 47)	QSSPSVASGYLS
FR2 (SEQ ID NO: 48)	WFQQKPGQPPKLLIY
CDR2 (SEQ ID NO: 49)	RASTLVS
FR3 (SEQ ID NO: 50)	GVPSRFKGS GSGTHFTLTISDVQCDDAATYYC
CDR3 (SEQ ID NO: 51)	AGAYSSRSDTT
FR4 (SEQ ID NO: 52)	FGGGTEVVVK

5 The antibodies of the present invention are not limited to comprising the sequences listed above (e.g., SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20). For example, in some embodiments, the C4.4a antibody comprises SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, a fragment thereof, or a peptide that is at least 60% identical (e.g., at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at

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least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, etc.) to SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In some embodiments, the fragment is between 20-60 amino acids, between 40-80 amino acids, between 60-100 amino acids, between 80-120 amino acids, between 100-140 amino acids, between 120-160 amino acids, between 140-180 amino acids, between 160-186 amino acids, between 160-190 amino acids, between 170-193 amino acids, etc. In some embodiments, the C4.4a antibody comprises a peptide that is at least 60% identical (e.g., at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, etc.) to a fragment (e.g., between 20-60 amino acids, between 40-80 amino acids, between 60-100 amino acids, between 80-120 amino acids, between 100-140 amino acids, between 120-160 amino acids, between 140-180 amino acids, between 160-186 amino acids, between 160-190 amino acids, between 170-193 amino acids, etc.) of one of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20.

In some embodiments, the C4.4a antibody may comprise a recombinant protein comprising two or more (e.g., three, four, five, six, seven, eight, nine, 10, 11, 12, etc.) peptides derived from any of: SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, a fragment (between 20-60 amino acids, between 40-80 amino acids, between 60-100 amino acids, between 80-120 amino acids, between 100-140 amino acids, between 120-160 amino acids, between 140-180 amino acids, between 160-186 amino acids, between 160-190 amino acids, between 170-193 amino acids, etc.) thereof, a peptide that is at least 60% identical (e.g., at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, etc.) to SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, and/or a peptide that is at least 60% identical (e.g., at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, etc.) to a fragment (e.g., between 20-60 amino acids, between 40-80 amino acids, between 60-100 amino acids, between 80-120 amino acids, between 100-140 amino acids, between 120-160 amino acids, between 140-180 amino acids, between 160-186 amino acids, between 160-190 amino acids, between 170-193 amino acids, etc.) of one of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. For example, the C4.4a antibody may comprise a first peptide section and a second peptide section, each selected



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from the above list (SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, a fragment (between 20-60 amino acids, between 40-80 amino acids, between 60-100 amino acids, between 80-120 amino acids, between 100-140 amino acids, between 120-160 amino acids, between 140-180 amino acids, between 160-186 amino acids, between 160-190 amino acids, between 170-193 amino acids, etc.) thereof, a peptide that is at least 60% identical (e.g., at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, etc.) to SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, and/or a peptide that is at least 60% identical (e.g., at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, etc.) to a fragment (e.g., between 20-60 amino acids, between 40-80 amino acids, between 60-100 amino acids, between 80-120 amino acids, between 100-140 amino acids, between 120-160 amino acids, between 140-180 amino acids, between 160-186 amino acids, between 160-190 amino acids, between 170-193 amino acids, etc.) of one of SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20.). In some embodiments, the first peptide section and second peptide section are contiguous. In some embodiments, the first peptide section and the second peptide section are separated by one or more additional amino acids (e.g., a linker). As an example, a C4.4a antibody may comprise a first peptide section corresponding to amino acids 1-110 of SEQ ID NO: 17 and another peptide section corresponding to amino acids 20-140 of SEQ ID NO: 18, possibly separated by a linker (e.g., a linker, e.g., a linker having 1 amino acid, 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, 6 amino acids, 8-10 amino acids, 10-20 amino acids, more than 20 amino acids, etc.). And, in some embodiments, said first peptide section may be downstream of said second peptide section.

Below are shown non-limiting examples of sequences that are at least 60% identical to SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. The present invention is in no way limited to these sequences or the particular amino acids that vary in the below sequences as compared to the original sequence. Note: "X" refers to any appropriate amino acid substitution.

