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[Continued on next page]

(54) Title: UPAR BINDING AGENTS AND METHODS OF USE THEREOF

(57) Abstract: The present disclosure relates to binding agents (e.g. antibodies) that bind to and/or modulate the activity of a urokinase plasminogen activator receptor (uPAR/CD87), compositions comprising the antibodies, and methods involving use of the antibodies or compositions.

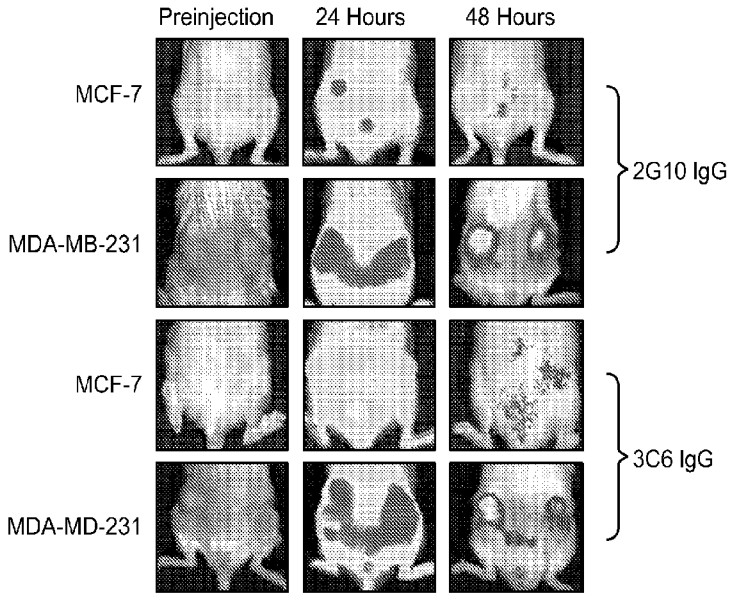


FIG. 12

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UPAR BINDING AGENTS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of U.S. provisional application serial no. 61/304,334, filed February 12, 2010, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant Nos. CA072006 and CA128765 awarded by the National Institutes of Health and Grant No. 073210 awarded by the National Institute of General Medical Sciences. The government has certain rights in the invention

INTRODUCTION

[0003] The urokinase plasminogen activator receptor (uPAR or CD87 (Cluster of Differentiation 87)) is a glycosylated protein of 45–55 kDa consisting of three homologous cysteine-rich domains. The protein is localized to the extracellular leaf of the plasma membrane through a glycosylphosphatidylinositol anchor. UPAR mediates a wide variety of cellular processes including inflammation, metastasis and invasion, tissue remodeling, angiogenesis, and cell adhesion.

[0004] Many of these processes are initiated by the highly specific binding of various ligands to membrane-bound uPAR at the cell surface. One such interaction is between uPAR and uPA, which mediates both extracellular and intracellular signaling events.

[0005] Binding of extracellular pro-uPA to uPAR facilitates its activation. In turn, uPA activates proteases, such as plasmin, which directly and indirectly degrade the extracellular matrix (ECM). Furthermore, plasmin can activate pro-uPA leading to a positive feedback loop that accelerates ECM degradation. uPAR is also able to act intracellularly by activating proliferative signal transduction pathways. uPAR is believed to directly associate with integrin family adhesion receptors in complexes that mediate RGD-independent cell signaling and migration. Accordingly, uPAR plays a role in the development of cancer and the metastasis of cancer.

SUMMARY

[0006] The present disclosure relates to agents (e.g. antibodies) that bind to and modulate the activity of urokinase plasminogen activator receptor (uPAR/CD87), compositions comprising the agents, and methods involving use of the compositions.

[0007] Also provided by the disclosure is an antibody comprising a heavy chain variable region comprising one or more Compelementary Determining Regions (CDRs); and/or a light chain variable region comprising one or more CDRs, in which the antibody competes with a ligand such as uPA and/or an integrin protein for uPAR binding. Methods for using the antibodies, combinations of different antibodies, and compositions thereof are also provided.

BRIEF DESCRIPTION OF FIGURES

[0008] Figure 1: Sequence homology for uPAR-binding Fabs identified by phage display. Panel A, The heavy and light chain protein sequences of the 22 unique clones were aligned to generate a percent identity tree diagram. Panel B, The sequences of the CDR loops of each unique Fab were aligned and shaded to indicate sequence identity. H1, H2, and H3 refer to heavy chain (V_H) CDR1, CDR2, and CDR3, respectively. Similarly, L1, L2, and L3 refer to light chain (V_L) CDR1, CDR2, and CDR3, respectively.

[0009] Figure 2: Binding of uPA to uPAR in the presence of Fab. The ratio of bound Fab in the presence of uPA to bound Fab in the absence of uPA is reported as a percentage.

[0010] Figure 3: IgG expression by transient transfection. Panel A, Fab sequences were grafted onto an IgG1 scaffold by independently sub-cloning the heavy and light chain sequences into pTT5-SP-H1. Panel B, SDS-PAGE analysis of purified antibodies is shown.

[0011] Figure 4: Equilibrium affinity determination of uPAR antibody interaction. Percent of maximal surface plasmon resonance response during analyte (uPAR) injection versus analyte concentration is shown. Curve fitting for 2E9 (open circle), 1A8 (open square), 2G10 (closed diamond), and 2B1 (x) yielded K_D values that are summarized in the table.

[0012] Figure 5: Detection of HEK cell surface uPAR with human anti-uPAR antibodies. (Panels A-D), white profiles represent staining with control whole human IgG; shaded profiles represent staining with human anti-uPAR antibody. The identity of the human anti-uPAR antibody is indicated within the shaded profile (Panel A = 1A8, Panel B = 2B1, Panel C = 2E9, Panel D = 2G10).

[0013] Figure 6: In vivo images of tumor xenografts in nude mice. Tumor was labeled using 2G10 IgG or visualized via luciferase-expressing cells. Upper left: mouse with MDA-MB-231 tumor. Upper right: mouse with MDA-MB-231 tumor labeled with 2G10 IgG. Lower left: mouse with MCF-7/Luc+ tumor. Lower right: mouse with MCF-7 tumor labeled with 2G10 IgG. Bottom: mouse with MCF-7/Luc+ tumor contacted with luciferin for visualization. Circled regions point to areas of high signal from labeled 2G10 IgG or luciferase activity.

[0014] Figure 7: Inhibition of uPA/uPAR mediated invasion and signaling in H1299 cells. Panel A, The results of Matrigel invasion experiment are expressed as percent inhibition of the invasion

observed with no treatment control. Panel B depicts the results of an experiment testing for the effect of antibodies on the phosphorylation of ERK (extracellular signal-regulated kinases).

[0015] Figure 8: Determination of 3C6 as a putative uPAR/β1 integrin antagonist. Panel A depicts the effects of antibody on the phosphorylation of ERK. Panel B shows adhesion assays in which 2G10 (uPAR/uPA antagonist) is directly compared with 3C6 (uPAR/β1 integrin antagonist). Panel C is a normalized graph comparing the adhesion of antibody treatment on the two different ECM coating.

[0016] Figure 9: 3C6 binds to cell surface uPAR and abrogates uPAR association with α5β1 integrin. Panel A depicts the results of a binding experiment to uPAR-expressing cells. Panel B depicts the results of an Immunoprecipitation experiment to investigate the effects of antibodies on uPAR's association with β1 integrin.

[0017] Figure 10: A combined treatment using 2G10 and 3C6 cells results in a synergistic effect of decreasing the invasive potential of H1299 through Matrigel/Collagen I and Collagen I. Experiments were carried out using H1299 cells pre-treated with antibodies and seeded on the Collagen I-coated (panel A) or Matrigel/Collagen I-coated (panel B) top membrane of a 24-well Transwell plate.

[0018] Figure 11: Binding of 2G10 Fab to cancer cell lines expressing high levels of uPAR (MDA-MB-231) and to another cancer cell line expressing low levels of uPAR (MCF-7). Fitc fluorescence indicates amount of antibody bound. Cells were also analyzed in the absence of antibodies.

[0019] Figure 12: In vivo images of immune-compromised mice that have been injected with two different cancer cell lines. The cancer cell line expressing high levels of uPAR is MDA-MB-231 and the cancer cell line expressing low levels of uPAR is MCF-7. After palpable tumors appeared, two nmol of labeled 2G10 IgG or 3C6 IgG were injected into the mice.

[0020] Figure 13: Fluorescence micrographs of MDA-MB-231 cells stained with Alexa-Fluor 488-labeled 2G10 Fab (Panel A) or fitc-labeled 3C6 Fab (Panel B). Cells were also stained with DAPI. Panel C, 2G10 Fab staining of paraffin-embedded breast tumor.

[0021] Figure 14: Amira-processed representation of an MDA-MB-231-xenograph mouse imaged with ^{111}In-DOTA- 2G10 at 48 hr post-injection of 2G10 IgG (250 μCi). The CT skeletal image can be seen in white. Dark gray represents the tumors. Panel A, ventral; Panel B, sagittal view at 90°; Panel C, dorsal; Panel D, sagittal view at 180°. Arrows point to the location of where the antibody binds, which corresponds to the location of the tumors.

[0022] Figure 15: A flow cytometry graph demonstrating the cytostatic states of MDA-MB-231 cells treated or untreated with 2G10 Fab.

[0023] Figure 16: Epitope mapping experiments for 3C6 using alanine-scanned uPAR mutants (panel A); front (top) and back (bottom) representation of the uPAR structure. Arrow points to the integrin binding site (panel B).

DETAILED DESCRIPTION OF EMBODIMENTS

[0024] The present disclosure relates to agents (e.g. antibodies, aptamers, and/or peptides, etc.) that bind to urokinase plasminogen activator receptor (uPAR), compositions comprising the agents, and methods involving use of the compositions. The agents disclosed herein and methods of use can modulate uPAR by disrupting binding of uPAR to other proteins.

[0025] Certain antibodies disclosed herein were found by screening a human Fab phage display library for uPAR binding. Several studies of the antibody and uPAR reveal that the antibody comprises features that prevent binding of ligand such as uPA and/or integrin proteins to uPAR as well as other features that render the antibodies specific for cells expressing uPAR. The data presented herein support the application of the agents (e.g. antibodies) that disrupt uPAR binding in methods and compositions, including the diagnosis and treatment of multiple types of human diseases (e.g. cancer).

[0026] Methods of screening are also provided to identify or engineer a uPAR-binding agent that specifically inhibits/disrupt uPAR binding to its partners.

[0027] Kits containing one or more compositions of the present disclosure, as well as those with instructions for use in a method of the present disclosure also are provided.

[0028] Before the present invention and specific embodiments of the invention are described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0029] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. That the upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

[0030] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, exemplary methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0031] It must be noted that as used herein and in the appended claims, the singular forms "a", "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an antigen" includes a plurality of such antigens and reference to "the peptide" includes reference to one or more peptides and equivalents thereof known to those skilled in the art, and so forth.

[0032] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DEFINITIONS

[0033] When describing the compositions, pharmaceutical formulations containing such, and methods of producing and using such compositions, the following terms have the following meanings unless otherwise indicated. It should also be understood that any of the moieties defined forth below may be substituted with a variety of substituents, and that the respective definitions are intended to include such substituted moieties within their scope.

[0034] The terms "polypeptide" and "protein" are used interchangeably throughout the application and mean at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides, peptides, and fragments thereof. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures. Thus "amino acid", or "peptide residue", as used herein means both naturally occurring and synthetic amino acids. For example, homo-phenylalanine, citrulline and noreleucine are considered amino acids for the purposes of the invention. "Amino acid" also includes imino acid residues such as proline and hydroxyproline. The side chains may be in either the (R) or the (S) configuration. Normally, the amino acids are in the (S) or L-configuration, except for glycine. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard *in vivo* degradation. Naturally occurring amino acids may be used and the protein may be a cellular protein that is either endogenous or expressed recombinantly. In some cases, the proteins of the present invention may be synthesized using any protein *in vivo* or *in vitro* protein synthesis technique understood in the art. The terms "polypeptide" and "protein" include fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions

with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; fusion proteins with detectable fusion partners, e.g., fusion proteins including as a fusion partner a fluorescent protein, β-galactosidase, luciferase, etc.; and the like. Polypeptides may be of any size, and the term "peptide" refers to polypeptides that are 5-50 residues (e.g., 8-20 residues) in length. In some cases, proteins may be modified by covalent or non-covalent attachment of other peptide or non-peptide molecules including but not limited to one or more molecules or compositions comprised of fluorescent dyes, polyethylene glycol or other polymer, biotin, enzymes, radionuclides, MRI contrast agents, therapeutics, or chemotherapeutics as described in more detail below.

[0035] By "nucleic acid" herein is meant either DNA or RNA, or molecules which contain both deoxy- and ribonucleotides. Nucleic acid may be naturally occurring or synthetically made, and as such, includes analogs of naturally occurring polynucleotides in which one or more nucleotides are modified over naturally occurring nucleotides.

[0036] The term, "endogenous", as used herein, refers to biomolecules, such as proteins, that are naturally-occurring within an organism.

[0037] The term "carrier" as used in the context of a carrier conjugated to an antibody includes a peptide or protein carrier, a non-peptide or protein carrier (e.g. a non-peptide polymer).

[0038] The term "cell surface antigen" (or "cell surface epitope") refers to an antigen (or epitope) on surface of a cell that is extracellularly accessible during at least one cell cycle or developmental stage of the cell, including antigens that are extracellularly accessible during all stages of the cell cycle. "Extracellularly accessible" in this context refers to an antigen that can be bound by an antibody provided outside the cell without need for permeabilization of the cell membrane.

[0039] The term "chemotherapy" as used herein refers to use of an agent (e.g., drug, antibody, etc.), particularly an agent(s) that is selectively destructive to a cancerous cell, in treatment of a disease, with treatment of cancer being of particular interest.

[0040] A "cancer cell" as used herein refers to a cell exhibiting a neoplastic cellular phenotype, which may be characterized by one or more of, for example, abnormal cell growth, abnormal cellular proliferation, loss of density dependent growth inhibition, anchorage-independent growth potential, ability to promote tumor growth and/or development in an immunocompromised non-human animal model, and/or any appropriate indicator of cellular transformation. "Cancer cell" may be used interchangeably herein with "tumor cell" or "cancerous cell", and encompasses cancer cells of a solid tumor, a semi-solid tumor, a primary tumor, a metastatic tumor, and the like.

[0041] The term "conjugated" generally refers to a chemical linkage, either covalent or non-covalent, usually covalent, that proximally associates one molecule of interest with second molecule of interest.

[0042] The terms “antigen” and “epitope” are well understood in the art and refer to the portion of a macromolecule (e.g., a polypeptide) which is specifically recognized by a component of the immune system, e.g., an antibody or a T-cell antigen receptor. As used herein, the term "antigen" encompasses antigenic epitopes, e.g., fragments of an antigen which are antigenic epitopes. Epitopes can be recognized by antibodies in solution, e.g. free from other molecules. Epitopes can be recognized by T-cell antigen receptor when the epitope is associated with a class I or class II major histocompatibility complex molecule.

[0043] The terms "derivative" and “variant” refer to without limitation any compound or antibody which has a structure or sequence derived from the compounds and antibodies of the present disclosure and whose structure/sequence is sufficiently similar to those disclosed herein and based upon that similarity, would be expected, by one skilled in the art, to exhibit the same or similar activities and utilities as the claimed and/or referenced compounds or antibody. In some cases, variants may be 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more homologous or identical at least one compound or antibody of the present disclosure.

[0044] The term "effective amount" of a composition as provided herein is intended to mean a non-lethal but sufficient amount of the composition to provide the desired utility. For instance, for eliciting a favorable response in a subject to treat a disorder or infection, the effective amount is the amount which eliminates or diminishes the symptoms associated with the disorder, e.g., so as to provide for control of cancer metastasis, to eliminate cancer cells, decrease bacterial or viral infection. As will be pointed out below, the exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition or disease that is being treated, the particular composition used, its mode of administration, and the like. Thus, it is not possible to specify an exact "effective amount." However, an appropriate effective amount may be determined by one of ordinary skill in the art using only routine experimentation.

[0045] The term “immunotherapy” refers to treatment of disease (e.g., viral or bacterial infection, or cancer) by modulating an immune response to a disease antigen. In the context of the present application, immunotherapy refers to providing an antibacterial and/or anti-cancer immune response in a subject by administration of an antibody (e.g., a monoclonal antibody).

[0046] The term “in combination with” as used herein refers to uses where, for example, a first therapy is administered during the entire course of administration of a second therapy; where the first therapy is administered for a period of time that is overlapping with the administration of the second therapy, e.g. where administration of the first therapy begins before the administration of the second therapy and the administration of the first therapy ends before the administration of the second therapy ends; where the administration of the second therapy begins before the administration of the first therapy

and the administration of the second therapy ends before the administration of the first therapy ends; where the administration of the first therapy begins before administration of the second therapy begins and the administration of the second therapy ends before the administration of the first therapy ends; where the administration of the second therapy begins before administration of the first therapy begins and the administration of the first therapy ends before the administration of the second therapy ends. As such, "in combination" can also refer to regimen involving administration of two or more therapies. "In combination with" as used herein also refers to administration of two or more therapies which may be administered in the same or different formulations, by the same or different routes, and in the same or different dosage form type.

[0047] The term "isolated" is intended to mean that a compound is separated from all or some of the components that accompany it in nature. "Isolated" also refers to the state of a compound separated from all or some of the components that accompany it during manufacture (e.g., chemical synthesis, recombinant expression, culture medium, and the like).

[0048] The term "antibody" refers to a polypeptide composed of complementarity determining regions (CDRs) that confer specific binding affinity of the polypeptide for an antigen. "Antibody" encompasses polyclonal and monoclonal antibody preparations where the antibody may be of any class of interest (e.g., IgM, IgG, and subclasses thereof), as well as preparations including hybrid antibodies, altered antibodies, covalently modified antibodies, $F(ab')_2$ fragments, F(ab) molecules, Fv fragments, single chain fragment variable displayed on phage (scFv), single chain antibodies (e.g. single-chain Fab), single domain antibodies, affibodies, diabodies, chimeric antibodies, human antibodies, humanized antibodies, and functional fragments thereof which exhibit immunological binding properties of the parent antibody molecule. The antibodies described herein may be detectably labeled, *e.g.*, with a radioisotope, an enzyme which generates a detectable product, a fluorescent protein, and the like. The antibodies may be further conjugated to other moieties, such as a cytotoxic molecule or other molecule (e.g., to provide for delivery of an anti-cancer drug to a cancer cell), members of specific binding pairs, *e.g.*, biotin (member of biotin-avidin specific binding pair), and the like. The antibodies may also be bound to a support (e.g., a solid support), such as a polystyrene plate or bead, test strip, and the like.

[0049] Antibodies can include the kappa and lambda light chains and the alpha, gamma (IgG_1, IgG_2, IgG_3, IgG_4), delta, epsilon and mu heavy chains or equivalents in other species. Full-length immunoglobulin "light chains" (usually of about 25 kDa or about 214 amino acids) comprise a variable region of about 110 amino acids at the NH_2-terminus and a kappa or lambda constant region at the COOH-terminus. Full-length immunoglobulin "heavy chains" (of about 50 kDa or about 446 amino acids), similarly comprise a variable region (of about 116 amino acids) and one of the aforementioned heavy chain constant regions, e.g., gamma (of about 330 amino acids).

[0050] Light or heavy chain variable regions are generally composed of a "framework" region (FR) interrupted by three hypervariable regions, also called CDRs. The extent of the framework region and CDRs have been precisely defined (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services, 1991, and Lefranc et al. IMGT, the international ImMunoGeneTics information system®. Nucl. Acids Res., 2005, 33, D593-D597)). A detailed discussion of the Kabat numbering system is provided on the World Wide Web at kabatdatabase.com/index.html. CDR and framework sequences may also be defined by the Chothia numbering system. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs. The CDRs are primarily responsible for binding to an epitope of an antigen.

[0051] The term "monoclonal antibody" refers to an antibody composition having a homogeneous antibody population. The term is not limited by the manner in which it is made. The term encompasses whole immunoglobulin molecules, as well as Fab molecules, F(ab')2 fragments, Fv fragments, single chain fragment variable displayed on phage (scFv), fusion proteins comprising an antigen-binding portion of an antibody and a non-antibody protein, and other molecules that exhibit binding properties of the parent monoclonal antibody molecule. Methods of making polyclonal and monoclonal antibodies are known in the art and described more fully below.

[0052] The term "specific binding of an antibody" or "antigen-specific antibody" in the context of a characteristics of an antibody refers to the ability of an antibody to preferentially bind to a particular antigen that is present in a homogeneous mixture of different antigens. In certain embodiments, a specific binding interaction will discriminate between desirable and undesirable antigens (or "target" and "non-target" antigens) in a sample, in some embodiments more than about 10 to 100-fold or more (e.g., more than about 1000- or 10,000-fold). The affinity between an antibody and antigen when they are specifically bound in an antibody-antigen complex can be characterized by a K_D (dissociation constant) of less than 10^{-6} M, less than 10^{-7} M, less than 10^{-8} M, less than 10^{-9} M, less than 10^{-9} M, less than 10^{-11} M, or less than about 10^{12} M or less.