SEQ ID NO:	Description	Sequence (note: X refers to any appropriate amino acid substitution)
61	Sequence at least 90% identical to SEQ ID NO: 17	<b>MX</b> TGLRWLLL <b>VX</b> VLKGVQCQS <b>LEX</b> SGGRL <b>XX</b> PDETL <b>XL</b> TCTVSGFSLNTVAIS <b>XVRQX</b> PGKGLEWIGFIHPTVN TYYARW <b>XX</b> KGRFTISRASSTTV <b>XL</b> KVTS <b>LT</b> FEDAATYFC VRGN <b>XX</b> HYDIW <b>XP</b> GT <b>LV</b> TV <b>SX</b> GQPKAPSVFPL <b>XPCCX</b> DTPSSTVTL <b>XL</b> CLV <b>KXX</b> YLPEPVTVTWNS <b>XTL</b> TNGV <b>RTF</b> PS
62	Sequence at least 80% identical to SEQ ID NO: 18	<b>MX</b> TRAPTQ <b>XL</b> GLL <b>XLWXPXARCXF</b> ELTQT <b>PSLVSXAV</b> <b>GXT</b> VTISCQS <b>XQSVYSXNYLAXYQQKPXQRPKXLIYK</b> <b>XSXLXSXXPSRFXSGSXTXFTLTISDLECADAXTYXC</b> QSYYGV <b>SXDSNAFXGGTEVXVKGDPVXPTVLIFPXSX</b> <b>XLVXTGTVXIVCVXNKYFPDVTVXW XV</b>
63	Sequence at least 95% identical to SEQ ID NO: 19	METGLRWLLL <b>VX</b> VLKGVQCQ <b>EXLEX</b> SGGGLVKPGGT LTLTCTAS <b>XF</b> SLISTYYICWVRQAPGKGLEWIGCIPLSH SVSWY <b>XN</b> WVNGRFSIS <b>KTSSTXV</b> TLKMASLTDADTAT YFC <b>XR</b> GSSGWGVDSKLWGP <b>GT</b> LVTV <b>XSG</b> QPKAPSV FPL <b>XPCC</b> GDTPSSTVTLGCLVKGYLPEPVTVTWNSGT LTNGV <b>RTFPS</b>
64	Sequence at least 70% identical to SEQ ID NO: 20	<b>MX</b> TR <b>AX</b> TQ <b>LLGLXXXWLPXAPXAA</b> XL <b>TQT</b> XSPV <b>XA XV</b> <b>XXTVXINXQSSPXVASGYLSXFXQKPXQPPXLLIYRXX</b> TL <b>XSGXPSR</b> FKG <b>XXSG</b> THFTLTISDVQ <b>XXXXATYYXXX</b> <b>XYSSRXDXTXGXXTEVXXKGD</b> P <b>XXPTX</b> LIFPP <b>AXDQV</b> ATG <b>XVTIXCVAXKYFPXVTXTWXX</b>

As previously discussed, the present invention includes any cDNA that encodes any peptide disclosed herein, e.g., a cDNA that encodes any of the aforementioned antibody sequences (or any antibody sequence according to the present invention).

**Epitope Regions**

The C4.4a antibody binds specifically to a particular sequence or region of C4.4a, e.g., a C4.4a epitope. Non-limiting examples of sequences or regions containing an epitope to which the antibody may bind include the uPAR-like domain 1 (e.g., SEQ ID NO: 3, SEQ ID NO: 53), the uPAR-like domain 2 (e.g., SEQ ID NO: 4, SEQ ID NO: 57), fragments thereof (e.g., between 5-10 amino acids, between 10-20 amino acids, between 10-30 amino acids, between 10-40 amino acids, between 20-40 amino acids, between 20-50 amino acids, between 30-50 amino acids, between 30-60 amino acids, between 30-70 amino acids, between 40-70 amino acids, between 40-80 amino acids, between 50-80 amino acids, between 50-90 amino acids, between 60-90 amino acids, between 50-100 amino acids, between 60-100 amino acids, etc.), peptides that are at least 60% identical (e.g., at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, etc.) to the uPAR-like domain 1 (e.g., SEQ ID NO: 3, SEQ ID NO: 53) or the uPAR-like domain 2 (e.g., SEQ ID NO: 4, SEQ ID NO: 57), peptides that are at least 60% identical to fragments of the uPAR-like domain 1 or the uPAR-like domain 2, etc. The uPAR-like domain 1 is not limited to amino acids 31-116 of C4.4a. For example, in some embodiments, the uPAR-like domain 1 comprises amino acids 33-126 of C4.4a (SEQ ID NO: 53), amino acids 32-116, amino acids 31-126, amino acids 31-125, amino acids 31-124, amino acids 31-123, amino acids 31-122, amino acids 31-121, amino acids 31-120, amino acids 31-119, amino acids 31-118, amino acids 31-118, amino acids 31-117, amino acids 31-115, amino acids 31-114, amino acids 31-113, amino acids 31-112, amino acids 31-111, amino acids 31-110, amino acids 32-110, amino acids 32-112, amino acids 32-114, amino acids 32-118, amino acids 32-119, amino acids 32-120, amino acids 33-128, amino acids 31-127, amino acids 34-119, amino acids 36-122, etc. The uPAR-like domain 2 is not limited to amino acids 139-224 of C4.4a. For example, in some embodiments, the uPAR-like domain 2 comprises amino acids 140-222 of C4.4a (SEQ ID NO: 53), amino acids 139-215, amino acids 139-216, amino acids 139-217, amino acids 139-218, amino acids 139-219, amino acids 139-220, amino acids 139-222, amino acids 139-223, amino acids 139-225, amino acids 139-226, amino acids 139-227, amino acids 138-215, amino acids 138-216, amino acids 138-217, amino acids 138-218, amino acids 138-219, amino acids 138-220, amino acids 138-222, amino acids 138-223, amino acids 138-225, amino acids 138-226, amino acids 138-227, amino acids 140-215, amino acids 140-216, amino acids 140-217, amino acids 140-218, amino acids 140-219, amino acids 140-220, amino acids 140-223,

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amino acids 140-224, amino acids 140-225, amino acids 140-226, amino acids 140-227, etc.

Further, the epitope is not limited to regions within the uPAR-like domains. In some embodiments, amino acids around the uPAR-like domains may also be part of the epitope.

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Non-limiting examples of regions containing epitopes to which the C4.4a antibodies may bind are shown below:

SEQ ID NO:	Amino Acid Length	Epitope Sequence
3	86	LECYSCVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIHGQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRCNAK (amino acids 31-116 of C4.4a)
53	94	CYSCVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIHGQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRCNAKLNLTSRALDP (amino acids 33-126 of C4.4a)
54	55	PNKMKTVKCAPGVDVCTEAVGAVETIHGQFSLAVRGCGSGLPGKNDRGLDLHGLL (amino acids 46-100 of C4.4a)
55	47	KTVKCAPGVDVCTEAVGAVETIHGQFSLAVRGCGSGLPGKNDRGLDL (amino acids 50-96 of C4.4a)
56	97	YSCVQKADDGCSPNKMKTVKCAPGVDVCTEAVGAVETIHGQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRCNAKLNLTSRALDPAGNE (amino acids 34-130 of C4.4a)
4	86	ECYSCVGLSREACQGTSPPVVSCYNASDHVYKGCFDGNVTLTAANVTVSLPVRGCVQDEFCTRDGVTGPGFTLSGSCCQGSRCNSD (amino acids 139-224 of C4.4a)

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SEQ ID NO:	Amino Acid Length	Epitope Sequence
57	83	CYSCVGLSREACQGTSPPVVSCYNASDHVYKGCFDGNVTLT AANVTVSLPVRGCVQDEFCTRDGVTGPGFTLSGSCCQGSRC N (amino acids 140-222 of C4.4a)
58	84	ECYSCVGLSREACQGTSPPVVSCYNASDHVYKGCFDGNVTL TAANVTVSLPVRGCVQDEFCTRDGVTGPGFTLSGSCCQGSRC CN (amino acids 139-222 of C4.4a)
59	71	CYSCVGLSREACQGTSPPVVSCYNASDHVYKGCFDGNVTLT AANVTVSLPVRGCVQDEFCTRDGVTGPGFT (amino acids 140-210 of C4.4a)
60	73	LSREACQGTSPPVVSCYNASDHVYKGCFDGNVTLTAAANVTVS LPVRGCVQDEFCTRDGVTGPGFTLSGSCCQG (amino acids 146-218 of C4.4a)

In some embodiments, the region containing the epitope comprises SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO 53, SEQ ID NO 54, SEQ ID NO 55, SEQ ID NO 56, SEQ ID NO 57, SEQ ID NO 58, SEQ ID NO 59, SEQ ID NO 60, a fragment (e.g., between 5-10 amino acids, between 10-20 amino acids, between 10-30 amino acids, between 10-40 amino acids, between 20-40 amino acids, between 20-50 amino acids, between 30-50 amino acids, between 30-60 amino acids, between 30-70 amino acids, between 40-70 amino acids, between 40-80 amino acids, between 50-80 amino acids, between 50-85 amino acids, between 60-85 amino acids, etc.) thereof, a peptide that is at least 60% identical (e.g., at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, etc.) to SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO 53, SEQ ID NO 54, SEQ ID NO 55, SEQ ID NO 56, SEQ ID NO 57, SEQ ID NO 58, SEQ ID NO 59, or SEQ ID NO 60, a peptide that is at least 60% identical (e.g., at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical, at

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least 85% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, etc.) to a fragment (e.g., between 5-10 amino acids, between 10-20 amino acids, between 10-30 amino acids, between 10-40 amino acids, between 20-40 amino acids, between 20-50 amino acids, between 30-50 amino acids, between 30-60 amino acids, between 30-70 amino acids, between 40-70 amino acids, between 40-80 amino acids, between 50-80 amino acids, between 50-85 amino acids, between 60-85 amino acids, etc.) of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO 53, SEQ ID NO 54, SEQ ID NO 55, SEQ ID NO 56, SEQ ID NO 57, SEQ ID NO 58, SEQ ID NO 59, or SEQ ID NO 60, or a combination thereof.

In some embodiments, the antibody binds to and/or the epitope comprises a C4.4a glycosylation site, e.g., amino acid 118, 163, 176, 183, or 326.