[0053] "Conservative amino acid substitution" refers to a substitution of one amino acid residue for another sharing chemical and physical properties of the amino acid side chain (e.g., charge, size, hydrophobicity/hydrophilicity). "Conservative substitutions" are intended to include but are not limited to substitution within the following groups of amino acid residues: gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr. Conservative amino acid substitutions in the context of an antibody disclosed herein are selected so as to preserve the interaction between the antibody and the protease of

interest. Other conservative substitutions that can preserve size, chemical property, and/or shape includes val, thr; asp, asn, glu, gln; leu, phe, tyr, trp; lys, leu; trp, phe, and tyr; and ala, val, tyr.

[0054] The term "pharmaceutically acceptable" refers to a material that is not biologically or otherwise undesirable, i.e., the material is of a medically acceptable quality and composition that may be administered to an individual along with the selected active pharmaceutical ingredient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

[0055] The term "pharmaceutically acceptable excipient" as used herein refers to any suitable substance which provides a pharmaceutically acceptable vehicle for administration of a compound(s) of interest to a subject. "Pharmaceutically acceptable excipient" can encompass substances referred to as pharmaceutically acceptable diluents, pharmaceutically acceptable additives and pharmaceutically acceptable carriers.

[0056] The term "purified" is intended to mean a compound of interest has been separated from components that accompany it in nature and provided in an enriched form. "Purified" also refers to a compound of interest separated from components that can accompany it during manufacture (e.g., in chemical synthesis, recombinant expression, culture medium, and the like) and provided in an enriched form. Typically, a compound is substantially pure when it is at least 50% to 60%, by weight, free from organic molecules with which it is naturally associated or with which it is associated during manufacture. Generally, the preparation is at least 75%, more usually at least 90%, and generally at least 99%, by weight, of the compound of interest. A substantially pure compound can be obtained, for example, by extraction from a natural source (e.g., bacteria), by chemically synthesizing a compound, or by a combination of purification and chemical modification. A substantially pure compound can also be obtained by, for example, enriching a sample having a compound that binds an antibody of interest. Purity can be measured by any appropriate method, e.g., chromatography, mass spectroscopy, HPLC analysis, polyacrylamide gel electrophoresis, etc.

[0057] The term "subject" is intended to cover humans, mammals and other animals which contain uPAR in any fashion. The terms "subject," "host," "patient," and "individual" are used interchangeably herein to refer to any mammalian subject for whom diagnosis or therapy is desired, particularly humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and so on.

[0058] In the context of cancer therapies and diagnostics described herein, "subject" or "patient" is used interchangeably herein to refer to a subject having, suspected of having, or at risk of developing a tumor. In some cases, the cancer is one associated with cancerous cells expressing an active and/or

dysregulated uPAR. Samples obtained from such subject are likewise suitable for use in the methods of the present disclosure.

[0059] As used herein, the terms "determining," "measuring," and "assessing," and "assaying" are used interchangeably and include both quantitative and qualitative determinations.

[0060] It is further noted that the claims may be drafted to exclude any optional or alternative element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely", "only" and the like in connection with the recitation of claim elements, or the use of a "negative" limitation.

[0061] All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed. To the extent a definition of a term set out in a document incorporated herein by reference conflicts with the definition of a term explicitly defined herein, the definition set out herein controls.

[0062] Examples of methods and compositions employable therein are described first in greater detail, followed by a review of the various specific compositions, formulations, kits and the like that may find use in the methods of the present disclosure, as well as a discussion of representative applications in which the methods and compositions of the present disclosure find use.

UPAR-BINDING AGENTS

[0063] The present disclosure provides uPAR-binding agents (e.g. anti-uPAR antibodies, also referred to as "uPAR antibodies"). Examples of uPAR-binding agents include but are not limited to aptamers (nucleic acids and/or peptides), antibodies, small molecules, and other biomolecules. Where the agent is an antibody, the antibody includes a whole antibody (e.g. IgG), an antigen-binding fragment thereof, single-chain Fabs, single chain Fv (e.g. diabodies or V_HH), Fab'2, minibody, and synthetic uPAR antibody that comprise portions of an antibody. uPAR, the target of the subject agents, is also known as urokinase plasminogen activator receptor, urokinase receptor, uPA receptor, or CD87 (Cluster of Differentiation 87). UPAR is composed of three different domains of the Ly-6/uPAR/alpha-neurotoxin family. All three domains are involved in high affinity binding of the primary ligand, urokinase. Besides

the primary ligand urokinase, uPAR interacts with several other proteins, including vitronectin, the uPAR associated protein (uPARAP) and the integrin family of membrane proteins.

[0064] As used herein, "uPAR" refers to urokinase plasminogen activator receptor, including those whose amino acid sequences that are at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% identical to the amino acid sequence of a naturally-occurring allelic variant and/or isoform thereof. Variants can also include mutations whose expression is associated with cancer. Many mammalian uPARs and their corresponding isoforms are known in the art. For example, the amino acid sequence of the longest human isoform is available as GenBank Accession No. NP_002650.1 and UniProt Accession No. Q03405.

[0065] Binding of ligands and/or integrins to uPAR is involved in signaling that can lead to proliferation. Certain signaling cascades that are initiated by activated uPAR mediate the regulation of cellular shape, adhesion, and mobility, and thus play a role in cell invasion. Accordingly, preventing ligands such as uPA and/or integrins (e.g. β1 integrins, such as α5β1 or α3β1) from binding to uPAR can reduce the effects of proliferative signaling cascades and those signals leading to angiogenesis. A subject binding agent can exhibit features that allow not only competitive binding with proteins (e.g. integrins and/or ligands) that bind to uPAR but also potent inhibition of uPAR-mediated cell signaling. UPAR-binding agents of the present disclosure can find use in a variety of applications, including use in various methods of treating a host suffering from a disease or condition associated with uPAR signaling, as well as in diagnosis of various diseases and conditions associated with uPAR expression. For example, a subject agent, such as an antibody, is specific for the integrin-binding site on uPAR and may be used to inhibit the proliferation or metastasis of cancer cells. More uses of a subject agent will be described later.

[0066] UPAR-expressing cells can serve as targets for the uPAR antibodies of the present disclosure. For example, uPAR-binding agents (e.g. antibodies) of the present disclosure can be used to bind human cells that express surface exposed uPAR. The binding may be specific so that cells that express uPAR are labeled using the subject antibody but cells that do not express uPAR are not. The uPAR expressed in cells may be endogenous, recombinants, naturally-occurring variants and isoforms, and/or a homolog of human uPAR (murine, rat, bovine, primates, etc.). Particularly, uPAR molecules that are expressed by cancer cells can be bound by the subject antibody. Such antibody may be useful in specifically labeling cancer cells (e.g. uPAR-positive cancer) for use in a diagnostic method, described in more detail below.

[0067] As a reference, an amino acid sequence of uPAR is provided below and can also be found in RSCB Protein Data Bank identified as 3BT1. Numbering system used in the present disclosure to refer to an amino acid residue position in uPAR would be in the context of the following amino acid sequence: LRCMQCKTNGDCRVEECALGQDLCRTTIVRLWEEGEELELVEKSCTHSEKTNRTLSYRTGLKITS

LTEVVCGLDLCNQGNSGRAVTYSRSRYLECISCGSSDMSCERGRHQSLQCRSPEEQCLDVVTHW IQEGEEGRPKDDRHLRGCGYLPGCPGSNGFHNNDTFHFLKCCNTTKCNEGPILELENLPQNGRQC YSCKGNSTHGCSSEETFLIDCRGPMNQCLVATGTHEPKNQSYMVRGCATASMCQHAHLGDAFS MNHIDVSCCTKSGCNHPDLDVQYR (SEQ ID NO:1)

[0068] The present disclosure provides uPAR agents (e.g. antibodies) that compete with and/or disrupt integrin binding to uPAR. Integrins encompass β1 integrins, such as α5β1 or α3β1. The agents thus find use in inhibiting integrin binding to cells (e.g., human cells expressing uPAR). For example, antibodies of clone 3C6 inhibit α5β1 and α3β1 integrin binding to uPAR. This inhibition may be due to the binding of the antibody to an epitope involved in the interaction between integrin and uPAR (e.g. integrin binding site) or to an epitope outside of the binding site so that uPAR is modified in a way to decrease uPAR's affinity to integrin (e.g. allosteric site). As such, a uPAR antibody of the present disclosure can compete with an antibody that binds to an epitope located in the integrin-binding site (e.g. α5β1 and/or α3β1 integrin binding site). One or more epitopes of an antibody of the present disclosure can be found in domain III, which corresponds to the amino acid sequence of uPAR from about amino acid residue position 192 to about position 275. Other epitopes outside of domain III may also contribute to the binding affinity of integrin or an antibody of the present disclosure to uPAR.

[0069] Antibodies of the present disclosure include those that can compete with an antibody that binds to an epitope including one or more of the following residues: D262, E208, E230, H249, and S156, all of which are located in domain III except for S156, which is located in domain II. For example, an antibody can bind to an epitope or compete with an antibody that binds to an epitope including residue E208. In another example, the epitope can include residue H249 and D262. Alternatively, the epitope includes E230 or S156. See example 16 and Figure 16 for detail.

[0070] The present disclosure also provides agents that compete with and/or inhibit uPA binding to uPAR. Urokinase-type plasminogen activator (uPA, also known as urokinase), an endogenous ligand of uPAR, is a member of a family of enzymes that exhibit protease activity described as EC 3.4.21.73 according to the IUMBM enzyme nomenclature. UPAR antibodies can decrease binding of uPA to uPAR by competitive inhibition, where the antibody binds to the same site of uPAR as uPA binds or at a different site outside of the uPA binding site (e.g. allosteric site), or by noncompetitive inhibition. Examples of antibodies that can inhibit uPA binding to uPAR include antibodies from clone 2E9 and antibodies from clone 2G10.

[0071] As such, a uPAR antibody of the present disclosure can compete with an antibody that binds to an epitope located in the uPA-binding site. One or more epitopes of a uPA-binding site can be found in domain I and/or domain II of uPAR. Domain I corresponds to an amino acid sequence of uPAR

from about amino acid residue position 1 to about position 80. Domain II corresponds to an amino acid sequence of uPAR from about amino acid residue position 91 to about position 191.

[0072] As noted above, antibody affinity for uPAR may be described by the dissociation constant, K_D. Antibodies of the present disclosure, for example, include those having a K_D for uPAR of less than about 1000nM, less than about 500nM, less than about 300nM, less than about 200nM, less than about 100nM, less than about 80 nM, less than about 60 nM, less than about 55 nM, less than about 50 nM, less than about 40 nM, less than about 30 nM, less than about 25 nM, less than about 20 nM, less than about 10 nM, less than about 5nM, less than about 2nM, less than about 1nM, less than about 750pM, less than about 500pM, less than about 300pM, less than about 200pM, less than about 100pM, or less than about 50pM. For example, the divalent IgG antibody derived from clone 2G10 has a K_D of about 40.5 nM. See Figure 4 for K_D values for other examples of antibodies of the present disclosure.

[0073] UPAR antibodies of the present disclosure include antibodies that facilitate a decrease in cellular signaling associated with uPAR ligand or integrin binding. Such antibodies can find use in, for example, decreasing cellular proliferation by binding to uPAR. Cellular signaling effects can be assessed by modulation of (e.g., a decrease in) phosphorylation levels of kinases associated with uPAR signaling, such as extracellular signal-regulated kinases (ERKs), mitogen activated kinases (MAPK), and/or microtubule-associated protein kinase. For example, antibodies of the present disclosure include those that can inhibit uPAR-dependent ERK phosphorylation and in turn, inhibit ERK activation. Antibodies of the present disclosure include those that can inhibit fibronectin-dependent ERK phosphorylation. Antibodies of the present disclosure include those that can facilitate inhibition of proliferation of cells by binding to cell-surface uPAR.

[0074] Antibodies of the present disclosure include those that can facilitate a decrease in invasion of uPAR-expressing cells into extracellular matrix and/or facilitate a decrease in adhesion of uPAR-expressing cells (e.g. fibronectin- or vitronecting-dependent adhesion). The ability of cells to invade is a phenotype correlated with the metastatic potential of cancer cells. For example, antibodies in Figure 1 (e.g. antibodies from clones 2E9, 2G10, and 3C6) facilitate inhibition of cancer cell invasion. Antibodies from clone 3C6 also facilitate a decrease in fibronectin-or vitronectin-dependent cell adhesion. Antibodies of the present disclosure include those that can find use in reducing migration of uPAR-expressing cancer cells.

Amino acid sequences

[0075] UPAR binding agents of the present disclosure include antibodies that bind an epitope in the ligand-binding region and/or integrin-binding region of uPAR. Several examples of a subject antibody are presented in Figure 1 and described below.

[0076] Antibodies of the present disclosure include antibodies having one, two, or three heavy chain CDRs about 85%, 90%, 95%, 98%, 99%, or 100% identical to V_H CDR1, V_H CDR2, or V_H CDR3, designated as H1, H2, and H3, respectively in Figure 1. Antibodies of the present disclosure include antibodies having one, two, or three light chain CDRs about 85%, 90%, 95%, 98%, 99%, or 100% identical to V_L CDR1, V_L CDR2, or V_L CDR3 designated as L1, L2, L3, respectively in Figure 1. All CDRs may be derived from the same antibody or be independently selected from different antibodies listed in Figure 1 and/or described below.

[0077] The V_H and V_L CDRs are separated by framework regions (FR). Amino acid sequences for FRs are exemplified by the FRs of the uPAR antibodies disclosed herein. uPAR antibodies include those containing FRs or other linkers having amino acid sequence that are different from the framework regions disclosed herein. Conservative amino acid substitutions may also be contemplated for any amino acid residue of CDR, framework regions, or linker regions. Other substitutions may be contemplated based on alignments provided in Figure 1, Panel B.

[0078] Optional linkers within a heavy chain or light chain polypeptide of an antibody may comprise amino acid residues or non-peptide polymers. The linkers may have a length of from about 1 to about 100 monomers, e.g., from about 2 to about 5, from about 7 to about 10, from about 10 to about 15, from about 15 to about 20, from about 20 to about 25, from about 25 to about 30, from about 30 to about 50, from about 50 to about 75, or from about 75 to about 100 monomers.

[0079] Examples of uPAR antibodies of the present disclosure include an antibody comprising a light chain polypeptide having an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or 100% amino acid sequence identity to a contiguous stretch of the amino acid sequence set forth as 3C6 V_L.

[0080] Examples of uPAR antibodies of the present disclosure include an antibody comprising a light chain polypeptide having an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or 100% amino acid sequence identity to a contiguous stretch of the amino acid sequence set forth as 2E9 V_L.

[0081] Examples of uPAR antibodies of the present disclosure include an antibody comprising a light chain polypeptide having an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or 100% amino acid sequence identity to a contiguous stretch of the amino acid sequence set forth as 2G10 V_L.

[0082] Examples of uPAR antibodies of the present disclosure include an antibody comprising a heavy chain polypeptide having an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or 100% amino acid sequence identity to a contiguous stretch of the amino acid sequence set forth as 3C6 V_H.

[0083] Examples of uPAR antibodies of the present disclosure include an antibody comprising a heavy chain polypeptide having an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or 100% amino acid sequence identity to a contiguous stretch of the amino acid sequence set forth as 2E9 V_H.

[0084] Examples of uPAR antibodies of the present disclosure include an antibody comprising a heavy chain polypeptide having an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, or 100% amino acid sequence identity to a contiguous stretch of the amino acid sequence set forth as 2G10 V_H.

[0085] Examples of uPAR antibodies of the present disclosure include an antibody comprising a light or a heavy chain polypeptide sequence as depicted in any of the antibodies listed in Figure 1. Such antibodies can also include any CDRs and framework regions (FRs) as the antibodies listed in Figure 1.

[0086] Examples of uPAR antibodies of the present disclosure include an antibody comprising a light chain polypeptide comprising one or more CDRs (CDR1, CDR2 or CDR3) of the variable region of a light chain polypeptide in Figure 1 and a heavy chain polypeptide comprising one or more CDRs (CDR1, CDR2, or CDR3) of the variable region of any heavy chain polypeptide in Figure 1. One or more amino acid residues in one or more of the CDRs set forth above may be deleted, inserted, or substituted in the subject antibody. Conservative substitutions may also be present.

[0087] UPAR antibodies of the present disclosure may be of any subclass (e.g. IgG, IgE, IgD, IgA, or IgM). The antibody may be fully human or may be a humanized monoclonal antibody. Chimeric antibodies composed of human and non-human amino acid sequences are also contemplated by the present disclosure. Antibodies of the present disclosure encompass antibodies and antibody fragments that are capable of exhibiting immunological binding properties of the antibodies described herein, e.g., antibodies that compete for binding of an epitope bound by any of the antibodies exemplified herein. Example of antibody fragments include, but are not limited to, Fab, Fab' and $F(ab')_2$, Fd, single-chain Fvs (scFv), single-chain immunoglobulins (e.g., wherein a heavy chain, or portion thereof, and light chain, or portion thereof, are fused), disulfide-linked Fvs (sdFv), diabodies, triabodies, tetrabodies, scFv, affibodies, minibodies, Fab minibodies, and dimeric scFv and any other fragments comprising a V_L and a V_H domain in a conformation such that a specific antigen binding region is formed. Antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entire or partial of the following: a heavy chain constant domain, or portion thereof, e.g., a CH1, CH2, CH3, transmembrane, and/or cytoplasmic domain, on the heavy chain, and a light chain constant domain, e.g., a C_{kappa} or C_{lambda} domain, or portion thereof on the light chain. Also included in the present disclosure are any combinations of variable region(s) and CH1, CH2, CH3, C_{kappa}, C_{lambda}, transmembrane

and cytoplasmic domains. One or more fragments of the antibody may also be provided as cyclized forms.

[0088] The disclosure also provides agents (e.g. antibodies) that are modified by conjugation to a moiety that can provide for a desired characteristic (e.g., increase in serum half-life, anti-cancer activity, etc.). Such antibody conjugates are described in more detail below.

Amino acid and nucleic acid sequences

[0089] UPAR-binding agents can comprise a contiguous amino acid sequence that is at least 80% identical to (e.g., at least 85%, at least 90%, at least 95%, at least 98%, or 100%) to a contiguous sequence of any sequences listed in Figure 1 and below.

[0090] 2B3 V_H:

QLQLQESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARPYSSSWYSVGNYGIDVWGQGTTVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:2)

[0091] 2B3 V_L:

QAVLTQPSSLSASPGASASLTCTLRRDIDIGTARIYWYQQKPGSPPQYLLNYKSDLYTEKASGVPS
RFSGSKDASANAGILLISGLQSEDEADYYCLIWHNNAWVFGGGTKLTVLGQPKAAPSVTLFPPSS
EELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQW
KSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:3)

[0092] 2B7 V_H:

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDY
AESVKSRIVINVDTSKNQFSLQLNSVTPEDTAAYYCARDPGGPLDDSFDIWGQGTMVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:4)

[0093] 2B7 V_L:

QSVVTQPPSVSGAPGQRVIISCTGSSSNIGAGFDVHWYQQLPGTVPKLLIYGNTNRPSGVPDRFSG
SKAGTSASLAITGLQAEDEADYYCQAYDDSLQGYVFGTGTKLTVVGQPKANPTVTLFPPSSEEL
QANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSH
RSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:5)

[0094] 2B8 V_H:

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSKSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDY
AVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCARDPGGPLDDSFDIWGQGTMVTVSSASTK

GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICYVNHKPSNTKVDKKVEPKSC (SEQ ID NO:6)

[0095] 2B8 V_L:

LDVVMTQSPLSLPVTPGEPASISCRSSQSLLRSNGYNYLDWYLQKPGQSPQLLIYLGSTRASGVPD
RFSGSGSGTDFTLKISRVEAEDVGVYYCMQAFQTPLTFGGGTKMEIKRTVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:7)

[0096] 2B11 V_H:

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDY
AVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCARDSGLGSDYFDYWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:8)

[0097] 2B11 V_L:

LDIQMTQSPPSLSASVGDRVTITCQAPHDIKNNLNWYQQKPGKAPKLLIFDASNLETGVPSRFSGS
GSGTNFVLTISSLQPEDIATYYCQQFDDLPLTFGGGTKVDMKRTVAAPSVFIFPPSDEQLESGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC (SEQ ID NO:9)

[0098] 2D5 V_H:

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDY
AVSVKSRIIINPDTSKNQFSLQLNSVTPEDTAVYYCARDPGGPLDDSFDIWGQGTMVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:10)

[0099] 2D5 V_L:

LDIQLTQSPSTLSASVGDRVTITCRASQTISSSLAWYQQKPGKAPNLLIYKASTLEGGVPSRFSGSG
SGTEFTLTISSLQPEDFATYYCQQSYTTPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC (SEQ ID NO:11)

[00100] 2E7 V_H:

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDY
AVSVKSRIIINPDTSKNQFSLQLNSVTPEDTAVYYCARDPGGPLDDSFDIWGQGTMVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:12)

[00101] 2E7 V_L:

LDIQLTQSPPSLSASVGDRVTITCQAPHDIKNNLNWYQQKPGKAPKLLIFDASNLETGVPSRFSGS

GSGTNFVLTISSLQPEDIATYYCQQFHDLPLTFGGGTKVDMKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)

[00102] 2E9 V_H:

QVQLQESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDEDYDYVWGSYRQYPSRYWGQGTLVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:14)

[00103] 2E9 V_L:

QSVLTQPPSVSVSPGQTASITCSGDNLGYKYASWYQQKPGQSPVLIIYQDKKRPSGIPERFSGSNS
GNTATLTISGTQAMDEADYYCQAWDSSTSVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANK
ATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSC
QVTHEGSTVEKTVAPTECS (SEQ ID NO:15)