The present invention is not limited to clone S42 and clone S20. The present invention also features a C4.4a antibody (e.g., a monoclonal antibody) or C4.4a binding fragment, wherein the C4.4a antibody or binding fragment has the same epitopic specificity as an antibody selected from the group consisting of clone S42H9L5 and clone S20H1L1. For example, the C4.4a antibody may have a different sequence than S42 and/or S20, but the C4.4a antibody may have the same or similar specificity for the epitope(s) of S42 and/or S20.

As previously discussed, C4.4a antibodies disclosed herein may be produced by immunizing a host with a C4.4a protein (e.g., an immunogen as previously discussed) or related peptide. The host may include but is not limited to a mouse, a rat, a rabbit. In some embodiments, the host model is a mouse model, a rat model, a rabbit (e.g., New Zealand white rabbit), donkey, sheep; however the host is not limited to these examples. In some embodiments, recombinant C4.4a antibodies may be produced using a host cell expression system. Examples of host cell expression systems include but are not limited to HEK293 cells or derivatives thereof, mammalian cells (e.g., CHO, HELA, SP20, etc.), insect cells, yeast (e.g., *P. pastoris*), plant cells (e.g., tobacco), prokaryotic systems (e.g., *E. coli*), etc. The present invention is not limited to the expression systems disclosed herein.

The present invention also features a labeled tissue sample (e.g., a formalin-fixed paraffin-embedded sample), wherein the tissue sample is labeled with a C4.4a antibody according to the present invention. The tissue sample may be obtained by a method according to the present invention.

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The present invention also features a kit comprising a C4.4a antibody according to the present invention, e.g., a kit for immunohistochemistry, e.g., for use on a formalin-fixed paraffin-embedded sample, wherein the C4.4a antibody is adapted for immunohistochemistry. Kits for IHC are well known to one of ordinary skill in the art. In some embodiments, the kit comprises a detection system (e.g., a chromogenic system, a fluorescence system, any other appropriate system) for making the C4.4a antibody visible. In some embodiments, the kit comprises any other appropriate reagents, e.g., a secondary antibody directed to the C4.4a antibody, buffers, etc.

10 In some embodiments, the antibody is adapted for use in immunohistochemistry (IHC) assays. In some embodiments, the antibody is adapted for use assays other than IHC assays, e.g., for western blotting or other antibody-related applications.

As previously discussed, the present invention also features methods of detecting C4.4a using the antibodies disclosed herein, as well as methods for detecting C4.4a-associated cancers using the C4.4a antibodies disclosed herein.

20 The present invention also features a method (e.g., automated method, manual method) of detecting C4.4a. In some embodiments, the method comprises providing a sample, contacting the sample with a C4.4a antibody according to the present invention, and making the antibody visible (e.g., via a detection system such as a chromogenic system, a fluorescence system, any other appropriate system). Detecting the antibody may be indicative of the presence of C4.4a.

25 The present invention also features a method of diagnosing a C4.4a-associated tumor (e.g., lung tumor, cervical tumor, skin carcinoma, esophageal tumor). The method may comprise detecting C4.4a according to a method of the present invention. Detection of C4.4a may be indicative of the C4.4a-associated tumor.

30 The present invention also features a stainer machine programmed to perform a method according to the present invention. For example, the stainer machine comprises components for performing an automated immunohistochemistry method. The stainer machine may comprise a program that allows for the methods of the present invention to be performed. The present invention also features a closed system for detecting C4.4a, e.g., a closed automated system adapted to perform a method according to the present invention.

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Computers typically include known components, such as a processor, an operating system, system memory, memory storage devices, input-output controllers, input-output devices, and display devices. It will also be understood by those of ordinary skill in the relevant art that there are many possible configurations and components of a computer and may also include cache memory, a data backup unit, and many other devices. Examples of input devices include a keyboard, a cursor control devices (e.g., a mouse), a microphone, a scanner, and so forth. Examples of output devices include a display device (e.g., a monitor or projector), speakers, a printer, a network card, and so forth. Display devices may include display devices that provide visual information, this information typically may be logically and/or physically organized as an array of pixels. An interface controller may also be included that may comprise any of a variety of known or future software programs for providing input and output interfaces. For example, interfaces may include what are generally referred to as “Graphical User Interfaces” (often referred to as GUI’s) that provide one or more graphical representations to a user. Interfaces are typically enabled to accept user inputs using means of selection or input known to those of ordinary skill in the related art. The interface may also be a touch screen device. In the same or alternative embodiments, applications on a computer may employ an interface that includes what are referred to as “command line interfaces” (often referred to as CLI’s). CLI’s typically provide a text based interaction between an application and a user. Typically, command line interfaces present output and receive input as lines of text through display devices. For example, some implementations may include what are referred to as a “shell” such as Unix Shells known to those of ordinary skill in the related art, or Microsoft Windows Powershell that employs object-oriented type programming architectures such as the Microsoft .NET framework.