[00104] 2G10 V_H:

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDY
AVSVKSRIIINPDTSKNQFSLQLNSVTPEDTAVYYCARDPGGPLDDSFDIWGQGTMVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:16)

[00105] 2G10 V_L:

LDVVMTQSPLSLPVTPGEPASISCRSSQSLLRSNGYNYLDWYLQKPGQSPQLLIYLGSIRASGVPD
RFSGSGSGTDFTLRISRVEAEDVGVYYCMQALQTPFTFGQGTKLEIKRTVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:17)

[00106] 2G12 V_H:

EVQLVDTGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDWGRNIAVAGTLDYWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:18)

[00107] 2G12 V_L:

LSYELTQPPSVSVSPGQTASITCSGDKLGQKYVSWYQQRPGQSPLLVIFQDDKRPSGIPERISGSNS
GHTATLTISATQAMDEAEYFCQAWDSNTAPYVFGTGTQVTVLSQPKANPTVTLFPPSSEELQAN
KATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYS
CQVTHEGSTVEKTVAPTECS (SEQ ID NO:19)

[00108] 3C6 V_H:

QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLK SRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGRRFGDFDYWGQGTLVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:20)

[00109] 3C6 V_L:

QPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPPGIPERFSGSN SGNTATLTISRVEAGDEADYYCQVWDSSSDHSPFGTGTKVTVLGQPKANPTVTLFPPSSEELQAN KATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYS CQVTHEGSTVEKTVAPTECS (SEQ ID NO:21)

[00110] 3C7 V_H:

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDY AVSVKSRIIINPDTSKNQFSLQLNSVTPEDTAVYYCARDPGGPLDDSFDIWGQGTMVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:22)

[00111] 3C7 V_L:

LDIQLTQSPPSLSASVGDRVTITCQAPHDIKNNLNWYQQKPGKAPKLLIFDASNLETGVPSRFSGS GSGTNFVLTISSLQPEDIATYYCQQFDDLPLTFGGGTKVDMKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHEVYAC EVTHQGLSSPVTKSFNRGEC (SEQ ID NO:23)

[00112] 3D6 V_H:

QVQLQQSGPGLVNPSQTLSVTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDY AVSVKSRITIKPDTSKNQFSLQLNSVTPDDTAVYYCARDPGGSLDDSFDIWGQGTTVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:24)

[00113] 3D6 V_L:

LDIQMTQSPPSLSASVGDRVTITCQAPHDIKNNLNWYQQKPGKAPKLLIFDASNLETGVPSRFSGS GSGTNFVLTISSLQPEDIATYYCQQFDDLPLTFGGGTKVDMKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC (SEQ ID NO:25)

[00114] 3H7 V_H:

QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDY AVSVKSRITIKPDTSKNQFSLQLNSVTPDDTAVYYCARDPGGSLDDSFDIWGQGTMVTVSSASTK

GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:26)

[00115] 3H7 V_L:
LDVVMTQSPLSLPVTPGEPASISCRSSQSLLRSNGYNYLDWYLQKPGQSPQLLIYLGSTRASGVPD
RFSGSGSGTDFTLKISRVEAEDVGVYYCMQAFQTPLTFGGGTKMEIKRTVAAPSVFIFPPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:27)

[00116] 4B6 V_H:
QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDY
AVSVKSRIIINPDTSKNQFSLQLNSVTPEDTAVYYCARDPGGPLDDSYDIWGQGTMVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:28)

[00117] 4B6 V_L:
LEIVLTQSPLSLPVTPGEPASISCRSSQSLLRSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDR
FSGSGSGTDFTLKISRVEAEDVGVYYCMQAFQTPLTFGGGTKMEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:29)

[00118] 4C1 V_H:
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSISASGGSTDYADSV
KGRFTISRDNSKNTLYLQMSSLRAEDTAVYYCVKERPDYDFWSAFDPWGQGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:30)

[00119] 4C1 V_L:
LDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVP
DRFSGSGSGTDFTLKISRVEAEDVGVYYCMQGTHWPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK
HKLYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:31)

[00120] Where the agent is an IgG antibody, the V_H of the IgG may contain an additional Fc region at the C-terminus. The Fc region may comprise a contiguous amino acid sequence that is at least 80% identical to (e.g., at least 85%, at least 90%, at least 95%, at least 98%, or 100%) to a contiguous sequence of
LLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY

KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:32).

[00121] Certain CDRs are listed separately in the table below. Other CDRs can be found in Figure 1, panel B.

[00122] Table 1 Complementarity determining regions of 3C6 and 2G10 according to the Kabat database.

Light Chain	3C6	2G10
CDR1	GGNNIGSKSVH (SEQ ID NO:33)	RSSQSLLRSNGYNYLD (SEQ ID NO:34)
CDR2	DDSDRPP (SEQ ID NO:35)	LGSIRAS (SEQ ID NO:36)
CDR3	QVWDSSSDHSP (SEQ ID NO:37)	MQALQTPFT (SEQ ID NO:38)
Heavy Chain		
CDR1	GGSFSGYYWSW (SEQ ID NO:39)	GDSVSSNSAAWN (SEQ ID NO:40)
CDR2	EINHSGSTNYNPSLKS (SEQ ID NO:41)	RTYYRSKWYNDYAVSVKS (SEQ ID NO:42)
CDR3	GRRFGDFDY (SEQ ID NO:43)	DPGGPLDDSFDI (SEQ ID NO:44)

Recombinant antibody

[00123] The agents of the present disclosure may be an antibody produced by recombinant methods. Such antibodies can be produced by expression of a polynucleotide having a nucleotide sequence encoding a polypeptide that is at least 80% identical to (e.g., at least 85%, at least 90%, at least 95%, at least 98%) to a contiguous sequence of any antibody listed in Figure 1 and/or of any sequence listed above. The percent identity of nucleic acids is based on the shorter of the sequences compared. Well known programs such as BLASTN (2.0.8) (Altschul et al. (1997) *Nucl. Acids. Res.* 25:3389-3402) using default parameters and no filter may be employed to make a sequence comparison. Examples of nucleic acids encoding the antibodies of the present disclosure are discussed later below.

[00124] Methods for producing recombinant antibodies are known in the art. For example, the nucleic acids encoding the antibody, or at least a CDR of a heavy chain polypeptide or at least a CDR of a light chain polypeptide, are introduced directly into a host cell, and the cell incubated under conditions sufficient to induce expression of the encoded antibody. The recombinant antibody may be glycosylated by an endogenous glycosyl-transferase in the host cells, unglycosylated, or may have an altered glycosylation pattern.

[00125] Recombinant antibodies include chimeric antibodies. Chimeric antibodies are immunoglobulin molecules comprising human and non-human portions. More specifically, the antigen

combining region (or variable region) of a humanized chimeric antibody is derived from a non-human source (e.g. murine), and the constant region of the chimeric antibody (which confers biological effector function to the immunoglobulin) is derived from a human source. The chimeric antibody can have the antigen binding specificity of the non-human antibody molecule and the effector function conferred by the human antibody molecule. A large number of methods of generating chimeric antibodies are well known to those of skill in the art. An alternative approach is the generation of humanized antibodies by linking the CDR regions of non-human antibodies to human constant regions by recombinant DNA techniques. See Queen et al., *Proc. Natl. Acad. Sci. USA* 86: 10029-10033 (1989).

Human antibodies

[00126] The uPAR-binding agent may be a fully human antibody. Human antibodies are primarily composed of characteristically human polypeptide sequences. A subject human antibody can be produced by a wide variety of methods (see, e.g., Larrick et al., U.S. Patent No. 5,001,065). Human antibodies may be derived from a fully human Fab phage display library, as described in de Haard et al. (1999) *Journal of Biological Chemistry. 274, 18218-18230*

[00127] Human antibodies can also be produced initially in trioma cells (descended from three cells, two human and one mouse). Genes encoding the antibodies are then cloned and expressed in other cells, particularly non-human mammalian cells. The general approach for producing human antibodies by trioma technology has been described by Ostberg et al. *Hybridoma* 1983, 2: 361-367, Ostberg, U.S. Patent No. 4,634,664, and Engelman et al., U.S. Patent No. 4,634,666. Triomas have been found to produce antibody more stably than ordinary hybridomas made from human cells.
Accordingly, the present disclosure contemplates a DNA molecule comprising a nucleic acid sequence encoding an antibody that binds to uPAR (e.g. a nucleic acid encoding 2E9, 2G10, or 3C6). Nucleic acid sequences will be described later below.

Conjugates

[00128] uPAR-binding agents of the present disclosure can be modified by chemical conjugation to a moiety of interest. For example, an agent may be conjugated to a second molecule of a different type (e.g. nucleic acid to a non-nucleic acid, or a peptide to a non-peptide). Where the agent is an antibody, the antibody conjugated to a second molecule is referred to as an "antibody conjugate." A subject antibody conjugate may be useful for modifying the growth of cells, particularly cancer cells. The compositions containing the agents can encompass aggregates of conjugates, as they are readily taken up by cells.

[00129] Conjugated agents retain a desired activity, while exploiting properties of the second molecule of the conjugate to impart an additional desired characteristic. For example, a subject agent (e.g. antibody) can be conjugated to a second molecule that aids in solubility, storage or other handling properties, cell permeability, half-life, controls release and/or distribution such as by targeting a particular

cell (e.g., neurons, leucocytes etc.) or cellular location (e.g., lysosome, endosome, mitochondria etc.), tissue or other bodily location (e.g., blood, neural tissue, particular organs etc.). Other examples include the conjugation of a dye, fluorophore or other detectable labels or reporter molecules for assays, tracking and the like. More specifically, a subject antibody can be conjugated to a second molecule such as a peptide, polypeptide, dye, fluorophore, luciferase, nucleic acid, carbohydrate, lipid and the like, such as the attachment of a lipid moiety, including N-fatty acyl groups such as N-lauroyl, N-oleoyl, fatty amines such as dodecyl amine, oleoyl amine, and the like.

[00130] The present disclosure further provides a conjugated agent that comprises a moiety that modifies cellular uptake relative to unconjugated material. The conjugate may exhibit increased cellular uptake relative to unconjugated material. In alternative embodiments, the conjugate exhibits decreased cellular uptake relative to unconjugated material. In this aspect, the efficiency of cellular uptake can be increased or decreased by linking to small organic or inorganic molecules, polymers, peptides or proteins that facilitate, or inhibit endocytosis. For example, a given antibody can be linked to a ligand for a target receptor or large molecule that is more easily engulfed by endocytotic mechanisms, such as another antibody. The antibody or other ligand can then be internalized by endocytosis and the payload released by acid hydrolysis or enzymatic activity when the endocytotic vesicle fuses with lysosomes. As such, the conjugate may be one that increases endocytosis relative to unconjugated agent. To decrease cellular uptake, the conjugate can include a ligand that retains the antibody on the surface of a cell, which can be useful as a control for cellular uptake, or in some instances decrease uptake in one cell type while increasing it in others.

[00131] Other features of a conjugated agent may include one where the conjugate reduces toxicity relative to unconjugated agent. Another feature is that the conjugate may target a cancer cell more efficiently than an unconjugated material. Additional examples include an antibody of the present disclosure conjugated with one or more molecules that complement, potentiate, enhance or can otherwise operate synergistically in connection with the antibody of the present disclosure. For instance, where the agent is an antibody, the antibody can optionally have attached an anti-cancer drug for delivery to a site of a cancer or bacterial cell to further facilitate cell killing or clearance, e.g., an anti-proliferation moiety (e.g., VEGF antagonist, e.g., an anti-VEGF antibody or aptamer), a toxin (e.g., an anti-cancer toxin, e.g., ricin, Pseudomonas exotoxin A, and the like), radionuclide (e.g. 90Y, 131I, 177L, 10B for boron neutron capture, and the like), anti-cancer drugs (e.g. doxorubicin, calicheamicin, maytansinoid DM1, auristatin caupecitabine, 5-fluorouricil, leucovorin, irinotercan, and the like), and/or can optionally be modified to provide for improved pharmacokinetic profile (e.g., by PEGylation, hyperglycosylation, and the like).

[00132] The present disclosure contemplates uPAR-binding agents to encompass recombinant fusion antibody that is modified to include a heterologous protein, i.e., is linked to a polypeptide that is

not part of the antibody derived from a clone. For example, a 3C6 heavy chain polypeptide or 2G10 light chain polypeptide, a 2E9 antibody fragment, or any combination thereof may be joined to a reporter protein or to a protein having a desired anti-cancer effect. The reporter protein may be a fluorescent protein or a luciferase. The antibody may also be conjugated to a second antibody (or at least an antigen-binding portion thereof), e.g., an antibody that specifically binds an angiogenic or proliferative factor, such as an antibody that is directed against vascular enthothelial growth factor (VEGF), which is key mediator of angiogenesis, where the antibody targets the conjugate to specific cancer cells and the anti-VEGF antibody inactivates VEGF thus inhibiting angiogenesis. Methods for producing a fusion protein of interest when provided a nucleic acid sequence are well known in the art.

[00133] UPAR-binding agents may also be detectably labeled, either directly or indirectly. Direct labels include radioisotopes (e.g., ^{125}I; ^{35}S, ^{111}In, ^{99m}Tc, and the like); enzymes whose products generate a signal (e.g., luciferase, β-galactosidase, horse radish peroxidase, alkaline phosphatase, and the like); fluorescent labels (e.g., fluorescein isothiocyanate, rhodamine, phycoerythrin, and the like); fluorescence emitting metals, e.g., ^{152}Eu, or others of the lanthanide series, attached to the antibody through metal chelating groups such as EDTA; chemiluminescent compounds, e.g., luminol, isoluminol, acridinium salts, and the like; bioluminescent compounds, e.g., luciferin; fluorescent proteins; or MRI contrast agents and the like. Indirect labels include second antibodies specific for a subject antibody, wherein the second antibody is labeled as described above; and members of specific binding pairs, e.g., biotin-avidin, and the like.

Polyethylene glycol (PEG)-modified antibodies

[00134] Examples of conjugates include agents (e.g. antibodies) modified to contain one or more poly(ethylene glycol) (PEG) moieties. Such antibodies are referred to as "PEGylated agents." Wherein the agent is an antibody, the antibodies include PEGylated antibodies, e.g., PEGylated recombinant antibodies that bind specifically to uPAR. Methods and reagents suitable for PEGylation of an antibody are well known in the art. In general, PEG suitable for conjugation to an antibody is generally soluble in water at room temperature, and has the general formula $R(O\text{-}CH_2\text{-}CH_2)_nO\text{-}R$, where R is hydrogen or a protective group such as an alkyl or an alkanol group, and where n is an integer from 1 to 1000. Where R is a protective group, it generally has from 1 to 8 carbons.

[00135] The PEG may have at least one hydroxyl group modified to generate a functional group that is reactive with an amino group, e.g., an epsilon amino group of a lysine residue, a free amino group at the N-terminus of a polypeptide, or any other amino group such as an amino group of asparagine, glutamine, arginine, or histidine.

[00136] PEG may also be derivatized so that it is reactive with free carboxyl groups in the antibody polypeptide. Suitable derivatives of PEG that are reactive with the free carboxyl group at the carboxyl-terminus of a heavy chain or light chain polypeptide include, but are not limited to PEG-amine, and hydrazine derivatives of PEG (e.g., PEG-NH-NH_2).

[00137] Additional derivatives of PEG comprises a terminal thiocarboxylic acid group, -COSH, which selectively reacts with amino groups to generate amide derivatives. In other embodiments, the PEG comprises a reactive ester such as an N-hydroxy succinimidate at the end of the PEG chain. Such an N-hydroxysuccinimidate-containing PEG molecule reacts with select amino groups at particular pH conditions such as neutral 6.5-7.5.

[00138] The PEG can be conjugated directly to an amino acid residue of the antibody, or through a linker. In some embodiments, a linker is added to an antibody polypeptide, forming a linker-modified antibody polypeptide. Such linkers provide various functionalities, e.g., reactive groups such sulfhydryl, amino, or carboxyl groups to couple a PEG reagent to the linker-modified antibody polypeptide.
The PEG may be conjugated to the antibody polypeptide is linear. In other embodiments, the PEG conjugated to the antibody polypeptide is branched. Branched PEG derivatives such as those known in the art, e.g., "star-PEG's" and multi-armed PEG's.

PHARMACEUTICAL COMPOSITIONS

[00139] The present disclosure provides pharmaceutical compositions containing one or more uPAR-binding agents (e.g. antibodies). The compositions of the present disclosure encompass those that contain more than one type of agents (e.g. antibodies). The composition may contain at least two, at least three, at least four or more different types of agents (e.g. antibodies). Where the agents in the subject compositions are antibodies, the antibodies may differ in their amino acid sequence, modification by conjugation, affinity, epitopes of uPAR bound, and/or effects on cell signaling mediated by uPAR. For example, the composition may contain a first antibody that competes with integrins (e.g. β1 integrins) binding to uPAR and a second antibody that competes with urokinase for binding to uPAR. Alternatively, a composition may contain a first antibody that binds to uPAR and competes with urokinase binding to uPAR and a second antibody that binds to uPAR and competes with urokinase binding to uPAR and does or does not compete with the binding of the first antibody. An example of a composition with combined antibodies is one that contains both antibodies from clone 3C6 and antibodies from clone 2G10 and/or 2E9.

[00140] In a related example, the composition can contain a first binding agent (e.g. anti-uPAR antibody) that inhibits a first uPAR signaling pathway; and a second binding agent (e.g. anti-uPAR antibody) inhibits a second uPAR signaling pathway. The different signaling pathways of uPAR affected

by the one or more binding agents may cross-talk. "Cross-talk" as used herein, refers to different signaling pathways in which one or more signal components are shared, such that a signal inducing condition can activate multiple responses and/or signaling pathways. A subject composition containing one or more agents that inhibit one or more signaling pathways can synergistically inhibit cell adhesion, proliferation, and/or migration of cancer cells. For example, one signaling pathway that can be inhibited by a binding agent is mediated by uPA binding to uPAR, while another pathway is mediated by integrin (e.g. a β1 integrin) binding to uPAR.

[00141] Other agents that may be included in the subject compositions include agents useful for treating a condition. For example, combination therapies discussed later below may use subject compositions containing one or more drug in addition to the one or more subject antibodies.

[00142] Pharmaceutical compositions can include a pharmaceutically acceptable excipient, which can be a solution such as an aqueous solution (e.g., a saline solution). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like.

[00143] An antibody of the present disclosure can be formulated for parenteral administration for use in the methods described below. Where an antibody is administered as a liquid injectable (such as in those embodiments where they are administered intravenously, intraarterially, or directly into a tissue), an antibody formulation may be provided as a ready-to-use dosage form, or as a reconstitutable storage-stable powder or liquid composed of pharmaceutically acceptable carriers and excipients.

[00144] Pharmaceutical compositions can also contain one or more of: a salt, e.g., NaCl, MgCl, KCl, $MgSO_4$, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; glycerol; and the like.

[00145] The concentration of an agent (e.g. antibody) in the pharmaceutical formulations can vary from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected and the patient's needs. The resulting compositions may be in the form of a solution, suspension, tablet, pill, capsule, powder, gel, cream, lotion, ointment, aerosol or the like.

[00146] Compositions of the present disclosure can include a therapeutically effective amount of a subject agent (e.g. antibody), as well as any other compatible components, as needed. By "therapeutically effective amount" is meant that the administration of that amount to an individual, either

in a single dose, as part of a series of the same or different antibody or compositions, is effective to provide a desired effect (e.g., inhibition of cell proliferation). The therapeutically effective amount can be adjusted in connection with dosing regimen and diagnostic analysis of the subject's condition (e.g., monitoring for the present or absence of a cell surface epitopes using an antibody specific for uPAR) and the like.

[00147] The amount of composition administered to an animal, e.g., a human, in the context of the present disclosure should be sufficient to effect a prophylactic or therapeutic response in the animal over a reasonable time frame, and varies depending upon the goal of the administration, the health and physical condition of the individual to be treated, age, the degree of resolution desired, the formulation of the antibody composition, the treating clinician's assessment of the medical situation, and other relevant factors. One skilled in the art will also recognize that dosage will depend on a variety of factors including the strength of the particular compound employed, the condition of the animal, and the body weight of the animal, as well as the severity of the illness and the stage of the disease. The size of the dose will also be determined by the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular compound. Thus it is expected that the amount will fall in a relatively broad range, but can nevertheless be routinely determined through various features of the subject such as those features noted above.

[00148] Also, suitable doses and dosage regimens can be determined by comparisons to anticancer agents that are known to affect the desired growth inhibitory response. Such dosages include dosages which result in the low dose inhibition of cell growth, without significant side effects. In proper doses and with suitable administration of certain compounds, the compounds of the present disclosure can provide for a wide range of intracellular effects, e.g., from partial inhibition to essentially complete inhibition of cell growth. Dosage treatment may be a single dose schedule or a multiple dose schedule (e.g., including ramp and maintenance doses). As indicated below, a subject composition may be administered in conjunction with other agents, and thus doses and regiments can vary in this context as well to suit the needs of the subject.

METHODS OF PRODUCTION

[00149] Wherein the agent is an antibody, the antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, antibody may be made from E. coli or mammalian cells containing expression cassettes encoding whole antibodies or Fabs. The antibody may also be isolated from hybridoma cells derived from an animal host immunized with an immunogenic composition containing uPAR.

[00150] Anti-uPAR antibodies, including antigen binding fragments of anti-uPAR antibodies, may also be produced by genetic engineering. Where the protein is produced using recombinant techniques, the proteins may be produced as an intracellular protein or as an secreted protein, using any suitable construct and any suitable host cell, which can be a prokaryotic or eukaryotic cell, such as for example a bacterial (e.g. *E. coli*) or a yeast host cell, respectively.

[00151] Examples of eukaryotic cells that may be used as host cells include yeast cells, insect cells, mammalian cells, and/or plant cells. Where mammalian host cells are used, the cells may include one or more of the following: human cells (e.g. Hela, 293, H9 and Jurkat cells); mouse cells (e.g., NIH3T3, L cells, and C127 cells); primate cells (e.g. Cos 1, Cos 7 and CV1) and hamster cells (e.g., Chinese hamster ovary (CHO) cells).

[00152] Vectors, each containing one heavy chain gene and one light chain gene retaining the initial antigen specificity, may be produced by insertion of appropriate sections of the nucleic acids encoding the antibodies into the expression vectors. A library of clones which co-express a heavy and light chain (comprising for example an intact antibody, an Fab fragment or an antigen binding fragment of an antibody molecule) can also be generated. The vectors that carry these genes may be co-transfected into a host (e.g. bacteria, insect cells, mammalian cells, or other suitable protein production host cell). Alternatively, the heavy and light chain may be inserted into a single vector and transfected into a host (e.g. bacteria, insect cells, mammalian cells, or other suitable protein production host cell).

[00153] Methods for introduction of genetic material into host cells include, for example, transformation, electroporation, conjugation, calcium phosphate methods, cationic peptide-based methods, polyethyleneimine-based methods, and the like. The method for transfer can be selected so as to provide for stable expression of the introduced antibody-encoding nucleic acid, such as by for example allowing selection for an antibiotic resistance marker (e.g. using gentimycin, ampicillin, kanamycin, G418 and the like), or a metabolism marker (e.g. selection for glutamine synthesis in glutamine free medium with or without methionine sulfoximine, or selection for DHFR with or without methotrexate). The antibody-encoding nucleic acid can be provided as an inheritable episomal element (e.g., plasmid) or can be genomically integrated. A variety of appropriate vectors for use in production of an antibody of interest are available commercially. When antibody gene synthesis is induced in the transfected host, the heavy and light chain proteins self-assemble to produce active antibodies that can be detected by assaying binding with the antigen or immunogen and isolated using techniques known in the art.

[00154] Further examples of techniques which can be used to produce single-chain Fvs and other antibodies include those described in Huston et al., *Methods in Enzymology* 1991, 203:46-88; and Skerra et al. (1988) *Science* 240:1038-1040. Antibodies can be humanized using a variety of techniques known in the art, veneering or resurfacing, and chain shuffling. Isolation and purification of antibodies can be

accomplished using techniques known in the art, and can provide for antibody-containing preparations at least 50% to 60%, by weight, free from organic molecules with which the antibody is naturally associated or with which it is associated during manufacture.

Nucleic acid

[00155] The present disclosure contemplates cells expressing a uPAR antibody as disclosed herein, e.g., by expression of heavy and light chain-encoding, or heavy and light chain fragment encoding, expression cassettes. Examples of encoding nucleic acids include a nucleic acid encoding a polypeptide comprising one or more CDRs at least about 85%, 90%, 95%, 98%, 99%, or 100% identical to those CDRs depicted in Figure 1. In another example, the antibody has one or more light and heavy chain complementarity determining region (CDR) polypeptide sequences at least about 85%, 90%, 95%, 98%, 99%, or 100% identical to those light and heavy chain CDR polypeptide sequences depicted in Figure 1.

[00156] An example of nucleic acid sequence encoding a heavy chain of an antibody that binds to uPAR includes SEQ ID NO:\\ and NO:\\. An example of nucleic acid sequence encoding a light chain of an antibody that binds to uPAR includes SEQ ID NO:\\ and NO:\\. The disclosure further contemplates recombinant host cells containing an exogenous polynucleotide encoding at least a CDR of a heavy chain polypeptide or at least a CDR of a light chain polypeptide of the subject antibody.

[00157] Wherein the subject agents are encoded by a nucleic acid (e.g. to produce a recombinant antibody), the nucleic acid can comprise a contiguous nucleic acid sequence that is at least 80% identical to (e.g., at least 85%, at least 90%, at least 95%, at least 98%, or 100%) to a contiguous sequence of any sequences listed below.

[00158] 1A8 V_H:

GCCCAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACT
CACCTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGC
AGTCCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTATAA
TGATTATGCAGTATCTGTGAAAAGTCGAATAACCATCAACCCAGACACATCCAAGAACCAGT
TCTCCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTACAAGAGAT
CCGGGGGGGGCTCTCGATGATAGTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTC
AAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTG
GGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTC
GTGGAACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAG
GACTCTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTAC
ATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAAT
CTTGT (SEQ ID NO:45)

[00159] 1A8 V_L:

CTTGATGTTGTGATGACTCAGTCTCCAGCCTCCCTGTCTGTATCTGTAGGAGACAGAGTCACC
CTCACTTGCCAGGCGAGTCAGGTCATTAACAACCACTTAAATTGGTATCAACAACAACCAGG
GAAAGCCCCTAAGCTCCTGGTCTACGATGCATCCAATCTGGAAACAGGGGTCCCATCAAGGT
TCAGTGGAAGTGGATCTGGGACAGATTTTACTTTCACCATCAGCGGCCTGCAGCCTGAAGAT
ATTGCAACATATTACTGTCAACAGTCTGATAATCTCCCGCTCACTTTCGGCGGAGGGACCAA
GCTAGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC
AGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC
AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAG
AGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
ACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:46)

[00160] 1D5 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGCGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATATTACAGGTCCAAGTGGTATAATGA
TTATGCAGAATCTGTGAAAAGTCGAATAGTCATCAACGTAGACACATCCAAGAACCAGTTCT
CCCTGCAGTTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCAAGAGATCCG
GGGGGGCCTCTCGATGATAGTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAG
CGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
T (SEQ ID NO:47)

[00161] 1D5 V_L:

CTTGAAATTGTGATGACACAGTCTCCAGTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTC
CATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCGTAATAATGGATACAACTATTTGGATTGGT
ACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCC
GGGGTCCCTGACAGGTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATCAGCA
GAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCTCTACAAACTCCATTCACT
TTCGGCCCTGGGACCAAAGTGGATATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTT
CCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTT
CTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAGTCGGGTAACTCC

CAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGA
CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGG
CCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:48)

[00162] 1F6 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTATAATGA
TTATGCAGTATCCGTGAAAAGTCGAATAATTATCAACCCAGACACATCCAAGAACCAGTTCT
CCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCAAGAGATCCG
GGGGGGCCTCTCGATGATAGTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAG
CGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCTACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
T (SEQ ID NO:49)

[00163] 1F6 V_L:

CTTGATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCGTAGTAATGGATACAACTATTTGGATTGGTA
CCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTATTCGGGCCTCCG
GGGTCCCTGACAGGTTCAGTGGCAGTGGATCGGGCACAGATTTTACACTGAGAATTAGCAG
AGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCTCTACAAACCCCGTTCACTT
TTGGCCAGGGGACCAAGCTGGAGATCAAGCGAACTGTGGCTGCACCATCTGTCTTCATCTTC
CCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTC
TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCC
AGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGAC
GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGC
CTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:50)

[00164] 1G9 V_H:

CAGGTGCAGCTGCAGGAGTCCGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCAC
CTGCACTGTCTCTGGTGGCTCCATCAGCAGTAGTAGTTACTACTGGGGCTGGATCCGCCAGC
CCCCAGGGAAGGGGCTGGAGTGGATTGGGAGTATCTATTATAGTGGGAGCACCTACTACAA
CCCGTCCCTCAAGAGTCGAGTCACCATATCCGTAGACACGTCCAAGAACCAGTTCTCCCTGA
AGCTGACCTCTGTGACCGCCGCAGACACGGCTGTGTATTACTGTGCGAGACTAAACGCCCAC

CCGATTTACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTC
AAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTG
GGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTC
GTGGAACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAG
GACTCTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTAC
ATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAAT
CTTGT (SEQ ID NO:51)

[00165] 1G9 V_L:

CTTGAAATTGTGCTGACTCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCAC
CCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCTACTTAGCCTGGTACCAACAGAAACCTG
GCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAACAGGGCCACTGGCATCCCAGCCAGG
TTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGA
TTTTGCAGTTTATTACTGTCAGCAGCGTAGCAACTGGCCTCCGATGTACACTTTTGGCCAGGG
GACCAAGCTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTG
ATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGA
GAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTG
TCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA
AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG
CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:52)

[00166] 2B1 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGTGCCATCTCCGGGGACAGTGTCTCTAGTAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGGAGGACATACTACAGGTCCAAGTGGTATTATGAT
TATGCAGTCTCTGTGAAAGGTCGAATAACCTTCACCCCAGACACATCCAAGAACCAGGTCTC
CCTGCACCTGAACGCTGTGACTCCCGAGGACACGGCTATGTATTACTGTGCAAGAGATCCGG
GGGGGCCTCTCGATGATAGTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAGC
GCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGG
CACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGA
ACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTC
TACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTG
CAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGT
(SEQ ID NO:53)

[00167] 2B1 V_L:

CTTGACATCCAGTTGACCCAGTCTCCACCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACT

ATCACTTGCCAGGCGCCTCACGACATTAAGAACAATTTAAATTGGTATCAACAGAAACCAGG
GAAAGCCCCTAAACTCCTGATCTTCGACGCATCTAATTTGGAGACGGGAGTCCCATCAAGAT
TCAGTGGAAGTGGATCTGGGACAAATTTTGTGCTCACCATCAGCAGCCTGCAGCCTGAAGAT
ATTGCAACTTATTACTGTCAACAGTTTCATGATCTCCCGCTCACTTTCGGCGGAGGGACCAA
GGTAGACATGAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC
AGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC
AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAG
AGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
ACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:54)

[00168] 2B3 V_H:

CAGCTGCAGCTGCAGGAGTCGGGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGCTCCA
GGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACTACGCAG
ACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAA
ATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGACCCTATAGCAGCA
GCTGGTACAGCGTTGGGAACTACGGTATAGACGTCTGGGGCCAAGGGACCACGGTCACCGT
CTCAAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCT
CTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGT
GTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCT
CAGGACTCTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC
TACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCA
AATCTTGT (SEQ ID NO:55)

[00169] 2B3 V_L:

CAGGCTGTGCTGACTCAGCCGTCTTCCCTCTCTGCATCTCCTGGAGCATCAGCCAGTCTCACC
TGCACCTTACGCAGAGACATTGATATTGGAACCGCCAGGATTTACTGGTACCAACAGAAGCC
AGGGAGCCCCCCCCCAGTATCTCCTGAACTACAAATCAGACTTGTACACGGAGAAGGCCTCTG
GAGTCCCCAGCCGCTTCTCTGGATCCAAGGATGCTTCGGCCAATGCAGGCATTTTGCTCATCT
CTGGGCTCCAGTCTGAGGATGAGGCTGACTATTACTGTCTGATTTGGCACAACAATGCTTGG
GTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCAC
TCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAA
GTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATGGCAGCCCCGTCAAGGC
GGGAGTGGAGACCACCAAACCCTCCAAACAGAGCAACAACAAGTACGCGGCCAGCAGCTAC

CTGAGCCTGACGCCCGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATG
AAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCA (SEQ ID NO:56)

[00170] 2B7 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGCGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATATTACAGGTCCAAGTGGTATAATGA
TTATGCAGAATCTGTGAAAAGTCGAATAGTCATCAACGTAGACACATCCAAGAACCAGTTCT
CCCTGCAGTTGAACTCTGTGACTCCCGAGGACACGGCTGCGTATTACTGTGCAAGAGATCCG
GGGGGGCCTCTCGATGATAGTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAG
CGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
T (SEQ ID NO:57)

[00171] 2B7 V_L:

CAGTCTGTCGTGACGCAGCCGCCCTCAGTGTCTGGGGCGCCAGGTCAGAGGGTCATCATCTC
CTGCACTGGGAGCAGCTCCAACATCGGGGCAGGCTTTGATGTACACTGGTATCAGCAGCTTC
CAGGAACAGTCCCCAAACTCCTCATCTATGGTAACACAAATCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGGCTGGCACCTCAGCCTCCCTGGCCATCACTGGGCTCCAGGCTGA
GGATGAGGCTGATTATTACTGCCAGGCTTATGACGACTCCCTGCAAGGTTATGTCTTCGGCA
CAGGGACCAAGTTAACCGTCGTCGGTCAGCCCAAGGCCAACCCCACTGTCACTCTGTTCCCG
CCCTCCTCTGAGGAGCTCCAAGCCAACAAGGCCACACTAGTGTGTCTGATCAGTGACTTCTA
CCCGGGAGCTGTGACAGTGGCCTGGAAGGCAGATGGCAGCCCCGTCAAGGCGGGAGTGGAG
ACCACCAAACCCTCCAAACAGAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGCCTGA
CGCCCGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCAC
CGTGGAGAAGACAGTGGCCCCTACAGAATGTTCA (SEQ ID NO:58)

[00172] 2B8 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGTGCCATCTCCGGGGACAGTGTCTCTAGCAAGAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTATAATGA
TTATGCAGTATCTGTGAAAAGCCGAATAACCATCAACCCAGACACATCCAAGAACCAGTTCT
CCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCAAGAGATCCG
GGGGGGCCTCTCGATGATAGTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAG

CGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCTACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
T (SEQ ID NO:59)

[00173] 2B8 V_L:

CTTGATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCGTAGTAATGGATACAACTATTTAGATTGGTA
CCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTACTCGGGCCTCCG
GGGTCCCTGACAGGTTCAGTGGCAGTGGATCGGGCACAGATTTTACACTGAAAATCAGCAG
AGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCTTTTCAAACTCCGCTCACTTT
CGGCGGAGGGACCAAGATGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCC
CGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCT
ATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCA
GGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACG
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC
TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:60)

[00174] 2B11 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTATAATGA
TTATGCAGTATCTGTGAAAAGTCGAATAACCATCAACCCAGACACATCCAAGAACCAGTTCT
CCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCAAGAGATTCG
GGACTGGGGTCAGACTACTTTGACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCAAGCGC
CTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCA
CAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAAC
TCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTA
CTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCA
ACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGT
(SEQ ID NO:61)

[00175] 2B11 V_L:

CTTGACATCCAGATGACCCAGTCTCCACCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCAC
TATCACTTGCCAGGCGCCTCACGACATTAAGAACAATTTAAATTGGTATCAACAGAAACCAG

GGAAAGCCCCTAAACTCCTGATCTTCGACGCATCTAATTTGGAGACGGGAGTCCCATCAAGA
TTCAGTGGAAGTGGATCTGGGACAAATTTTGTGCTCACCATCAGCAGCCTGCAGCCTGAAGA
TATTGCAACTTATTACTGTCAACAGTTTGATGATCTCCCGCTCACTTTCGGCGGAGGGACCAA
GGTAGACATGAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC
AGTTGGAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC
AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAG
AGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
ACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:62)

[00176] 2D5 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTATAATGA
TTATGCAGTATCCGTGAAAAGTCGAATAATTATCAACCCAGACACATCCAAGAACCAGTTCT
CCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCAAGAGATCCG
GGGGGGCCTCTCGATGATAGTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAG
CGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
T (SEQ ID NO:63)

[00177] 2D5 V_L:

CTTGACATCCAGTTGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGGGACAGAGTCACC
ATTACTTGCCGGGCCAGTCAGACTATAAGTAGTTCGTTGGCCTGGTATCAGCAGAAACCAGG
GAAAGCCCCTAACCTCCTGATCTATAAGGCGTCTACATTAGAAGGTGGGGTCCCCTCGCGTT
TCAGCGGCAGTGGATCTGGGACAGAATTCACTCTCACCATCAGCAGCCTGCAGCCTGAAGAT
TTTGCAACTTACTACTGTCAACAGAGTTACACTACCCCGCTCACTTTCGGCGGAGGGACCAA
GGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC
AGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTACCCCAGAGAGGCC
AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAG
AGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
ACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:64)

[00178] 2E7 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGTGCCATCTCCGGGGACAGTGTCTCTAGTAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGGAGGACATACTACAGGTCCAAGTGGTATTATGAT
TATGCAGTCTCTGTGAAAGGTCGAATAACCTTCACCCCAGACACATCCAAGAACCAGGTCTC
CCTGCACCTGAACGCTGTGACTCCCGAGGACACGGCTATGTATTACTGTGCAAGAGATCCGG
GGGGGCCTCTCGATGATAGTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAGC
GCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGG
CACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGA
ACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTC
TACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTG
CAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGT
(SEQ ID NO:65)

[00179] 2E7 V_L:

CTTGACATCCAGTTGACCCAGTCTCCACCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACT
ATCACTTGCCAGGCGCCTCACGACATTAAGAACAATTTAAATTGGTATCAACAGAAACCAGG
GAAAGCCCCTAAACTCCTGATCTTCGACGCATCTAATTTGGAGACGGGAGTCCCATCAAGAT
TCAGTGGAAGTGGATCTGGGACAAATTTTGTGCTCACCATCAGCAGCCTGCAGCCTGAAGAT
ATTGCAACTTATTACTGTCAACAGTTTCATGATCTCCCGCTCACTTTCGGCGGAGGGACCAA
GGTAGACATGAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC
AGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC
AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAG
AGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
ACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:66)

[00180] 2E9 V_H:

CAGGTGCAGCTGCAGGAGTCGGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCA
GGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAG
ACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAA
ATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAGATGAGGATTATG
ATTACGTTTGGGGGAGTTATCGACAATACCCCAGTCGCTACTGGGGCCAGGGAACCCTGGTC
ACCGTCTCAAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGA

CGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCA
GACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAG
CCCAAATCTTGT (SEQ ID NO:67)

[00181] 2E9 V_L:

CAGTCTGTGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGACAGACAGCCAGCATCAC
CTGCTCTGGAGATAATTTGGGGTATAAATATGCTTCCTGGTATCAGCAGAAGCCAGGCCAGT
CCCCTGTGCTGATCATCTATCAAGATAAGAAGCGGCCCTCTGGGATCCCTGAGCGATTCTCT
GGCTCCAACTCTGGGAACACAGCCACTCTGACCATCAGCGGGACCCAGGCTATGGATGAGG
CTGACTATTACTGTCAGGCGTGGGACAGCAGCACTTCTGTGGTATTCGGCGGAGGGACCAAG
CTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCACCCTCCTCTGAG
GAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGT
GACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCC
TCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGAGCAGT
GGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGAC
AGTGGCCCCTACAGAATGTTCA (SEQ ID NO:68)

[00182] 2G10 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTATAATGA
TTATGCAGTATCCGTGAAAAGTCGAATAATTATCAACCCAGACACATCCAAGAACCAGTTCT
CCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCAAGAGATCCG
GGGGGGCCTCTCGATGATAGTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAG
CGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
T (SEQ ID NO:69)

[00183] 2G10 V_L:

CTTGATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCGTAGTAATGGATACAACTATTTGGATTGGTA
CCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTATTCGGGCCTCCG
GGGTCCCTGACAGGTTCAGTGGCAGTGGATCGGGCACAGATTTTACACTGAGAATTAGCAG

AGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCTCTACAAACCCCGTTCACTT
TTGGCCAGGGGACCAAGCTGGAGATCAAGCGAACTGTGGCTGCACCATCTGTCTTCATCTTC
CCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTC
TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCC
AGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGAC
GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGC
CTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:70)

[00184] 2G12 V_H:

GAGGTGCAGCTGGTGGACACTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCA
GGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAG
ACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAA
ATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTATTGTGCGAAAGATTGGGGAAGAA
ATATAGCAGTGGCTGGTACCCTTGACTACTGGGGCCAGGGCACCCTGGTCACCGTCTCAAGC
GCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGG
CACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGA
ACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTC
TACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTG
CAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGT
(SEQ ID NO:71)

[00185] 2G12 V_L:

CTTTCCTATGAGCTGACTCAGCCACCCTCAGTGTCGGTGTCCCCAGGACAGACAGCCAGCAT
TACCTGCTCTGGAGATAAATTGGGACAAAAGTATGTTTCATGGTATCAGCAGAGGCCAGGCC
AGTCTCCTCTACTGGTCATCTTTCAAGATGACAAGCGGCCCTCAGGGATCCCTGAGCGAATC
TCTGGCTCCAACTCTGGGCACACAGCCACTCTGACCATCAGCGCGACCCAGGCTATGGATGA
GGCTGAGTATTTCTGTCAGGCGTGGGACAGTAACACTGCCCCTTATGTCTTCGGAACTGGGA
CCCAGGTCACCGTCCTAAGTCAGCCCAAGGCCAACCCCACTGTCACTCTGTTCCCGCCCTCCT
CTGAGGAGCTCCAAGCCAACAAGGCCACACTAGTGTGTCTGATCAGTGACTTCTACCCGGGA
GCTGTGACAGTGGCCTGGAAGGCAGATGGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCA
AACCCTCCAAACAGAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGCCTGACGCCCGA
GCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG
AAGACAGTGGCCCCTACAGAATGCTCT (SEQ ID NO:72)

[00186] 3C6 V_H:

CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCCCTCAC

CTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGATCCGCCAGCCCCCAG
GGAAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAAGCACCAACTACAACCCGTC
CCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGA
GCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGAGGCAGAAGGTTCGGGGA
TTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCAAGCGCCTCCACCAAGGGCCCAT
CGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGC
CTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAG
CGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTAGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCA
GCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGT (SEQ ID NO:73)