Those of ordinary skill in the related art will appreciate that interfaces may include one or more GUI’s, CLI’s or a combination thereof. A processor may include a commercially available processor such as a Celeron, Core, or Pentium processor made by Intel Corporation, a SPARC processor made by Sun Microsystems, an Athlon, Sempron, Phenom, or Opteron processor made by AMD Corporation, or it may be one of other processors that are or will become available. Some embodiments of a processor may include what is referred to as multi-core processor and/or be enabled to employ parallel processing technology in a single or multi-core configuration. For example, a multi-core architecture typically comprises two or more processor “execution cores”. In the present example, each execution core may perform as an independent processor that enables parallel execution of



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multiple threads. In addition, those of ordinary skill in the related will appreciate that a processor may be configured in what is generally referred to as 32 or 64 bit architectures, or other architectural configurations now known or that may be developed in the future.

5 A processor typically executes an operating system, which may be, for example, a Windows type operating system from the Microsoft Corporation; the Mac OS X operating system from Apple Computer Corp.; a Unix or Linux-type operating system available from many vendors or what is referred to as an open source; another or a future operating system; or some combination thereof. An operating  
10 system interfaces with firmware and hardware in a well-known manner, and facilitates the processor in coordinating and executing the functions of various computer programs that may be written in a variety of programming languages. An operating system, typically in cooperation with a processor, coordinates and executes functions of the other components of a computer. An operating system  
15 also provides scheduling, input-output control, file and data management, memory management, and communication control and related services, all in accordance with known techniques.

System memory may include any of a variety of known or future memory storage devices that can be used to store the desired information and that can be accessed  
20 by a computer. Computer readable storage media may include volatile and non-volatile, removable and non-removable media implemented in any method or technology for storage of information such as computer readable instructions, data structures, program modules, or other data. Examples include any commonly available random access memory (RAM), read-only memory (ROM), electronically  
25 erasable programmable read-only memory (EEPROM), digital versatile disks (DVD), magnetic medium, such as a resident hard disk or tape, an optical medium such as a read and write compact disc, or other memory storage device. Memory storage devices may include any of a variety of known or future devices, including a compact disk drive, a tape drive, a removable hard disk drive, USB or flash drive,  
30 or a diskette drive. Such types of memory storage devices typically read from, and/or write to, a program storage medium such as, respectively, a compact disk, magnetic tape, removable hard disk, USB or flash drive, or floppy diskette. Any of these program storage media, or others now in use or that may later be developed, may be considered a computer program product. As will be appreciated, these  
35 program storage media typically store a computer software program and/or data. Computer software programs, also called computer control logic, typically are

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stored in system memory and/or the program storage device used in conjunction with memory storage device. In some embodiments, a computer program product is described comprising a computer usable medium having control logic (computer software program, including program code) stored therein. The control logic, when  
5 executed by a processor, causes the processor to perform functions described herein. In other embodiments, some functions are implemented primarily in hardware using, for example, a hardware state machine. Implementation of the hardware state machine so as to perform the functions described herein will be apparent to those skilled in the relevant arts. Input-output controllers could include  
10 any of a variety of known devices for accepting and processing information from a user, whether a human or a machine, whether local or remote. Such devices include, for example, modem cards, wireless cards, network interface cards, sound cards, or other types of controllers for any of a variety of known input devices. Output controllers could include controllers for any of a variety of known display  
15 devices for presenting information to a user, whether a human or a machine, whether local or remote. In the presently described embodiment, the functional elements of a computer communicate with each other via a system bus. Some embodiments of a computer may communicate with some functional elements using network or other types of remote communications. As will be evident to those skilled in the relevant art, an instrument control and/or a data processing  
20 application, if implemented in software, may be loaded into and executed from system memory and/or a memory storage device. All or portions of the instrument control and/or data processing applications may also reside in a read-only memory or similar device of the memory storage device, such devices not requiring that the instrument control and/or data processing applications first be loaded through  
25 input-output controllers. It will be understood by those skilled in the relevant art that the instrument control and/or data processing applications, or portions of it, may be loaded by a processor, in a known manner into system memory, or cache memory, or both, as advantageous for execution. Also, a computer may include one  
30 or more library files, experiment data files, and an internet client stored in system memory. For example, experiment data could include data related to one or more experiments or assays, such as detected signal values, or other values associated with one or more sequencing by synthesis (SBS) experiments or processes. Additionally, an internet client may include an application enabled to access a  
35 remote service on another computer using a network and may for instance comprise what are generally referred to as "Web Browsers". In the present example, some commonly employed web browsers include Microsoft Internet Explorer available

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from Microsoft Corporation, Mozilla Firefox from the Mozilla Corporation, Safari from Apple Computer Corp., Google Chrome from the Google Corporation, or other type of web browser currently known in the art or to be developed in the future. Also, in the same or other embodiments an Internet client may include, or  
5 could be an element of, specialized software applications enabled to access remote information via a network such as a data processing application for biological applications.