[00187] 3C6 V_L:

CAGCCTGTGCTGACTCAGCCCCCCTCGGTGTCAGTGGCCCCAGGAAAGACGGCCAGGATTAC
CTGTGGGGGAAACAACATTGGAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGCCAG
GCCCCTGTGCTGGTCGTCTATGATGATAGCGACCGGCCCCCAGGGATCCCTGAGCGATTCTC
TGGCTCCAATTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGGGATGAG
GCCGACTATTACTGTCAGGTGTGGGATAGTAGTAGTGATCACTCCCCCTTCGGAACTGGGAC
CAAGGTCACCGTCCTAGGTCAGCCCAAGGCCAACCCCACTGTCACTCTGTTCCCGCCCTCCT
CTGAGGAGCTCCAAGCCAACAAGGCCACACTAGTGTGTCTGATCAGTGACTTCTACCCGGGA
GCTGTGACAGTGGCCTGGAAGGCAGATGGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCA
AACCCTCCAAACAGAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGCCTGACGCCCGA
GCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAG
AAGACAGTGGCCCCTACAGAATGCTCT (SEQ ID NO:74)

[00188] 3C7 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTATAATGA
TTATGCAGTATCCGTGAAAAGTCGAATAATTATCAACCCAGACACATCCAAGAACCAGTTCT
CCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCAAGAGATCCG
GGGGGGCCTCTCGATGATAGTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAG
CGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT

GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
T (SEQ ID NO:75)

[00189] 3C7 V_L:

CTTGACATCCAGTTGACCCAGTCTCCACCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACT
ATCACTTGCCAGGCGCCTCACGACATTAAGAACAATTTAAATTGGTATCAACAGAAACCAGG
GAAAGCCCCTAAACTCCTGATCTTCGACGCATCTAATTTGGAGACGGGAGTCCCATCAAGAT
TCAGTGGAAGTGGATCTGGGACAAATTTTGTGCTCACCATCAGCAGCCTGCAGCCTGAAGAT
ATTGCAACTTATTACTGTCAACAGTTTGATGATCTCCCGCTCACTTTCGGCGGAGGGACCAA
GGTAGACATGAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC
AGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC
AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAG
AGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACGAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
ACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:76)

[00190] 3D6 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAACCCCTCGCAGACCCTCTCAGTCAC
ATGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGGAGGACATACTACAGGTCGAAGTGGTATAATGA
TTATGCAGTATCTGTGAAAAGTCGAATAACCATCAAACCAGACACATCCAAGAACCAGTTCT
CCCTGCAGCTGAACTCTGTGACTCCCGACGACACGGCTGTGTATTACTGTGCAAGAGATCCG
GGGGGGTCTCTCGATGATTCTTTTGATATCTGGGGCCAAGGGACCACGGTCACCGTCTCAAG
CGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
T (SEQ ID NO:77)

[00191] 3D6 V_L:

CTTGACATCCAGATGACCCAGTCTCCACCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCAC
TATCACTTGCCAGGCGCCTCACGACATTAAGAACAATTTAAATTGGTATCAACAGAAACCAG
GGAAAGCCCCTAAACTCCTGATCTTCGACGCATCTAATTTGGAGACGGGAGTCCCATCAAGA
TTCAGTGGAAGTGGATCTGGGACAAATTTTGTGCTCACCATCAGCAGCCTGCAGCCTGAAGA
TATTGCAACTTATTACTGTCAACAGTTTGATGATCTCCCGCTCACTTTCGGCGGAGGGACCAA
GGTAGACATGAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC

AGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC
AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAG
AGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
ACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:78)

[00192] 3F7 V_H:

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCCTCGGAGACCCTGTCCCTCA
CTTGCACTGTCTCTGGTGGCTCCTTCAGCAGTTACTACTGGAGCTGGATCCGGCAGCCCCCA
GGGAAGGGACTGGAGTGGATTGGGTATATTTCTGACAGTGGGAGCACCAACTACAACCCCT
CCCTCCAGTCTCGAGTCACCATATCATTAGACACGTCCAAGAACCAGTTCTCCCTGAAACTG
AACTCTGTGACCGCCACAGACACGGCCGTGTATTACTGTGCGAGAGGCCCGCCTATTCATGA
TTACGTTTGGGGGAGTTATCGCCGCCCCTCGCGAGAATATGATATCTGGGGCCAAGGGACAA
TGGTCACCGTCTCAAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCA
AGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACC
GGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCC
TACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGC
ACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAG
TTGAGCCCAAATCTTGT (SEQ ID NO:79)

[00193] 3F7 V_L:

CTTAATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCTCCGGGGAAGACGGTTACTATC
TCCTGCACCCGCAGCAGTGGCAGCGTTGCCAGCAACTATGTCCACTGGTACCAGCAGCGACC
GGGCAGTTCCCCCTCCATTCTAATCCATGAGTTTAACATAAGACCCTCTGGGGTCCCTGATCG
GTTCTCAGGCTCCATCGACAGCTCCTCCAACTCTGCCTCCCTCACCATCTCTGGACTGACGAC
TGAGGACGAGGCTGATTACTATTGTCAGTCTTCTGTCAACAACCTTCAATGGGTGCTCGGCG
GAGGGACCAAGCTGACCGTCCTGGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCA
CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTA
CCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAG
ACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGCCTGA
CGCCTGAGCAGTGGAAGTCCCACAAAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCAC
CGTGGAGAAGACAGTGGCCCCTACAGAATGTTCA (SEQ ID NO:80)

[00194] 3H7 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCGAAGTGGTATAATGA

TTATGCAGTATCTGTGAAAAGTCGAATAACCATCAAACCAGACACATCCAAGAACCAGTTCT
CCCTGCAGCTGAACTCTGTGACTCCCGACGACACGGCTGTGTATTACTGTGCAAGAGATCCG
GGGGGGTCTCTCGATGATTCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAG
CGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
T (SEQ ID NO:81)

[00195] 3H7 V_L:

CTTGATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCGTAGTAATGGATACAACTATTTAGATTGGTA
CCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTACTCGGGCCTCCG
GGGTCCCTGACAGGTTCAGTGGCAGTGGATCGGGCACAGATTTTACACTGAAAATCAGCAG
AGTGGAGGCTGAAGATGTTGGGGTTTATTACTGCATGCAAGCTTTTCAAACTCCGCTCACTTT
CGGCGGAGGGACCAAGATGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCC
CGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCT
ATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCA
GGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACG
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC
TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:82)

[00196] 4B6 V_H:

CAGGTACAGCTGCAGCAGTCAGGTCCAGGACTGGTGAAGCCCTCGCAGACCCTCTCACTCAC
CTGTGCCATCTCCGGGGACAGTGTCTCTAGCAACAGTGCTGCTTGGAACTGGATCAGGCAGT
CCCCATCGAGAGGCCTTGAGTGGCTGGGAAGGACATACTACAGGTCCAAGTGGTATAATGA
TTATGCAGTATCCGTGAAAAGTCGAATAATTATCAACCCAGACACATCCAAGAACCAGTTCT
CCCTGCAGCTGAACTCTGTGACTCCCGAGGACACGGCTGTGTATTACTGTGCAAGAGATCCG
GGGGGGCCTCTCGATGATAGTTATGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAG
CGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGG
GCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
CTACTCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTG
T (SEQ ID NO:83)

[00197] 4B6 V_L:

CTTGAAATTGTGCTGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCGTAGTAATGGATACAACTATTTAGATTGGTA
CCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCCG
GGGTCCCTGACAGGTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATCAGCAG
AGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCTTTTCAAACTCCGCTCACTTT
CGGCGGAGGGACCAAGATGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCC
CGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCT
ATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCA
GGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACG
CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC
TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:84)

[00198] 4C1 V_H:

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCA
GGGAAGGGGCTGGAGTGGGTCTCAAGTATTAGTGCTAGTGGTGGTAGCACAGACTACGCAG
ACTCCGTGAAGGGCAGATTCACCATCTCCAGAGACAATTCCAAGAACACTCTGTATCTTCAA
ATGAGCAGTCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGTGAAAGAGCGTCCGGATT
ACGATTTTTGGAGTGCGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCAAGCGCC
TCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCAC
AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACT
CAGGCGCCCTGACCAGCGGCGTCCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTAC
TCCCTCAGCAGCGTAGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGT (SEQ
ID NO:85)

[00199] 4C1 V_L:

CTTGATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCGGCCTCC
ATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTAATGGATACAACTATTTGGATTGGTA
CCTGCAGAAGCCGGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCCTCCG
GGGTCCCTGACAGGTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATCAGCAG
AGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGGTACACACTGGCCTCCGACTT
TTGGCCAGGGGACCAAGCTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTC
CCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTC
TATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCC

AGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGAC
GCTGAGCAAAGCAGACTACGAGAAACACAAACTCTACGCCTGCGAAGTCACCCATCAGGGC
CTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO:86)

[00200] The Fc region optionally present at the C-terminus of the heavy chain can be encoded by a contiguous nucleotide sequence having 80% identity to (e.g., at least 85%, at least 90%, at least 95%, at least 98%, or 100%) to a contiguous sequence of the following DNA sequence:
TTGCTAGCACCcTcCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAA
GGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCcTGACCAGCGGCGTCC
ACACCTTCCCGGCTGTcCTACAGTCCTCCGGACTCTACTCCCTCAGCAGCGTAGTGACCGTGC
CCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC
AAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCC
CAGCACCTGAACTCCTGGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACC
CTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCC
TGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCG
CGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGG
ACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCAT
CGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCC
CCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTA
TCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGgACAAG
AGCAGGTgGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGgCTCTGCACaAcCAC
TACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:87).

DIAGNOSTIC METHODS

[00201] The present disclosure provides methods of detecting uPAR in a biological sample *in situ* or isolated from a subject. Since certain cancers (e.g. metastatic cancer) overexpress uPAR, detection of uPAR can aid in diagnosis, choice of therapy, and prognosis. The subject method generally involves contacting a sample containing a cell with a subject agent (e.g. antibody); and directly or indirectly detecting binding of the subject agent (e.g. antibody) to a cell in the sample. The cell can be *in vitro*, where the cell is in a biological sample obtained from a patient suspected of having, or known to have uPAR-positive cells (e.g. cancer cells), a patient undergoing treatment, or a patient being tested for susceptibility to treatment. The cell can be *in vivo*, e.g., the cell is in a patient suspected for having cancer cells, a patient undergoing treatment, or a patient being tested for susceptibility to treatment.

[00202] Antibodies that bind uPAR can be used to detect uPAR-expressing cells in a biological sample of a subject having or suspected of having cells expressing a detectable level of uPAR (e.g. cancer cells) using anti-uPAR antibodies in immunodiagnostic techniques known in the art. The present disclosure provides antibodies suitable for the purpose of detection of uPAR-expressing cancer cells. Some examples of cancer cells that can be detected using the subject antibodies include cancer cells of the breast. An example of a breast cancer cell that can be detected by the subject method includes triple negative cancer cells that are negative for Her2/neu, estrogen receptor, and progesterone receptor (Pal SK et al. (2009) *Maturitas* 63:269-274; Ahmad A et al. (2009) *Journal of Cellular Biochemistry* 108:916-925). Other cancer that can be detected include cancers in the ovaries, prostate, testes, colon, rectum, lung, brain, blood, bone, marrow, or any other organ or tissue in the body, including but not limited to leukaemias, fibrosarcomas, and glioblastomas.

[00203] Such diagnostics can be useful to identify patients amenable to the therapies disclosed herein, and/or to monitor response to therapy.

[00204] Suitable immunodiagnostic techniques include, but are not necessarily limited to, both *in vitro* and *in vivo* methods (e.g. imaging). The phrase "*in vivo* imaging" as used herein refers to methods of detecting the presence of a detectable protein (e.g. detectably labeled 3C6) in whole, live mammal. Optically detectable, such as fluorescent antibodies and luciferases-conjugated antibodies, or radioactively labeled agents may be detected by *in vivo* imaging. *In vivo* imaging may be used provide 2-D as well as 3-D images of a mammal. Charge-coupled device cameras, photodiodes, avalanche photodiodes, photomultiplier tubes, CMOS, or 3D tomographers may used to carry out *in vivo* imaging. For example, Burdette JE (2008) *Journal of Mol. Endocrin.* 40: 253-261 reviews the uses of computed tomography, magnetic resonance imaging, ultrasonography, positron emission tomography, single-photon emission computed tomography, etc., for *in vivo* imaging. Methods for using a detectable label for real-time imaging of luciferase expression in live animals can be readily adapted for use in the subject methods disclosed herein (e.g., Greer LF et al. (2002) *Luminescence* 17: 43-74). *In vivo* imaging of fluorescent proteins in live animals is described in, e.g., Hoffman (2002) *Cell Death and Differentiation* 9:786-789. In some embodiments, *in vivo* imaging may be performed by detecting a label that emits light at a wavelength designed to penetrate living tissue. Such labels include long wavelength emitting fluorescent dyes or proteins such as infrared and near infrared dyes or proteins including but not limited to dyes or proteins that emit in the range of about 600nm to about 800nm, about 650 nm to about 800nm, or about 700nm to about 800 nm. Alternatively, labels designed to emit light that penetrates living tissue may include non fluorescent reagents including but not limited to red-shifted luciferases.

[00205] *In vivo* imaging can also involve computed tomography, magnetic resonance imaging, ultrasonography, positron emission tomography, single-photon emission computed tomography (SPECT),

etc. (See Burdette JE (2008) *Journal of Mol. Endocrin.*, 40: 253-261 for detail). SPECT can also be used with an integrated x-ray CAT (CT) scanner (SPECT/CT) in the subject methods. The information from many *in vivo* imaging methods as those described above can provide 3D distribution of the antibodies in the subject. See Example 14 for more detail.

[00206] Where the cell detected using the subject method is *in vivo*, the method can determine the presence or absence of a particular uPAR-positive cell and/or the location of the uPAR-positive cell in a patient. For example, the subject method can help determine if a cancer cell positive for uPAR has migrated away from the original tumor, the presence or absence of a cancer cell positive for uPAR in the lymph nodes, and/or can help identify lymph nodes containing uPAR-positive cells. In some embodiments, the method may be used to track the progress of anti-cancer treatments including anti-cancer treatments directed toward uPAR positive cancer cells, for example by detecting any decrease or increase in tumor size *in vivo*. The method can involve administering the subject antibody via local injection, e.g. at a tumor site or site suspected of having cells expressing uPAR or by administering the antibody systemicly including but not limited to infusion (e.g. arterial or venous infusion), or injection (e.g. intravenous, intraarterial, intrathecal, intreacranial, subcutaneous, intramuscular, or other method of injection known in the art).

[00207] Where the methods are *in vitro*, the biological sample can be any sample in which uPAR may be present, including but not limited to tissues, whole cells, and extracts thereof. For example, the assay can involve detection of uPAR on cells in a histological tissue sample. For example, the tissue sample may be fixed (e.g., by formalin treatment) and may be provided embedded in a support (e.g., in paraffin) or frozen unfixed tissue.

[00208] Assays can take a wide variety of forms, such as competition, direct reaction, or sandwich type assays. Examples of assays include Western blots; agglutination tests; enzyme-labeled and mediated immunoassays, such as enzyme-linked immunosorbent assays (ELISAs); biotin/avidin type assays; radioimmunoassays; immunoelectrophoresis; immunoprecipitation, fluorescence activated cell sorting, and the like. The reactions generally include detectable labels such as fluorescent, chemiluminescent, radioactive, enzymatic labels or dye molecules, or other methods for detecting the formation of a complex between antigen in the sample and the antibody reacted therewith.

[00209] The assays can involve separation of unbound antibody in a liquid phase from a solid phase support to which antigen-antibody complexes are bound. Solid supports which can be used include substrates such as nitrocellulose (e.g., in membrane or microtiter well form); polyvinylchloride (e.g., sheets or microtiter wells); polystyrene latex (e.g., beads or microtiter plates); polyvinylidine fluoride; diazotized paper; nylon membranes; activated beads, magnetically responsive beads, and the like.

[00210] Where a solid support is used, the solid support is usually first reacted with a solid phase component (e.g., an anti-uPAR antibody) under suitable binding conditions such that the component is sufficiently immobilized to the support. Sometimes, immobilization to the support can be enhanced by first coupling the antibody to a protein with better binding properties, or that provides for immobilization of the antibody on the support with out significant loss of antibody binding activity or specificity. Suitable coupling proteins include, but are not limited to, macromolecules such as serum albumins including bovine serum albumin (BSA), keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, and other proteins well known to those skilled in the art. Other molecules that can be used to bind antibodies to a support include polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and the like, with the proviso that the molecule used to immobilize the antibody does not adversely impact the ability of the antibody to specifically bind antigen. Such molecules and methods of coupling these molecules to the antibodies are well known to those of ordinary skill in the art.

[00211] After reacting the solid support with the solid phase component, any non-immobilized solid-phase components are removed from the support by washing, and the support-bound component is then contacted with a biological sample suspected of containing uPAR under suitable binding conditions. After washing to remove any non-bound ligand, a secondary binder moiety is added under suitable binding conditions, wherein the secondary binder is capable of associating selectively with the bound ligand. The presence or absence of the secondary binder can then be detected using techniques well known in the art.

[00212] Alternatively, antibodies may be coupled to the beads non-covalently for example through contacting beads or other solid surface covalently attached to protein-A, protein-G, protein-L, or an antibody that recognizes the Fc region of one or more of the subject antibodies with one or more of the subject antibodies. The beads or other solid surface may then be contacted with the tissue, cell or extract to be tested, alternatively washed, collected (e.g. by centrifugation), and analyzed to determine the presence or absence of antibody-antigen complexes.

[00213] An ELISA method can be used, wherein the wells of a microtiter plate are coated with a subject anti-uPAR antibody. A biological sample containing or suspected of containing uPAR (e.g., a tumor cell expressing active uPAR), is then added to the coated wells. After a period of incubation sufficient to allow antibody binding, the plate(s) can be washed to remove unbound moieties and a detectably labeled secondary binding molecule added. The secondary binding molecule is allowed to react with any captured antigen, the plate washed and the presence or absence of the secondary binding molecule detected using methods well known in the art.

[00214] Where desired, the presence or absence of bound uPAR from a biological sample (e.g., uPAR-expressing cells) can be readily detected using a secondary binder comprising an antibody directed against the antibody ligands. For example, a number of anti-bovine, anti-rabbit, anti-equine, anti-rat, anti-mouse, and anti-human immunoglobulin (Ig) molecules are known in the art which can be readily conjugated to a detectable enzyme label, such as horseradish peroxidase, alkaline phosphatase or urease, using methods known to those of skill in the art. An appropriate enzyme substrate is then used to generate a detectable signal. In other related embodiments, competitive-type ELISA techniques can be practiced using methods known to those skilled in the art.

[00215] Assays can also be conducted in solution, such that the antibodies and uPAR form complexes under precipitating conditions. For example, the antibody can be attached to a solid phase particle (e.g., an agarose bead or the like) using coupling techniques known in the art, such as by direct chemical or indirect coupling. The antibody-coated particle is then contacted under suitable binding conditions with a biological sample suspected of containing uPAR to provide for formation of particle-antibody-uPAR complex aggregates which can be precipitated and separated from the sample using washing and/or centrifugation. The reaction mixture can be analyzed to determine the presence or absence of antibody-antigen complexes using any of a number of standard methods, such as those immunodiagnostic methods described above.

[00216] Assays can also be conducted in solution by fluorescence activated cell sorting FACS. For example, a biological sample known to comprise, or suspected of comprising, uPAR may be contacted with an antibody of the present invention. The subject antibody may be directly labeled (e.g. fluorescently labeled) or indirectly labeled (e.g. via a secondary antibody) as described herein or generally known in the art. The biological sample may then be counted, and in some cases sorted with a FACS machine. In some cases, fixed cells may be counted or sorted, in other cases, live cells may be counted or sorted.

[00217] The test sample used in the diagnostics assays can be any sample in which uPAR may be present, including but not limited to, cells and tissues, and extracts thereof. In some embodiments, particularly as in embodiments involving detection of cancer cells, it may be desirable to conduct the assay using a sample from the subject to be diagnosed that contains intact, living cells. uPAR detection can then be assessed on an extracellular surface of the cells.

[00218] Diagnostic assays can also be conducted *in situ*. For example, anti-uPAR antibodies can be detectably labeled, administered to a subject suspected of having a cancer characterized by cell surface expression of uPAR, and bound detectably labeled antibody detected using imaging methods available in the art, including but not limited to those *in vivo* imaging methods described herein.

[00219] The diagnostic assays described herein can be used to determine whether a subject has a cancer that is more or less amenable to therapy using antibody-based therapy, as well as monitor the progress of treatment in a subject. It also may be used to assess the course of other combination therapies. Thus, the diagnostic assays can inform selection of therapy and treatment regimen by a clinician.

[00220] UPAR can be detected by detection of specific binding of an antibody, e.g., a monoclonal antibody (mAb) that has the antigen-binding specificity of antibodies as those listed in Figure 1. For example, the 3C6-reactive antigen, 2E9-reactive antigen, and/or 2G10-reactive antigen may be present on the cell surface of a cancer cell. The antigen can also be detected in a permeabilized test cell. For example, a test cancer cell that exhibits a pattern of staining with a 3C6 antibody (or an antibody having the antigen binding specificity of 3C6) that is distinct from a pattern of antibody staining in a normal cell is identified as a cancerous cell that exhibits a 3C6-reactive antigen. Such cancers are thus amenable to therapy with an antibody that specifically binds the 3C6-reactive antigen (e.g., the 3C6).