A network may include one or more of the many various types of networks well known to those of ordinary skill in the art. For example, a network may include a  
10 local or wide area network that may employ what is commonly referred to as a TCP/IP protocol suite to communicate. A network may include a network comprising a worldwide system of interconnected computer networks that is commonly referred to as the Internet, or could also include various intranet architectures. Those of ordinary skill in the related arts will also appreciate that  
15 some users in networked environments may prefer to employ what are generally referred to as “firewalls” (also sometimes referred to as Packet Filters, or Border Protection Devices) to control information traffic to and from hardware and/or software systems. For example, firewalls may comprise hardware or software elements or some combination thereof and are typically designed to enforce  
20 security policies put in place by users, such as for instance network administrators, etc.

### **EXAMPLES**

The following examples describe non-limiting examples of experiments using methods, compositions, and systems of the present invention.

#### **Example 1:**

IHC protocol on mouse xenografts: DISCOVERY XT protocol used on mouse xenograft tissues (SCC-T9 - strong; H293-T3 -moderate; MCF-7 -weak; PC3-negative) using DISCOVERY XT platform. Briefly, selects Std CC1 cell conditioning for antigen retrieval, dilute anti-C4.4a antibody clone SP246 (a  
30 S42H9L5 clone) at 1:400 (3 µg/ml) in antibody diluent (Catalog Number 251-018, Ventana), incubates the primary antibody for 16 min at room temperature, selects standard ChromoMap DAB detection. Lastly, target antigen is detected using a chromogenic substrate (DAB), followed with hematoxylin counterstaining for 1 minute. See FIG. 1A, FIG. 1B.

**Example 2:**

IHC protocol on human tissues: Standard ultraView Universal DAB Detection Kit protocol is used on human tissues (skin, skin squamous cell carcinoma, cervix, and esophagus) using BenchMark Ultra platform (Ventana Medical System). Briefly, selects StdCC1 cell conditioning for antigen retrieval, dilutes anti-C4.4a antibody clone SP245 (a S20H1L1 clone) at 1:400 (3  $\mu$ g/ml) in antibody diluent (Catalog Number 251-018, Ventana), incubates the primary antibody for 16 min at room temperature, selects standard ultraView Universal DAB Detection protocol. Lastly, target antigen is detected using DAB, followed with hematoxylin counterstaining for 1 minute. See FIG. 2A, FIG. 2B.

As used herein, the term "about" refers to plus or minus 10% of the referenced number.

The abbreviation "aa" means "amino acid".

15

Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

20

Although there has been shown and described the preferred embodiment of the present invention, it will be readily apparent to those skilled in the art that modifications may be made thereto which do not exceed the scope of the appended claims. Therefore, the scope of the invention is only to be limited by the following claims. Reference numbers recited in the claims are exemplary and for ease of review by the patent office only, and are not limiting in any way. In some embodiments, the figures presented in this patent application are drawn to scale, including the angles, ratios of dimensions, etc. In some embodiments, the figures are representative only and the claims are not limited by the dimensions of the figures. In some embodiments, descriptions of the inventions described herein using the phrase "comprising" includes embodiments that could be described as

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“consisting of”, and as such the written description requirement for claiming one or more embodiments of the present invention using the phrase “consisting of” is met.

5 The reference numbers recited in the below claims are solely for ease of examination of this patent application, and are exemplary, and are not intended in any way to limit the scope of the claims to the particular features having the corresponding reference numbers in the drawings.

**CLAIMS:**

1. A monoclonal C4.4a antibody comprising a heavy chain variable region sequence comprising the amino acid sequence of SEQ ID NO: 17 and a light chain variable region sequence comprising the amino acid sequence of SEQ ID NO: 18.
2. A monoclonal C4.4a antibody comprising a heavy chain CDR1 sequence of SEQ ID NO: 23, a heavy chain CDR2 sequence of SEQ ID NO: 25, a heavy chain CDR3 sequence of SEQ ID NO: 27, a light chain CDR1 sequence of SEQ ID NO: 31, a light chain CDR2 sequence of SEQ ID NO: 33, and a light chain CDR3 sequence of SEQ ID NO: 35.
3. A monoclonal C4.4a antibody specific for an epitope within a uPAR-like domain 2, said antibody comprising a heavy chain variable region sequence comprising the amino acid sequence of SEQ ID NO: 17 and a light chain variable region sequence comprising the amino acid sequence of SEQ ID NO: 18.
4. The antibody of any one of claims 1-3, wherein the antibody is raised by immunizing a host with a peptide comprising SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, a fragment thereof, a peptide that is at least 90% identical to SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 4, a peptide that is at least 90% identical to a fragment of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 4, or a combination thereof.
5. The antibody of claim 4, wherein the fragment is at least 20 amino acids in length.
6. The antibody of claim 4, wherein the host is a rabbit host.
7. The antibody of any one of claims 1-3, wherein the antibody binds to an epitope within a region, said region comprising SEQ ID NO: 4, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, a fragment thereof, a peptide that is at least 90% identical to SEQ ID NO: 4, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, or SEQ ID NO: 60, a

peptide that is at least 90% identical to a fragment of SEQ ID NO: 4, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, or SEQ ID NO: 60, or a combination thereof.