[00221] The above-described assay reagents, including the antibodies generated by immunization with uPAR according to the methods described previously, can be provided in kits, with suitable instructions and other necessary reagents, in order to conduct immunoassays as described above. The kit can also contain, depending on the particular immunoassay used, suitable labels and other packaged reagents and materials (i.e. wash buffers and the like). Standard immunoassays, such as those described above, can be conducted using these kits.

THERAPEUTIC METHODS

[00222] The uPAR-binding agents (e.g. antibodies) of the present disclosure can find use as therapeutic for treatment of proliferative disorders that are mediated by uPAR-expressing cells. For example, one or more uPAR-binding agents (e.g. antibody) can be used in a therapy for a uPAR-expressing cancer (including prevention and post-diagnosis therapy) or diagnostics for cancers in which cancer cells express uPAR. Subjects having, suspected of having, or at risk of developing a uPAR-expressing cancer are contemplated for therapy and diagnosis described herein. Samples obtained from such subject are likewise suitable for use in the methods of the present disclosure.

[00223] By "treatment" is meant that at least an amelioration of the symptoms associated with the condition afflicting the host is achieved, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g. symptom, associated with the condition being treated. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, e.g., prevented from happening, or stopped, e.g. terminated, such that the host no longer suffers from the condition, or at least the symptoms that characterize the condition. Thus treatment includes: (i) prevention, that is, reducing the risk of development of clinical symptoms,

including causing the clinical symptoms not to develop, e.g., preventing disease progression to a harmful state; (ii) inhibition, that is, arresting the development or further development of clinical symptoms, e.g., mitigating or completely inhibiting an active disease, e.g., so as to decrease tumor load, and/or to decrease the cancer metastases. Such treatment also includes situations where the pathological condition, or the progression of a pathological condition towards a more advanced disease state, or at least symptoms associated therewith, is reduced, or slowed down. In some cases, treatment includes situations wherein the mean time for survival between a patient population undergoing treatment comprising the administration of one or more subject antibodies and a control population not undergoing treatment is greater. In some cases, the increase in mean time for survival may be statistically significant.

[00224] A variety of hosts are treatable according to the methods. Generally such hosts are "mammals" or "mammalian," where these terms are used broadly to describe organisms which are within the class mammalia, including the orders carnivore (e.g., dogs and cats), rodentia (e.g., mice, guinea pigs, and rats), and primates (e.g., humans, chimpanzees, and monkeys). In many embodiments, the hosts will be humans. In some cases, the host may be a rodent (e.g. mouse, rat, or guinea pig) that is athymic, nude, or otherwise immune impaired. In some cases, the host may represent a xenotrophic cancer model in which human or other mammalian cancer cells from another species are introduced into the host, and then one or more subject antibodies are administered to treat the resulting tumor.

[00225] In the methods of cancer treatment, administering of one or more agents (e.g. antibodies) specific for uPAR facilitates a reduction in proliferation of cancerous cells and/or in inhibition of metastasis of cancer cells exposed to the antibody. The method involves administering to the subject an effective amount of a pharmaceutically acceptable formulation that contains one or more agents (e.g. antibodies) specific for uPAR. The agent can have the effect of retarding or otherwise arresting cell growth and/or metastasis. The effects of the agent on cancer cells can be dose dependent, and thus adjustable.

[00226] In a related embodiment, the subject being treated possesses cells expressing overly active or overly abundant uPAR relative to a noncancerous cell. The uPAR can be expressed on the cell surface, such as on a cancer cell. This aspect can be beneficial in the context of the methods of the present disclosure in that cells expressing or presenting uPAR can be more amenable to treatment with a binding-antibody of the present disclosure. The antibody can be administered to a subject, for example, where therapy is initiated at a point where presence of the uPAR is not detectable, and thus is not intended to be limiting. It is also possible to initiate antibody therapy prior to the first sign of disease symptoms, at the first sign of possible disease, or prior to or after diagnosis of a disease.

Cancer

[00227] The The uPAR-binding agent (e.g. antibody) compositions may be used in an anti-cancer therapy in treatment of cancers that express uPAR on an extracellularly accessible cell surface.

[00228] The presence of uPAR and other members of the plasminogen activation system in normal human tissue appear to be transient and low abundance. It is prevalent only in abnormal cells, such as cancer cells including metastasizing cancer cells. Since expression of high levels of uPAR exists predominantly in cancer cells, treatment with subject compositions can be used to detect the presence and localize cancer growth and can block cancer growth. It should be noted that while uPAR may be expressed at higher levels on a cancer cell compared to a non-cancerous cell, this is not a limitation of the therapies disclosed herein.

[00229] The subject compositions described herein can be administered to a subject (e.g. a human patient) to, for example, reduce proliferation cancerous cells, e.g., to reduce tumor size, reduce tumor load, decrease metastatic potential (e.g. reduce cancer cell migration) and/or improve the clinical outcome in patients. In other words, the compositions can be used to reduce cell growth, cell division, and/or decrease the invasiveness of cancer cells, e.g., by decreasing any signaling events leading up to cancer metastasis. Some ways of decreasing cancer invasiveness involve reducing the ability of cancer cells to leave the original cancerous site, reducing the ability of cancer cells to migrate, and the ability of cancer cells to adhere to areas of the body after migration.

[00230] Cancers particularly amenable to antibody therapy can be identified by examining markers of cellular proliferation (e.g., Ki-67 antigen) and/or by examining the presence / accessibility of the uPAR bound by one or more subject antibodies (e.g. 3C6, 2G10, 2E9) or by other antibodies specific for uPAR (e.g., as in an *in vitro* assay).

Types of cancer

[00231] Where the anti-cancer therapy comprises administration of an antibody composition described previously, the anti-cancer therapy can be particularly directed to cancer cells. For example, one or more subject antibodies (e.g. 3C6, 2G10, 2E9) may bind a uPAR-expressing cancer cell. As illustrated in the examples, 3C6, 2G10, and 2E9 are highly effective in binding as well as inhibiting various functional activities of uPAR.

[00232] Examples of cancers presenting uPAR include but not limited to cancer cells of epithelial origin. Some examples are squamous carcinomas, hematological neoplasms, gastric cancer, lymph node, colorectal cancer, pancreatic cancer, hepatic cancer, and immunological disorders. Other more specific examples of cancer include breast (e.g. triple negative breast tumor), ovarian, prostate, lung, leukaemias, fibrosarcomas, glioblastomas, and prostate cancer, as discussed above.

Combination therapies

[00233] The therapeutic methods described herein can include administration of a uPAR agent (e.g. antibody) in combination with one or more other therapies. The combination therapy below can provide for additive or synergistic benefits relative to a regimen in which only one therapy is administered.

[00234] An example of combination therapy involves administering more than one type of agent (e.g. antibody) to a subject. As described above for pharmaceutical compositions, the therapeutic method may involve administering at least one, at least two, at least three or more different types of antibodies simultaneously or sequentially, including for example one or more subject antibodies. The antibodies may differ in the epitopes of uPAR to which they bind. The method, for example, may involve administering antibodies from clone 3C6 and antibodies from clone 2G10 and/or 2E9 to a subject in need of therapy. The antibodies may also bind the same or overlapping epitopes of uPAR. The method for example may involve administering two or more antibodies that each inhibit the interaction between uPAR and uPA, or two or more antibodies that each inhibit the interaction between uPAR and an integrin, or two or more antibodies that each inhibit the interaction between uPAR and vitronectin, or two or more antibodies that each inhibit the interaction between uPAR and uPARAP, or the method may involve administering two or more antibodies that bind to uPAR but do not inhibit one or more of the foregoing interactions, or any combination thereof.

[00235] The combination therapy method can treat cancer in various ways. As noted above for the subject composition, the subject method can employ one or more agents that inhibit one or more uPAR signaling pathways. Where more than one signaling pathways are targeted by the agents, there can be a synergistic inhibition of cell adhesion, proliferation, and/or migration of cancer cells. For example, one signaling pathway that can be inhibited by a binding agent is mediated by uPA binding to uPAR, while another pathway is mediated by integrin (e.g. a $\beta 1$ integrin, such as such as $\alpha 5\beta 1$ or $\alpha 3\beta 1$) binding to uPAR.

[00236] Additional standard anti-cancer therapeutics that may or may not be administered in conjunction with a subject antibody, include but not limited to immunotherapy, chemotherapeutic agents and surgery (e.g., as those described further below). In addition, therapeutic administration of a subject antibody can also be post-therapeutic treatment of the subject with an anti-cancer therapy, where the anti-cancer therapy can be, for example, surgery, radiation therapy, administration of chemotherapeutic agents, and the like. Cancer therapy using a subject antibody in combination with immunotherapy that employs anti-uPAR antibodies is of particular interest.

[00237] For example, a subject antibody can be administered in combination with one or more chemotherapeutic agents (e.g., cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP)),

and/or in combination with radiation treatment and/or in combination with surgical intervention (e.g., pre- or post-surgery to remove a tumor), radiation therapy, bone marrow transplantation, biological response modifier treatment, and certain combinations of the foregoing. Radiation therapy includes, but is not limited to, X-rays or gamma rays that are delivered from either an externally applied source such as a beam, or by implantation of small radioactive sources.

[00238] Chemotherapeutic agents sutiable for use in combination (formulated either separately or with a uPAR antibody) can include a variety of agents. Examples of such agents are discussed in more detail below.

[00239] Chemotherapeutic agents may be non-peptidic (i.e., non-proteinaceous) compounds that reduce proliferation of cancer cells, and encompass cytotoxic agents and cytostatic agents. Non-limiting examples of chemotherapeutic agents include alkylating agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones.

[00240] Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (CYTOXAN™), melphalan (L-sarcolysin), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, uracil mustard, chlormethine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.

[00241] Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, 10-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatine, and gemcitabine.

[00242] Suitable natural products and their derivatives, (e.g., vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (TAXOL®), docetaxel (TAXOTERE®), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, *e.g.* vincristine, vinblastine, vinorelbine, vindesine, *etc.*; podophyllotoxins, *e.g.* etoposide, teniposide, *etc.*; antibiotics, *e.g.* anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, *etc.*; phenoxizone biscyclopeptides, *e.g.* dactinomycin; basic glycopeptides, *e.g.* bleomycin; anthraquinone glycosides, *e.g.* plicamycin (mithramycin); anthracenediones, *e.g.*

mitoxantrone; azirinopyrrolo indolediones, *e.g.* mitomycin; macrocyclic immunosuppressants, *e.g.* cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, *etc.*; and the like.

[00243] Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrazole, letrazole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

[00244] Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (e.g., NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (TAXOL®), TAXOL® derivatives, docetaxel (TAXOTERE®), thiocolchicine (NSC 361792), trityl cysterin, vinblastine sulfate, vincristine sulfate, natural and synthetic epothilones including but not limited to, eopthilone A, epothilone B, discodermolide; estramustine, nocodazole, and the like.

[00245] Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, *e.g.* prednisone, dexamethasone, *etc.*; estrogens and progestins, *e.g.* hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; *etc.*; and adrenocortical suppressants, *e.g.* aminoglutethimide; 17α-ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, Flutamide (Drogenil), Toremifene (Fareston), and ZOLADEX®. Estrogens stimulate proliferation and differentiation; therefore compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids may inhibit T cell proliferation.

[00246] Other chemotherapeutic agents include metal complexes, *e.g.* cisplatin (cis-DDP), carboplatin, *etc.*; ureas, *e.g.* hydroxyurea; and hydrazines, *e.g.* N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur; etc.. Other anti-proliferative agents of interest include immunosuppressants, *e.g.* mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); IRESSA® (ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)quinazoline); etc.

[00247] Proteosome inhibitors, and kinase inhibitors,

[00248] "Taxanes" include paclitaxel, as well as any active taxane derivative or pro-drug. "Paclitaxel" (which should be understood herein to include analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL®, TAXOTERE® (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3'N-desbenzoyl-3'N-t-butoxycarbonyl analogs of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art, or obtained from a variety of commercial sources,

including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from *Taxus brevifolia*; or T-1912 from *Taxus yannanensis*).

[00249] Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (e.g., TAXOTERE docetaxel, as noted above) and paclitaxel conjugates (e.g., paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylose).

[00250] Also included within the term "taxane" are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but not limited to, galactose and mannose derivatives; piperazino; taxane derivatives; 6-thio derivatives; sulfenamide derivatives; and taxol derivative. It may further include prodrugs of paclitaxel.

[00251] Where a combination therapy is administered, the therapy or treatment other than administration of antibody composition can be administered anywhere from simultaneously to up to 5 hours or more, e.g., 10 hours, 15 hours, 20 hours or more, prior to or after administration of a subject antibody. A subject antibody and other therapeutic intervention can be administered or applied sequentially, e.g., where a subject antibody is administered before or after another therapeutic treatment. Alternatively, a subject antibody and other therapy are administered simultaneously, e.g., where a subject antibody and a second therapy are administered at the same time, e.g., when the second therapy is a drug it can be administered along with a subject antibody as two separate formulations or combined into a single composition that is administered to the subject. Regardless of whether administered sequentially or simultaneously, as illustrated above, the treatments are considered to be administered together or in combination for purposes of the present disclosure.

Dosage

[00252] In the methods, an effective amount of an agent (e.g. a uPAR antibody) is administered to a subject in need thereof. For example, in some embodiments, a uPAR-binding agent can facilitate inhibition of growth and/or proliferation of a uPAR-expressing cancer cell. The amount administered can vary depending upon the goal of the administration, the health and physical condition of the individual to be treated, age, the degree of resolution desired, the formulation of a subject agent, the treating clinician's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. For example, the amount of subject agent employed to inhibit cancer cell growth is not more than about the amount that could otherwise be irreversibly toxic to the subject (i.e., maximum tolerated dose). In other cases the amount is around or even well below the toxic threshold, but still in an effective concentration range, or even as low as threshold dose.

[00253] Individual doses are typically not less than an amount required to produce a measurable effect on the subject, and may be determined based on the pharmacokinetics and pharmacology for absorption, distribution, metabolism, and excretion ("ADME") of the antibody, and thus based on the disposition of the composition within the subject. This includes consideration of the route of administration as well as dosage amount, which can be adjusted for parenteral (applied by routes other than the digestive tract for systemic or local effects) applications, for example. For instance, administration of a subject antibody is typically via injection and often intravenous, intramuscular, intratumoral, intracranial, intraarterial, intraocular, intrathecal, or a combination thereof.

[00254] A uPAR-binding agent (e.g. antibody) may be administered by infusion or by local injection. It also can be administered prior, at the time of, or after other therapeutic interventions, such as surgical intervention to remove cancerous cells. As noted above, a uPAR antibody can also be administered as part of a combination therapy, in which at least one of an immunotherapy, a cancer chemotherapy or a radiation therapy is administered to the subject (as described in detail above).

[00255] Disposition of the agent and its corresponding biological activity within a subject is typically gauged against the fraction of agent present at a target of interest. For example, an antibody once administered can accumulate with uPAR or other biological target that concentrates the material in cancer cells and cancerous tissue. Thus dosing regimens in which the antibody is administered so as to accumulate in a target of interest over time can be part of a strategy to allow for lower individual doses. This can also mean that, for example, the dose of antibody that are cleared more slowly in vivo can be lowered relative to the effective concentration calculated from in vitro assays (e.g., effective amount in vitro approximates mM concentration, versus less than mM concentrations in vivo).

[00256] As an example, the effective amount of a dose or dosing regimen can be gauged from the IC_{50} of a given antibody for inhibiting or binding uPAR. By "IC_{50}" is intended the concentration of a drug required for 50% inhibition in vitro. Alternatively, the effective amount can be gauged from the EC_{50} of a given antibody concentration. By "EC_{50}" is intended the plasma concentration required for obtaining 50% of a maximum effect in vivo.

[00257] In general, with respect to the uPAR-binding agents of the present disclosure, an effective amount is usually not more than 200X the calculated IC_{50}. Typically, the amount of an antibody that is administered is less than about 200X, less than about 150X, less then about 100X and many embodiments less than about 75X, less than about 60X, 50X, 45X, 40X, 35X, 30X, 25X, 20X, 15X, 10X and even less than about 8X or 2X the calculated IC_{50}. In one embodiment, the effective amount is about 1X to 50X of the calculated IC_{50}, and sometimes about 2X to 40X, about 3X to 30X or about 4X to 20X of the calculated IC_{50}. In other embodiments, the effective amount is the same as the calculated IC_{50}, and in certain embodiments the effective amount is an amount that is more than the calculated IC_{50}.

[00258] An effective amount may not be more than 100X the calculated EC_{50}. For instance, the amount of antibody that is administered is less than about 100X, less than about 50X, less than about 40X, 35X, 30X, or 25X and many embodiments less than about 20X, less than about 15X and even less than about 10X, 9X, 9X, 7X, 6X, 5X, 4X, 3X, 2X or 1X than the calculated EC_{50}. In one embodiment, the effective amount is about 1X to 30X of the calculated EC_{50}, and sometimes about 1X to 20X, or about 1X to 10X of the calculated EC_{50}. In other embodiments, the effective amount is the same as the calculated EC_{50}, and in certain embodiments the effective amount is an amount that is more than the calculated EC_{50}.

[00259] Effective amounts can readily be determined empirically from assays, from safety and escalation and dose range trials, individual clinician-patient relationships, as well as *in vitro* and *in vivo* assays such as those described herein and illustrated in the Experimental section, below.

[00260] The IC_{50} may be calculated by inhibiting the agent binding to uPAR (e.g. uPAR alone or complexed uPAR, such as uPAR with integrins) in vitro. This aspect can be carried out by assessing the ability of the agent of interest to inhibit 3C6 antibody binding to uPAR. In general, the procedure is carried out by standard ELISA in which the plates are coated with uPAR as described in the examples at a concentration of about 1 μg/ml, and then processed and employed as described in the experimental examples to determine inhibition of antibody binding and the IC_{50}. These agents and others suitable for various aspects of this purpose can be employed.

Routes of administration

[00261] In practicing the methods, routes of administration (path by which a subject agent is brought into a subject in need of therapy or diagnosis) may vary, where representative routes of administration for a subject antibody are described in greater detail below. A subject agent alone or in combinations described above can be administered systemically (e.g., by parenteral administration, e.g., by an intravenous route) or locally (e.g., at a local tumor site, e.g., by intratumoral administration (e.g., into a solid tumor, into an involved lymph node in a lymphoma or leukemia), administration into a blood vessel supplying a solid tumor, etc.).

[00262] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described.

[00263] The formulations of the present disclosure can also be made into aerosol formulations to be administered via inhalation. These aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like. They may also be formulated as pharmaceuticals for non-pressured preparations such as for use in a nebulizer or an atomizer.

[00264] Suppository formulations are also provided by mixing with a variety of bases such as emulsifying bases or water-soluble bases. Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams.

[00265] Unit dosage forms for rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit contains a predetermined amount of the composition containing the antibody compositions. Similarly, unit dosage forms for injection or intravenous administration may comprise the antibody in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present disclosure calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

METHODS OF SCREENING

[00266] A screening method of the present disclosure can be employed to screen for a binding-agent that binds uPAR. The method can involve contacting uPAR with a candidate agent and detecting binding of the candidate agent with uPAR. The method may also involve contacting uPAR with a candidate agent and contacting uPAR with one or more known ligands of uPAR before, after or during the step of contacting uPAR with a candidate agent and then detecting the binding of either the candidate agent with uPAR or the one or more ligands with uPAR or detecting the binding of both the candidate agent and the one or more uPAR ligands. The method may also involve the use of libraries of constructs encoding antibodies, aptamers, and/or libraries of small molecules to screen for a uPAR-binding agent. The binding agent may be selected for its potent inhibition of uPAR activities, inhibition of the expression of mature uPAR, and/or inhibition of the binding affinity for uPAR-interacting proteins (ligands and/or integrins, e.g. β1 integrins). The method may be executed according to methods known in the art.

[00267] Briefly, uPAR (e.g. uPAR alone or uPAR complexed with its ligand and/or integrins) is contacted with a candidate agent. The binding of the candidate agent to uPAR is measured to see if there

is a binding affinity for uPAR. The ability of the candidate agent to disrupt uPAR binding to ligands (e.g. uPA, vitronectin, and/or uPARAP) or to the members of the integrin family is also assessed. Candidate agents that are effective in disrupting binding of uPAR to its interacting partners (e.g. β1 integrins) are selected to be potential agents to be used in diagnostic and therapeutic compositions and methods of use. Candidate agents that can disrupt binding of uPAR to its interacting partners encompass those that can decrease the binding affinity of uPAR to its interacting partners either competitively or noncompetitively.

[00268] UPAR that may be used to screen for potential agent include uPAR as described previously. Exemplary uPAR to be used in the subject screening methods include but are not limited to full-length uPAR, mature uPAR, fragments of uPAR, such as a fragment of uPAR lacking the GPi anchor, uPAR alone or uPAR bound to one or more ligand or to members of the integrin family.

[00269] In an example of a screening method, uPAR (e.g. uPAR in the presence or absence of uPA or β1 integrins) may be immobilized on an ELISA plate or on beads through a covalent or non-covalent interaction, such as hydrophobic adsorption, biotin-avidin interaction, and Ni^{2+}-6xHis interaction. A population of candidate agents is then incubated with the immobilized uPAR, washed, and recovered. During selection, the bound candidate is recovered and identified. Multiple successive selection rounds ensure a selection of a candidate that acts as a specific binding agent for uPAR. Other methods such as plasma resonance, western blot, functional assays (e.g. invasiveness, protease activity, and/or phosphorylation of downstream targets), fluorescence activated cell sorting, etc. can also be used to screen and select for agents that can bind uPAR, or bind uPAR and inhibit its interaction to one or more ligands or β1 integrins. Where the method involves protease assay, a fluorgenic peptide or colorimetric substrate (e.g. spectrazyme UK) may be used in accordance with methods known in the art (e.g. Zimmerman et al. (1978) *PNAS* 75:750-753). By detecting a change in the amount of substrate cleaved or the rate at which the substrate is cleaved, an agent would be selected based on its ability to change the amount of protease activity bound to uPAR. Various assays employed in the screening method can involve comparing binding and/or activity of uPAR in the presence or absence of the candidate agents.

[00270] Candidate uPAR-binding agents may also be engineered so that the agent contains sites that are known to have affinity for either the ligand-binding site or the integrin-binding site.

[00271] Also contemplated by the present disclosure is a library of nucleic acid constructs encoding the candidate uPAR-binding agents described herein. The library encodes a plurality of candidate protease binding agents that may have one or more polypeptide regions in common with any antibody disclosed herein (e.g. framework region or a heavy chain CDR3)

KITS & SYSTEMS

[00272] Also provided are kits and systems that may find use in practicing the methods, as described above. For example, kits and systems may include one or more of the compositions described herein, such as an anti-uPAR antibody (e.g. 3C6, 2E9, or 2G10 or any antibody described herein), a nucleic acid encoding the same (especially a nucleic acid encoding a CDR of a heavy and/or light chain of 3C6, 2E9, or 2G10), or a recombinant cell containing the same. Other optional components of the kit include: buffers, etc., for administering the anti-uPAR antibody, and/or for performing a diagnostic assay. The recombinant nucleic acids of the kit may also have restrictions sites, multiple cloning sites, primer sites, etc to facilitate their ligation to constant regions of non-3C6, 2E9 or 2G10 encoding nucleic acids. The various components of the kit may be present in separate containers or certain compatible components may be precombined into a single container, as desired.

[00273] The kits and systems for practicing the methods may include one or more pharmaceutical formulations that include the antibody compositions described herein. As such, the kits may include a single pharmaceutical composition present as one or more unit dosages. In yet other embodiments, the kits may include two or more separate pharmaceutical compositions.

[00274] In addition to the above components, the kits may further include instructions for practicing the methods. These instructions may be present in the kits in a variety of forms, one or more of which may be present in or on the kit. One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in or on the packaging of the kit, in a package insert, etc. Yet another means would be a computer readable medium, e.g., diskette, CD, flash drive, thumb drive, etc., on which the information has been recorded. Yet another means that may be present is a website address which may be used via the internet to access the information at a removed site. Any convenient means may be present in the kits.

[00275] A kit may be provided for use in treating a host suffering from a cellular proliferative disease. This kit includes a pharmaceutical composition comprising antibody specific for uPAR, and instructions for the effective use of the pharmaceutical composition in a method of treating a host suffering from a cancerous condition by inhibiting the growth and/or metastasis of a cancer cell in a subject. Such instructions may include not only the appropriate handling properties, dosing regiment and method of administration, and the like, but can further include instructions to optionally screen the subject for uPAR associated with the disease. This aspect can assist the practitioner of the kit in gauging the potential responsiveness of the subject to treatment with an antibody of the present disclosure , including timing and duration of treatment relative to the type and growth stage of the cancer. Thus in another embodiment, the kit may further include an antibody or other reagent, such as 3C6, 2E9, and/or 2G10, for

detecting uPAR on an extracellularly accessible surface of a cancer cell. The kit may also include an antibody that contains a conjugate with a detectable label, such as a fluorophore.

[00276] The term "system" as employed herein refers to a collection of antibodies described herein and one or more second therapeutic agents, present in single or disparate compositions that are brought together for the purpose of practicing the methods. For example, separately obtained antibody specific to uPAR and chemotherapy dosage forms brought together and co-administered to a subject are a system according to the present disclosure.

[00277] The following examples further illustrate the present invention and should not be construed as in any way limiting its scope.

EXAMPLES

[00278] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

MATERIALS AND METHODS

[00279] The following methods and materials were used in the Examples below.

[00280] ***uPAR expression and purification.*** Human soluble uPAR cDNA (residues 1-277) was ligated into the insect cell expression vector pACgp67 (BD Biosciences). pACgp67 and Baculogold DNA (BD Biosciences) were co-transfected into *Spodoptera frugiperda* 9 (Sf9) cells using Lipofectamine™ (Invitrogen) according to the manufacturer's protocol. Infected cell culture supernatant was harvested seven days post-transfection.

[00281] uPAR was captured by antibody affinity chromatography, eluted, and then dialyzed overnight before purificatopm by fast protein liquid chromatography on a MonoQ (GE Life Sciences) column using a linear gradient from 0 to 1 M NaCl for elution.

[00282] ***Phage Display Library Construction***. A fully human naïve Fab phage display library was constructed using methods described by de Haard *et al.* (1999) *J Biol Chem* 274:18218-18230. Briefly, peripheral blood lymphocyte RNA was converted to cDNA. The resulting library was cloned into a phagemid vector, which fuses a C-terminal hexa-histidine and c-myc tag to the heavy chain. Large-scale phage rescue was performed using M13K07 helper phage.

[00283] ***Phage Display Panning***. Human soluble uPAR was immobilized to a Nunc Maxisorp™ 96-well microplate (eBioScience) at 10 µg/ml in 50 mM sodium carbonate pH 9.5 and unbound uPAR was removed by washing. uPAR-coated wells were then blocked with milk, washed, and a pre-blocked aliquot of the phage library was divided between the wells. Unbound phage were washed away, and bound phage were recovered by adding *Escherichia coli* (*E. coli*) TG1 cells. Infected TG1 cells were spread onto selection plates, grown overnight, and harvested by plate scraping. Phage were amplified with M13K07 helper phage infection in liquid culture. Fab-displaying phage were harvested from the culture supernatant and concentrated by PEG precipitation.

[00284] The 2nd and 3rd rounds of panning were conducted similarly to the 1st round, but were made increasingly stringent to remove weakly bound phage.

[00285] ***Expression of Fab into culture supernatants***. Phage-infected *E. coli* TG1 colonies were grown in selection media, and Fab expression was induced by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG; 1 mM final) to cultures showing log phase growth. Cultures were shaken overnight to induce periplasmic Fab expression, a minor portion of which leaks into the culture supernatant. Following overnight incubation, TG1 culture supernatants containing leaked Fabs were collected by centrifugation.

[00286] ***Preparation of periplasmic fraction***. Cell pellets from phage-infected TG1 cultures grown at the 96-well plate scale and induced for Fab expression by IPTG, were resuspended in 50 µl 100 mM Tris pH 8.0, 25% glucose, 100 µg/ml hen egg white lysozyme and shaken at room temperature for 30 minutes. 300 µl of ice-cold water was then added and mixed with vigorous pipeting. The periplasmic fraction was the clarified by centrifugation.

[00287] ***Fab purification.*** Individual Fab clones were expressed in *E. coli* BL21 cells (as described for TG1 cells). Periplasmic fractions were purified by immobilized nickel chelate chromatography using Chelating Sepharose™ (GE LifeScience) according to the manufacturer's protocol.

[00288] Purified protein was analyzed by SDS-PAGE, and the concentration was estimated with the BCA™ Protein Assay Kit (Pierce), using bovine serum albumin (BSA) standards. Each Fab was analyzed for expression by Western Blot using a Penta-His horseradish peroxidase (HRP) conjugate antibody (Qiagen) according to the manufacturer's protocol. The Western Blot was visualized on a Typhoon imager (GE LifeScience) using the ECL Plus™ chemiluminescent reagent (GE LifeScience).

[00289] ***uPAR ELISA.*** uPAR-binding Fabs were detected on a Nunc Maxisorp™ 96-well plate coated with 50 µl of 1 µg/ml uPAR. Fabs (either culture supernatant, periplasmic fraction, or purified protein at 22.5 µg/ml) were applied to the plate's wells, which were then washed. Bound Fabs were detected using 100 µg/ml of HRP-conjugated anti-myc antibody clone 9E10 (Roche). Three wells not

coated with uPAR were included to control for nonspecific Fab binding. For ELISA assays using culture supernatants, bound 9E10-HRP was detected using 1-Step™ Turbo-TMB ELISA (Pierce) for endpoint analysis at 450 nm according to the manufacturer's protocol. For all other experiments, bound 9E10-HRP was detected as the rate of change of the absorbance at 650 nm in the presence of TMB substrate.

[00290] ***Sequence Analysis***. The heavy and light chain expression cassettes of all 36 uPAR-binding clones were sequenced. The complementarity determining regions (CDRs) of the heavy and light chain sequences were aligned on the ClustalW2 server (Larkin et al. (2007) *Bioinformatics* 23:2947-2948).

[00291] ***Competitive ELISA***. 95 µl of each Fab was combined with 6 nM high molecular weight uPA (HMW-uPA) (American Diagnostica). The resulting mixture was incubated with the uPAR-coated microplate wells described in the previous section. Wells not coated with uPAR were included to control for any nonspecific binding of HMW-uPA. Wells coated with uPAR and incubated against all Fabs without HMW-uPA were included to control for nonspecific protease activity. Maximal uPA binding was determined by incubating HMW-uPA with uPAR-coated wells, without any Fab. Unbound Fabs and HMW-uPA were removed by washing. The amount of bound HMW-uPA was measured by assaying proteolytic activity in the treated wells using the chromogenic uPA substrate Spectrazyme® UK (American Diagnostica) and monitoring the rate of change of the absorbance at 405 nm. The wells were further assayed to detect the presence of bound Fab using 9E10-HRP as described in the previous section.

[00292] ***uPA activity in presence of Fabs***. Fabs were tested for direct inhibition of uPA in two ways. First, 1 µg/ml of HMW-uPA was incubated in uPAR-coated plates; unbound HMW-uPA was removed by washing, and Fabs were added to the wells at 25 µg/ml. The activity of HMW-uPA in the presence and absence of Fab was measured as described above. Second, 10 nM HMW-uPA and low molecular weight uPA (LMW-uPA) (American Diagnostica) were incubated in a microtiter plate in the presence and absence of 450 nM Fab. The activity of HMW- and LMW-uPA was measured in triplicate by assaying proteolytic activity as described above.

[00293] ***Human IgG1 Antibody Expression and Purification***. Heavy and light chain Fab sequences were amplified by PCR and separately cloned into vector pTT5-SP-H1, a modification of the pTT5 vector (National Research Council of Canada). Heavy and light chain expression vectors were transformed into NEB Turbo Competent *E. coli* (NEB) and large-scale plasmid preparations were performed using the Pure Yield Plasmid Midiprep system (Promega). The sequences of all full-length antibody expression clones were confirmed.

[00294] HEK-293-EBNA1 cells, a generous gift from Yves Durocher of the Canadian National Research Council, were adapted to GIBCO® FreeStyle™ 293 Expression Medium (Invitrogen) supplemented with 50 µg/ml G418. Heavy and light chain encoding pTT5 plasmids were co-transfected

into the cells with jetPEI™ (Polyplus) according to the manufacturer's protocol. Cells were incubated for four to five days post-transfection, after which the IgG-containing spent media was harvested. IgGs were purified on a Protein A agarose (Pierce) affinity column, eluted with 100 mM citrate pH 3.0, neutralized, dialyzed overnight against PBS, and stored at 4°C. IgG expression levels were determined using the Easy-Titer Human IgG Assay Kit (Pierce) and spectrophotometric readings at 280 nm.

[00295] ***Surface Plasmon Resonance***. The interaction affinities between uPAR and 1A8, 2B1, 2G10, and 2E9 were determined by equilibrium surface plasmon resonance (SPR) using a Biacore 1000. In order to abrogate the effect of avidity, antibodies were immobilized on the surface of a Biacore CM5 chip and soluble uPAR was flowed as the analyte. Four Biacore CM5 chip flow cells were sequentially treated, according to the manufacturer's protocol, with 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). 1A8, 2B1, 2E9 and 2G10 IgGs were each diluted to 5 µg/ml in 10mM sodium acetate pH 5.0 and then immobilized to separate flow cells to obtain approximately 2700 relative response units. The flow cells were blocked with 1 M ethanolamine pH 8.5 after antibody immobilization. A flow cell on each CM5 chip was immediately treated with 1 M ethanolamine pH 8.5 after EDC/NHS activation to provide a reference surface.

[00296] Soluble human uPAR was injected over flow cells at the following concentrations: 450 nM, 225 nM, 112.5 nM, 56.25 nM, 28.13 nM, 14.1 nM, 7 nM, 3.5 nM, 1.8 nM, and 0 nM. Bound uPAR was removed with 10 mM glycine pH 1.5. Instrument response values were recorded and imported into Scrubber2 (BioLogic Software) for analysis. Data were normalized using the double referencing method (26), and analyzed using a one site binding model as implemented in Scrubber2. Response values reached a stable plateau as judged by a change of less than 0.05% over the last minute of injection.

[00297] ***Flow Cytometry***. A confluent flask of either HEK 293 cells or HEK 293 uPAR cells was treated with TrypLE Express (Gibco). Harvested cells were re-suspended in Stain Buffer (BD Pharmingen) and either $5x10^5$ or $1x10^6$ cells were transferred to tubes for antibody staining. 1A8, 2B1, 2E9, 2G10, and whole human IgG (Sigma) were added to a final concentration of 5 µg/ml. 2G10 and 3C6 Fab were added to a final concentration of 50 µg/ml. All samples were incubated on a rotator at 4°C for 30 minutes after addition of antibody, harvested by centrifugation, and resuspended in 500 µl of Stain Buffer. The IgG samples were resuspended and incubated with 20 µl of fluorescein isothiocyanate (FITC)-conjugated mouse anti-human monoclonal antibody (BD Pharmingen), while the Fab samples were incubated with Alexa Fluor® 488-conjugated mouse anti-cMyc monoclonal antibody (AbD Serotec). $5x10^5$ cells were analyzed with a Beckton Dickinson FACSCalibur cytometer. Data analysis was performed with FlowJo version 7.2.4.

[00298] ***Mouse Xenograft Generation***. Subcutaneous MCF-7/Luc+ (MCF-7 cells expressing luciferase), and orthotopic MDA-MB-231/Luc+ tumor xenografts were generated using MCF7 and

MDA-MB-231-luc breast cancer cell lines. MDA-MB-231-luc cells are MDA-MB-231 cells which were modified to stably express luciferase, so that the tumor can also be imaged via bioluminescent detection of injected luciferin.

[00299] ***Fluorescent labeling of IgG.*** 2G10 IgG was labeled with Alexa Fluor 680 (Invitrogen, Carlsbad, CA) according to manufacturer's protocol. Protein was purified from unreacted dye on a Superdex 75 FPLC column (GE Healthcare, Little Chalfont, UK). Degree of labeling was determined using UV/Vis spectrometry as directed in manufacturer's protocol. An average of fifteen moles of dye per mole of 2G10 IgG was achieved.

[00300] ***Optical imaging of mice.*** Mice were handled, injected, and imaged as follows. Briefly, Alexa Fluor 680-labeled 2G10 IgG was administered by tail vein injection to achieve ~0.25 nmols IgG per mouse. Images were collected in fluorescent mode on an IVIS 50 using Living Image 2.50.2 software (Caliper Life Sciences, Hopkinton, MA) at set intervals. Two mice were imaged, each, for both the uPAR-positive tumors (MDA-MB-231) and uPAR-negative tumors (MCF-7). Bioluminescent images were also obtained as previously described. All *in vivo* studies were performed as directed under institutional approval.

[00301] ***Adhesion Assay.*** The cell adhesion assay was performed as described previously (Wei et al. (2007) *J Biol Chem* 282:3929-3939). Briefly, H1299 cells ($2x10^5$) were seeded onto fibronectin (FN)-coated (10 μg/ml) plates with or without the anti-uPAR Fabs (10 mg/ml), RGD peptide, or RAD peptide (0.4 mM). Attached cells were fixed with methanol and Giemsa stain was used for colorimetric analysis by measuring the optical density at 550 nm. FN was purchased from Sigma-Aldrich (St. Louis, MO). RGD and RAD peptides were purchased from Anaspec (San Jose, CA).

[00302] ***ERK Phosphorylation Assays.*** Serum-starved H1299 cells were washed with 50 mM glycine-HCl, 100 mM NaCl, pH 3.0 for three minutes to remove surface-bound endogenous uPA, and neutralized with 0.5 M HEPES, 0.1 M NaCl pH 7.5 for 10 minutes on ice. Cells were pre-treated with 10 μg/ml of 1A8, 2B1, 2E9, 2G10, or control human IgG for one hour at 37°C. Pro-uPA was added to 10 nM and incubated at 37°C for five minutes to initiate ERK activation. After incubation, cells were lysed in RIPA buffer (Pierce) supplemented with protease and phosphatase inhibitors (Sigma-Aldrich) and blotted for phospho- and total ERK (Cell Signaling). In the case of FN-stimulated ERK phosphorylation, cells were cultured on a FN- (10 μg/ml) coated surface for 30 minutes before lysis.

[00303] ***Invasion Assays.*** H1299 human lung cancer cells ($1x10^5$) were pre-treated with 1A8, 2B1, 2E9, 2G10, or control human IgG (each 10 μg/ml), and 2G10, 3C6, 2G10+3C6 Fab (each 10 μg/ml) for one hour at 37°C. Cells were then seeded on BD Biocoat™ Matrigel™ Invasion Chambers (BD Biosciences) with bottoms pre-coated FN, and then cultured overnight in serum-free DMEM containing 5 mg/ml BSA. Fetal bovine serum was added to the lower chamber. 24 hours later, the Matrigel and cells

on the membrane's top chamber side were removed, and cells on the membrane's bottom chamber side were fixed with methanol, stained with Giemsa, extracted in 10% acetic acid, and measured in a plate reader at 595 nm. All assays were performed in triplicate and the data expressed as percent inhibition by the antibodies: % Inhibition= $((OD_{595,\ Ab}-OD_{595,\ Ctrl})/OD_{595,\ Ab})x100$.

[00304] ***Anti-uPAR Co-immunoprecipitation.*** H1299 cells ($1x10^7$) were lysed in Triton lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl and 1% Triton X-100) supplemented with protease inhibitors (Sigma) and 1 mM PMSF. Clarified lysates were first incubated with anti-uPAR Fabs (10 μg/ml) at 4 °C for 1 hour, then with Penta-His Antibody (Qiagen) for 1 hour, and finally with 50 ⌈l of mixed Protein A- and Protein G-Agarose beads overnight. The immunoprecipitates were subjected to SDS-PAGE and Western blot analysis for uPAR and ⟨V integrin. The anti-uPAR monoclonal antibody (R2) was a kind gift from Michael Ploug (Finsen Lab, Copenhagen, Denmark). The anti-⟨V integrin polyclonal antibody was purchased from Chemicon (Temecula, CA).

EXAMPLE 1 PHAGE DISPLAY IDENTIFIES UPAR BINDING FABS

[00305] Prior to panning, the binding of non-denatured uPAR to a microplate surface was confirmed by detecting the binding of high molecular weight uPA (HMW-uPA) to the uPAR-coated surface. Binding of HMW-uPA was detected by the presence of specific proteolytic activity within a microplate well with the uPA substrate spectrazyme UK after incubating the uPAR-coated plate with HMW-uPA and stringently washing.

[00306] Fabs capable of binding human uPAR were obtained after three rounds of panning, in which washes to remove weakly bound Fab-displaying phage were made increasingly stringent. 384 independent clones were evaluated from the final round of panning. To confirm that these Fabs could be expressed in bacteria, culture supernatants (into which a small fraction of Fabs escaped after IPTG addition) were tested for the presence of Fab capable of binding to uPAR. From these 384 clones, 96 were selected for further analysis on the basis of reproducible uPAR binding. Periplasmic protein fraction were then prepared from the 96 clones. With these fractions, ELISA analysis gave stronger, more consistent signals compared to that of culture supernatants. Of the 96 clones, 36 candidates were confirmed as strong binders of uPAR, with an average signal greater than 8-fold over background.

EXAMPLE 2 SEQUENCE ANALYSIS AND SMALL-SCALE EXPRESSION IDENTIFIES UNIQUE FABS

[00307] The 36 candidates were sequenced and evaluated for expression at the 100 ml culture scale. Sequencing of the heavy and light chain expression cassettes revealed that 22 of the 36 candidates have unique Fab sequences. ClustalW alignment of these sequences yielded a percent identity

dendrogram with two distinct groups of antibodies defined by having a κ or λ light chain (Figure 1, panel A). Several sub-groups of highly related sequences are evident within the κ light chain group whereas eight antibodies with a relatively low degree of sequence similarity are evident within the λ light chain group. In Panel A of Figure 1, the number of identical clones is indicated in parentheses for redundant Fab sequences. The Fab subgroups, defined by their light chain identity (κ or λ), are also labeled. The vertical line indicates the 82% sequence identity threshold. Sequences that branch to the right of the 82% cut-off are considered equivalent.

[00308] Alignment of the six complementary determining regions (CDRs) of each unique Fab (Figure 1, panel B) shows that the CDR sequences determine the subgrouping pattern observed in the dendrogram of Figure 1, panel A. The lowest pairwise sequence identity between antibody CDRs was 22%. The name of each CDR loop is indicated above the alignment. Fab heavy and light chain protein sequences with greater than 82% sequence identity were grouped together (boxed).