8. The antibody of claim 7, wherein the fragment is at least 20 amino acids in length.
9. The antibody of any one of claims 1-8, wherein the antibody is produced in a host expression system.
10. A cDNA encoding the C4.4a antibody according to any one of claims 1-9.
11. A host cell expression system expressing the antibody according to any one of claims 1-9.
12. The host cell expression system of claim 11, wherein the host cells are HEK293 cells.
13. A kit for detecting C4.4a comprising the C4.4a antibody according to any one of claims 1-9.
14. The kit of claim 13, wherein the antibody is adapted for immunohistochemistry.
15. The kit of claim 13 further comprising a detection system for making the C4.4a antibody visible.
16. The kit of claim 15, wherein the detection system comprises a chromogenic detection system.
17. The kit of claim 15, wherein the detection system comprises a fluorescence detection system.

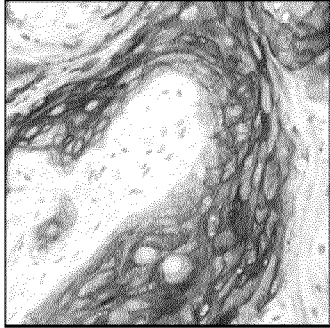
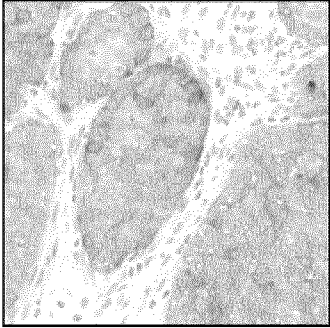
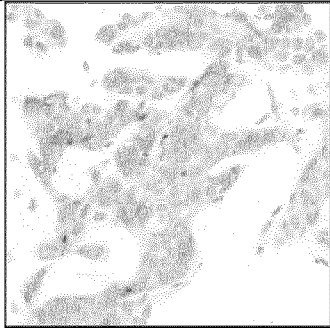
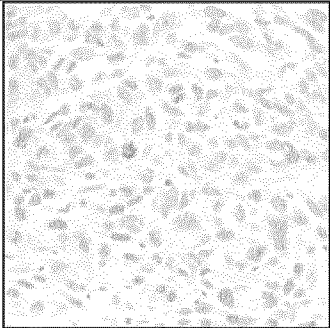
18. A method of detecting C4.4a, said method comprising:
  - a. contacting a sample with the C4.4a antibody according to any one of claims 1-9;  
and
  - b. making the antibody visible;wherein detecting the C4.4a antibody is indicative of the presence of C4.4a.
19. The method of claim 18, wherein the method is automated.
20. The method of claim 18, wherein the method is manual.
21. The method of any one of claims 18-20, wherein the step of making the antibody visible comprises contacting the antibody with a chromogenic detection system or a fluorescence detection system.
22. A method of producing the C4.4a antibody according to any one of claims 1-9, said method comprising immunizing an animal with a peptide comprising SEQ ID NO: 2, SEQ ID NO: 4, a fragment of SEQ ID NO: 2 comprising at least SEQ ID NO: 4, a peptide that is at least 90% identical to SEQ ID NO: 2, or SEQ ID NO: 4, a peptide that is at least 90% identical to the fragment of SEQ ID NO: 2 comprising at least SEQ ID NO: 4, or a combination thereof.
23. A method of diagnosing a C4.4a-expressing tumor, said method comprising the method of detecting C4.4a according to any one of claims 18-21, wherein detection of C4.4a is indicative of the C4.4a-expressing tumor.
24. A closed system for detecting C4.4a in a sample with the C4.4a antibody according to any one of claims 1-9, the system is automated and is adapted to perform the method according to any one of claims 18-21.



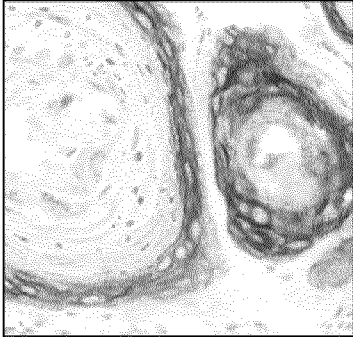
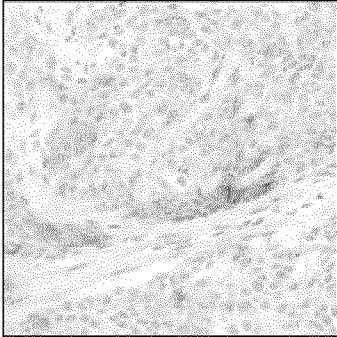
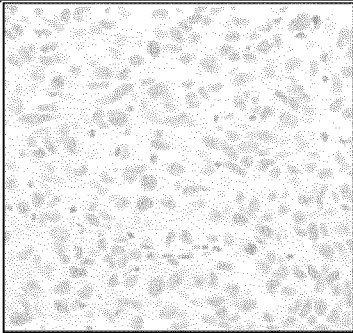
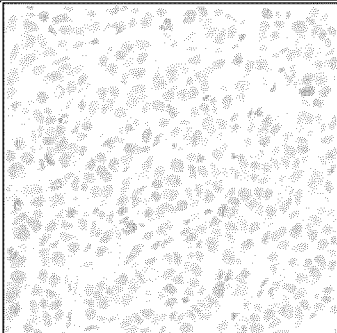
25. A system comprising:
  - a. a stainer machine;
  - b. a processor;
  - c. the C4.4a antibody according to any one of claims 1 to 9; and
  - d. a memory coupled to the processor, the memory stores computer-readable instructions that, when executed by the processor, cause the processor to perform operations comprising:
    - i. instructing the stainer machine to deposit the C4.4a antibody onto a sample; and
    - ii. instructing the stainer machine to deposit a detection reagent onto the sample so as to make the C4.4a antibody visible.
26. The system of claim 25, wherein the stainer machine comprises at least a dispenser for dispensing the C4.4a antibody onto the sample.
27. The system of claim 25, wherein the operations further comprise instructing the stainer machine to incubate the sample at a temperature sufficient for binding of the C4.4a antibody to its target.
28. The system of claim 27, wherein the stainer machine comprises a heat pad for incubating the sample.
29. The system of claim 28, wherein the operations further comprise instructing the stainer machine to wash the sample with a wash buffer before the detection reagent is deposited onto the sample.
30. The system of claim 29, wherein the stainer machine comprises a dispenser for dispensing a wash buffer onto the sample.

31. The system of claim 25, wherein the operations further comprise instructing the stainer machine to incubate the sample in a cell conditioning buffer prior to the depositing of the C4.4a antibody.
32. The system of claim 31, wherein the operations further comprise instructing the stainer machine to deposit a deparaffinizing buffer onto the sample prior to the depositing of the cell conditioning buffer.
33. The system of claim 32, wherein the operations further comprise instructing the stainer to bake the sample prior to the depositing of the deparaffinizing buffer onto the sample.
34. The system of claim 25, wherein the detection reagent comprises a secondary antibody, a chromogenic detection reagent, or a combination thereof.

**Figure 1A**

	SCC-T9 xenograft (+++ Ctr)	H293-T3 (++ Ctr)
S42H9L5		
	MCF-7 xenograft (+ Ctr)	PC3(- Ctr)
S42H9L5		

**Figure 1B**

	SCC-T9 xenograft (+++ Ctr)	H293-T3 (++ Ctr)
S20H1L1		
	MCF-7 xenograft (+ Ctr)	PC3(- Ctr)
S20H1L1		

**Figure 2A**

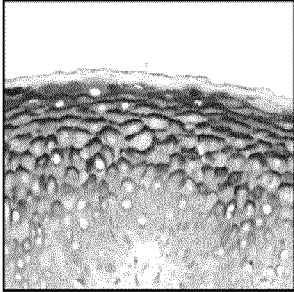
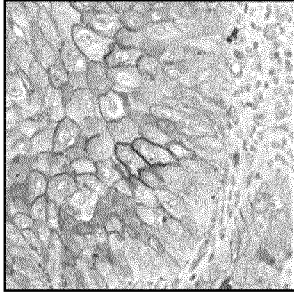
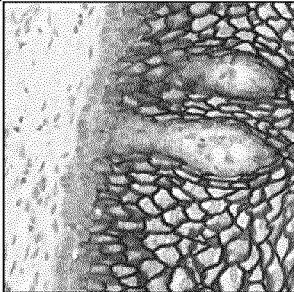
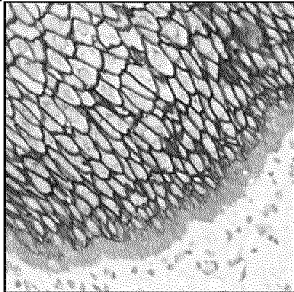

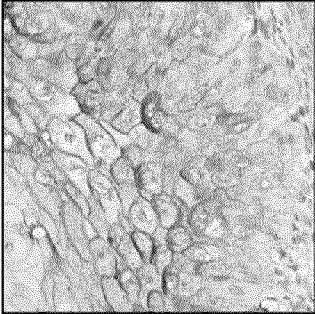
	Skin	Skin Squamous Cell Carcinoma
S42H9L5		
	Cervix	Esophagus
S42H9L5		

Figure 2B

	Skin	Skin Squamous Cell Carcinoma
S20H1L1		
	Cervix	Esophagus
S20H1L1	