[00309] The expression levels of the 22 unique Fabs in *E. coli* were determined after IPTG induction of 100 ml cultures. Histidine-tagged Fabs from the periplasmic fraction were obtained by osmotic shock, purified on a nickel chelating sepharose column, and analyzed for expression by Western blot. Asterisks indicate Fab clones that did not express in *E. coli* Rosetta-gami™ B cells. Small-scale expression of the remaining Fabs, with the exception of 2E9, yielded 250 μg/L of *E. coli* culture. Fab 2E9 expression yields were five fold lower.

[00310] Purified Fabs were further characterized by uPAR ELISA. Initial measurements of bound antibody exhibited a large variance between different Fabs, but control experiments measuring uPA binding to immobilized uPAR did not show similar variance suggesting that these differences reflect inherent disparities in binding mode or affinity between different Fabs.

[00311] The list of Fabs to further pursue was narrowed by clustering individual clones based on their sequences and bacterial expression abilities. Sequences with a sequence similarity greater than or equal to 82% were clustered together (Figure 1). From these groupings, A representative clone was selected from each group based on expression levels in *E. coli* Rosetta-gami™ B cells and is indicated to the left of the box, thus narrowing the list of Fabs to 12 clones for further analysis.

EXAMPLE 3 Competitive ELISA identifies 2E9 and 2G10 as the most competitive with uPA for uPAR binding.

[00312] Purified Fabs from the 12 remaining clones were analyzed for their ability to compete with uPA for binding to immobilized uPAR. The presence of uPA was measured by the amount of bound proteolytic activity in the presence and absence of each Fab (Figure 2). The presence of uPA was

determined by the amount of bound proteolytic activity and is reported as initial velocities from progress curves. Maximal uPA binding was determined by incubating uPA without Fab and is labeled "no Fab". Data is plotted left to right from Fabs that do not compete with uPA for uPAR binding to Fabs that show maximal competition. For 1A8 and 2B1, the amount of Fab bound to uPAR in the presence and absence of uPA was determined by ELISA. This assay identified 2E9 and 2G10 as competitors of the uPA/uPAR interaction. Controls showed that these antibodies did not directly inhibit uPA's proteolytic activity.

[00313] The competitive ELISA data also suggested that 1A8 and 2B1 do not compete with uPA for uPAR binding. To verify that these Fabs were not weak uPA competitors, the ratio of bound Fab in the presence of uPA to bound Fab in the absence of uPA was calculated (Figure 2, inset). The amount of Fab bound in the presence and absence of uPA was determined in the same uPAR coated well, therefore some loss of Fab is expected due to processing between measurements. This assay verified that 1A8 and 2B1 bound a non-uPA binding site on uPAR. The two strongest non-competitive binders, 1A8 and 2B1, and the two strongest competitive inhibitors, 2G10 and 2E9, were chosen for further analysis.

EXAMPLE 4 FULL-LENGTH IGG EXPRESSION IN MAMMALIAN CELLS PRODUCES ROBUST AMOUNTS OF ANTIBODY

[00314] The heavy and light chain sequences of 1A8, 2B1, 2G10, and 2E9 were cloned into the mammalian expression vector pTT5-SPH1 for high-level expression by transient transfection in HEK-293-EBNA1 cells. The plasmid map of this transient expression vector is shown in Figure 3. For a given antibody, both the pTT5-SP-H1-heavy chain vector and pTT5-SP-H1-light chain vector were co-transfected into HEK-293-EBNA1 cells for expression. Co-transfection of varying ratios of heavy and light chain expression plasmids revealed that an equal mass of heavy and light chain DNA, which corresponds to a slight excess of light chain plasmid particles in comparison to heavy chain ones, produced the highest level of antibody. A total DNA : PEI ratio of 1 µg: 4 µl, and sub-confluent maintenance of HEK 293-EBNA1 cells resulted in greater than 90% transfection efficiency. Optimal time to harvest post transfection was four to five days. Antibody expression yield was sequence dependant and varied between 20 mg/L to 100 mg/L of culture supernatant at the 1 ml scale, and between 10 mg/L and 50 mg/L in large scale trials (500 ml).

EXAMPLE 5 SURFACE PLASMON RESONANCE REVEALS LOW NM AFFINITIES FOR UPAR

[00315] The monovalent interaction affinity between uPAR and the antibodies 1A8, 2B1, 2G10, and 2E9 were determined by equilibrium surface plasmon resonance methods using a Biacore 1000.

Analysis of instrument response versus analyte (uPAR) concentration yielded monovalent dissociation constants in the nanomolar range (Figure 4).

EXAMPLE 6 FLOW CYTOMETRY SHOWS SPECIFIC LABELING OF UPAR EXPRESSING CELLS

[00316] The ability of the identified antibodies to bind uPAR, as it is presented on the cell surface, was analyzed by flow cytometry. HEK-293 cells stably expressing membrane-bound human uPAR were labeled with full-length anti-uPAR IgGs, or an isotype control. Anti-uPAR IgGs were detected with an anti-human Fc FITC-conjugated secondary antibody. Labeled cells were analyzed on a flow cytometer (Figure 5). To quantify the relative staining intensities of the human anti-uPAR antibodies, the same gate (horizontal line as shown in Figure 5) was applied to each sample. The % of cells staining positive for uPAR expression is indicated above the gate. All the antibodies tested indicated robust labeling of uPAR-expressing HEK-293 cells, but did not show labeling of the parental HEK-293 cells lacking uPAR expression.

[00317] Similar experiments were carried out for 2G10 Fab using cancer cell lines. MCF-7 (low uPAR expression) and MDA-MB-231 (high uPAR expression) are two breast cancer cell lines whose relative expression of uPAR were previously characterized. MCF-7 cells and MDA-MB-231 cells were contacted with 2G10, and the binding of 2G10 was able to discriminate between these two cell lines, consistent with the levels of uPAR (Figure 11).

EXAMPLE 7 IGG LABELS UPAR-POSITIVE TUMORS BUT NOT UPAR-NEGATIVE ONES

[00318] To test the binding of the antibodies *in vivo*, fluorescently labeled 2G10 IgG were used to image uPAR-expressing breast tumor xenografts in nude mice. Cancer cells lines known to express uPAR were pre-screened for 2G10 binding via flow cytometry. Two breast cell lines were chosen; MDA-MB-231 for its ability to be labeled by 2G10, and MCF-7 for its inability to be detectably labeled. As shown in representative mice in Figure 6, 2G10 IgG was able to label uPAR-expressing MDA-MB-231 tumor xenografts, but not MCF-7 ones. Although the figure shows the 24-hour time point for the MDA-MB-231, when the signal intensity was highest, the signal persisted over one week, indicating that these antibodies may have favorable pharmacokinetics.

[00319] Sections were taken from MDA-MB-231 tumor xenographs and embedded in paraffin. These sections were labeled with Alexa Fluor 488-labeled 2G10 Fab (Panel A) and FITC-labeled 3C6 Fab (Panel B) to probe for uPAR (Figure 13). Staining patterns of 2G10 and 3C6 differ and this difference suggests that 2G10 and 3C6 bind to different uPAR epitope. Ability of staining of 2G10 Fab to stain paraffin-embedded aggressive breast tumor is shown in Panel C of Figure 13. 2G10 was able to

bind to its uPAR epitope under the harsher preservation conditions of formalin fixation followed by paraffin embedding. 2G10 staining was found to be more intense in some cells than others. High-intensity-stained cells might be macrophages. These antibodies were found to be specific for uPAR because probing against paraffin-embedded engineered HEK 293 cell line that over-expresses membrane-bound uPAR results in a high binding signal while parent HEK 293 cell line does not.

[00320] Additional *in vivo* experiments were carried out using immune compromised mice implanted the high- or low-uPAR expressing cell lines (MDA-MB-231 and MCF-7, respectively) (Figure 12). After a period of time post-implantation for palpable tumors to appear, two nanomoles of labeled 2G10 or 3C6 IgG antibody were injected into the mice. After 48 hour post injection, photographs of the mice stained with the antibodies were taken. Uptakes of antibodies were observed in the uPAR expressing tumors derived from MDA-MB-231 cells, but not in the tumors derived from uPAR deficient cells (MCF-7). Both antibody antagonists 2G10 and 3C6 produced the same results (upper two rows and bottom two rows).

EXAMPLE 8 2E9 AND 2G10 DECREASE H1299 INVASION

[00321] H1299 cells have also been shown *in vitro* to migrate through, or invade, extracellular matrix components such as Matrigel in a manner that is dependent on uPA binding to uPAR (Tang et al. (2008) *J Cell Sci* 121:3747-3756). A strong *in vitro* Matrigel invasion phenotype is thought to correlate with the metastatic potential of a cancer cell *in vivo*. For the experiments shown in panel A of Figure 6, H1299 cells were pre-treated with antibodies (10 µg/ml): 2E9, 2G10, 2B1, and 1A8 before they were allowed to invade Matrigel for 24 hours. The cells that migrated through and attached to the bottom of the filter were fixed, stained with Giemsa, and extracted with 10% acetic acid. Cell invasiveness is evaluated by measuring $OD_{595\ nm}$. Analysis of the effects of antibodies 1A8, 2B1, 2G10, and 2E9 on Matrigel invasion by H1299 cells shows that 2G10 and 2E9 are both capable of inhibiting migration, whereas 1A8 and 2B1 are not (Figure 7, panel A).

EXAMPLE 9 2E9 AND 2G10 DECREASE UPA-DEPENDENT ERK PHOSPHORYLATION IN H1299 CELLS

[00322] The human lung cancer cell line H1299 exhibits pro-proliferative ERK phosphorylation and activation that is dependant on signaling events mediated by binding of uPA to uPAR . This cell line was used to test the ability of the anti-uPAR antibodies to inhibit uPAR-dependant pro-proliferative signals triggered by uPA binding. H1299 cells expressing endogenous uPAR were serum-starved, acid washed, pre-treated with antibodies (10 µg/ml), and then incubated with pro-uPA (10 nM). The lysates were immuno-blotted with anti-pERK (top panel) and anti-total ERK (bottom panel) (Figure 7, panel B).

[00323] The results demonstrate that antibodies 1A8 and 2B1 do not inhibit ERK phosphorylation under the conditions tested. However, 2E9 and 2G10, which compete with uPA binding to uPAR, are able to inhibit ERK phosphorylation.

EXAMPLE 10 3C6 DECREASES FN-DEPENDENT ERK PHOSPHORYLATION IN H1299 CELLS, AND ABROGATES THEIR FN- AND VN-DEPENDENT ADHESION

[00324] The activation of FN-dependent ERK phosphorylation in H1299 cells is dependent on the formation of the uPAR/β1/FN complex. To determine if any of the unique anti-uPAR Fabs interfere with the uPAR/β1 interaction, their ability to decrease ERK phosphorylation in H1299 cells seeded in FN-coated wells was tested. Similar to the experiments in Example 9, H1299 cells were serum-starved, acid washed, were pre-treated with Fabs (10 μg/ml): 2B1, 2B7, 2B11, 2D5, 2E9, 2G10, 2G12, 3C6, and 4C1, and cultured on a FN-coated surface (10 μg/ml) for 30 minutes before lysis. The lysates were immuno-blotted with anti-pERK (top) and anti-total ERK (bottom) (Figure 8, panel A). 3C6 was identified as able to significantly decrease FN-dependent ERK phosphorylation.

[00325] To further characterize the functional effects of 3C6, a FN adhesion assay was utilized. The β1/FN interaction can occur in a uPAR-independent context that is sensitive to antagonism by the RGD peptide, and in a uPAR-dependent context that is resistant to the RGD peptide (Wei et al. (2007) *J Biol Chem* 282:3929-3939). H1299 cells were seeded on FN-coated (10 μg/ml) or VN-coated (5 μg/ml) 96-well plates with or without anti-uPAR antibody, and RGD or RAD peptide. In the presence of both the RGD peptide and 3C6, H1299 adhesion to FN-coated wells was completely abrogated (Figure 8, panel B). The selectivity of this effect was verified by inclusion of RAD peptide and the Fab form of the uPA competitor, 2G10, as negative controls.

[00326] To determine if 3C6's ability to disrupt uPAR/β1 integrin-mediated adhesion is generalizable, the ability of uPAR/α3β1-mediated H1299 cell adhesion to VN was characterized. In an assay similar to the FN adhesion assay, it was found that 3C6 could also prevent the adhesion of H1299 cells to VN in the presence of RGD peptide (Figure 8, panel B), suggesting that 3C6 is able to specifically block the functions of uPAR complexes with multiple β1 integrins. As seen in panel C of Figure 8, a normalized graph comparing the adhesion for each antibody treatment on the two different ECM coatings was obtained by dividing the average reading from RGD-treated wells by that from RAD-treated wells. It was found that 3C6 treatment disrupts uPAR-mediated integrin adhesion at least four fold more than 2G10 treatment.

EXAMPLE 11 3C6 FAB BINDS UPAR OVER-EXPRESSING HEK CELLS

[00327] To confirm that 3C6 recognizes uPAR as displayed on a cell's surface, the same flow cytometry assay used to characterize the anti-uPAR IgGs was used here. Since the investigation of 3C6-dependent cellular effects was done with the Fab form of the antibody, this format of the antibody (e.g. Fab) was used for flow cytometry. 2G10 Fab was included as a benchmark for an antagonistic antibody's ability to bind cellular uPAR-expressing HEK-293 cells. HEK 293 cells over-expressing uPAR were stained with 3C6 and 2G10 to confirm 3C6's ability to bind cell surface uPAR. The results are shown in panel A of Figure 9. The dashed white profile represents staining with 2G10 Fab; the shaded profile represents staining with 3C6 Fab; the solid white profile represents no Fab staining, but inclusion of the AlexaFluor 488 conjugated secondary. The data indicate that 3C6 can bind to cells that over-express uPAR, albeit not as robustly as the 2G10 Fab.

EXAMPLE 12 3C6 PREVENTS THE ASSOCIATION OF UPAR AND α5β1 IN H1299 CELLS

[00328] To determine if 3C6 directly blocked uPAR's association with α5β1 integrin, 3C6 and 2G10 were used to immunoprecipitate uPAR from H1299 lysates. H1299 lysates were incubated with anti-uPAR Fab (2G10 or 3C6), Penta-His antibody, and Protein A/G agarose. The resulting immunoprecipitates were analyzed Western blot for both uPAR and α5β1 integrin.

[00329] The resulting immunoprecipitates were analyzed by Western blot for both uPAR and α5β1 integrin. The results indicate that 3C6 prevents uPAR's association with α5β1 integrin, while 2G10 does not (Figure 9, panel B).

EXAMPLE 13 3C6 DECREASES H1299 INVASION, AND 2G10 AND 3C6 SYNERGISTICALLY INHIBIT H1299 INVASION THROUGH CROSS-LINKED MATRICES

[00330] Migration is a complex phenomenon that requires modulation of adhesion and degradation of ECM. As shown in Figure 7, panel A, antagonism of the uPAR/uPA complex by 2E9 and 2G10 inhibits the invasion of H1299 cells. To determine if 3C6 has a similar effect on invasion by antagonizing the uPAR/β1 complex, the potential synergy of. 3C6 Fab with the uPA competitor Fab, 2G10, was tested for their ability to block cell invasion through Matrige/Collagen I or Collagen I. H1299 cells were pre-treated with antibodies (2G10, 3C6, and 2G10/3C6 at 5-10 μg/ml) before seeding on the Collagen I-coated (Figure 10, panel A) or Matrigel/Collagen I-coated (Figure 10, panel B) top membrane of a 24-well Transwell plate (10^5 cells/well in triplicate). Cells were incubated for 24 hours. The cells that migrated through and attached to the bottom of the filter were fixed, stained with Giemsa, and extracted with 10% acetic acid. Cell invasiveness is evaluated by measuring $OD_{595\ nm}$. The results are expressed as a

percentage of inhibition observed in the no treatment control. As shown in panel A of Figure 10, not only do 2G10 and 3C6 Fabs inhibit invasion through Collagen I, but combined dosage also exhibits a synergistic response.

[00331] Additionally, the invasion assay was repeated on a substrate comprised of both Matrigel and collagen I, to provide a matrix that contained more physiologically relevant cues for migration and ECM degradation. The results were consistent for what was observed on the collagen I coated inserts with concurrent 2G10 and 3C6 treatment resulting in a synergistic response (Figure 10, panel B).

EXAMPLE 14 RADIOLABELING DOTA-2G10 IGG WITH ^{111}IN AND IN VIVO SPECT/CT IMAGING

[00332] For radiolabeling, the DOTA-2G10 IgG was diluted to 2μM with 1X PBS. This corresponds to a weight by volume concentration of around 250μg/ml. A 200μl DOTA-2G10 IgG aliqout (50 μg of IgG) was incubated with 12μl of $^{111}InCl_3$ (2.59 mCi) in 0.01N HCl for 50 minutes at 37°C. Using radio TLC, the labeling efficiency of the ^{111}In with the DOTA chelate was determined to be 90%. The radiolabeled antibody was separated from unreacted $^{111}InCl_3$ by size-exclusion chromatography using a PD-10 column pre-equilibrated with 1X PBS buffer. 0.5 ml fractions were collected from the column and were assayed for the presence of radiolabeled IgG by radio TLC. Fractions with high radioactive purity were then injected into the tail vein of six-week old nude mice bearing MDA-MB-321 cancer xenografts of approximately 400mm^3 in size. Injection was done with 250 μCi of 2G10 IgG. The mice were then imaged at 48 hr using a Gamma Medica Ideas X-SPECT SPECT/CT scanner. CT and SPECT images were reconstructed and fused together using the software provided by the manufacturer. The data were then analyzed using Visage Imaging Amira software. A processed image of the MDA-MB-321 xenograph labeled with ^{111}In-DOTA-2G10 IgG is shown in Figure 14 with four different views. As indicated by regions that are dark gray, 2G10 specifically labeled uPAR-expressing tumors. Doral, ventral and saggital views of the MDA-MB-321 xenograph are also shown in various panels in Figure 14.

EXAMPLE 15 2G10 CAN INDUCE CYTOSTATIC STATE IN MDA-MB-231 CELLS.

[00333] MDA-MB-231 cells were treated with 1μM of 2G10 Fab for four days to assess any level of cell death or cytostatic properties using propidium iodide staining. Cytostatic properties refer the inhibition of growth and/or division of cells. As shown by the flow cytometry experiment in Figure 15, 2G10 induced a cytostatic state in the treated cells, trapping them in the G0/G1 cell cycle state.

EXAMPLE 16 EPITOPE MAPPING OF 3C6 ONTO ALANINE-SCANNED UPAR MUTANTS

[00334] To provide initial analysis for the binding epitope of integrin competitor antibody, 3C6, a flow cytometry based epitope mapping studying was conducted on alanine-scanned mutants of uPAR. Shown in Figure 16, panel A is a representation of the results. Each mutation is located in domain 3 of uPAR and affect 3C6 binding to lesser or greater extents. Accordingly, all the uPAR mutants considered are present in domain 3 of uPAR, which is implicated as the major contributor to β1 integrin binding. This site is located opposite side of the uPA binding site (Figure 16, panel B).

[00335] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

What is claimed is:

1. A binding agent that specifically binds urokinase-type plasminogen activator receptor (uPAR) wherein said agent competes with an integrin for binding to uPAR.

2. The binding agent of claim 1, wherein said integrin is a β1 integrin.

3. The binding agent of claim 2, wherein said integrin is α5β1 or α3β1 integrin.

4. The binding agent of claim 1, wherein said agent competes for binding to uPAR with an antibody from clone 3C6.

5. The binding agent of claim 1, wherein said agent comprises:

a) a V_HCDR1 comprising an amino acid sequence of a 3C6 V_HCDR1 as set forth in Figure 1;

b) a V_HCDR2 comprising an amino acid sequence of a 3C6 V_HCDR2 as set forth in Figure 1; and

c) a V_HCDR3 comprising an amino acid sequence of a 3C6 V_HCDR3 as set forth in Figure 1.

6. The binding agent of claim 5, wherein said agent comprises:

a) a V_L CDR1 comprising an amino acid sequence of a 3C6 V_L CDR1 as set forth in Figure 1;

b) a V_L CDR2 comprising an amino acid sequence of a 3C6 V_L CDR2 as set forth in Figure 1; and

c) a V_L CDR3 comprising an amino acid sequence of a 3C6 V_L CDR3 as set forth in Figure 1.

7. The binding agent of claim 6, wherein said agent comprises:

a) a heavy chain comprising an amino acid sequence of at least 85% amino acid sequence identity to the full length amino acid sequence of V_H of 3C6; and

b) a light chain comprising an amino acid sequence of at least 85% amino acid sequence identity to the full length amino acid sequence of V_L of 3C6.

8. An isolated antibody that specifically binds urokinase-type plasminogen activator receptor, wherein said antibody comprises:

a) a V_H CDR1 comprising an amino acid sequence of a 2E9 V_H CDR1 as set forth in Figure 1;

b) a V_H CDR2 comprising an amino acid sequence of a 2E9 V_H CDR2 as set forth in Figure 1; and

c) a V_HCDR3 comprising an amino acid sequence of a 2E9 V_HCDR3 as set forth in Figure 1.

9. The isolated antibody of claim 8, wherein said antibody comprises:

a) a heavy chain comprising an amino acid sequence of at least 85% amino acid sequence identity to the full length amino acid sequence of the V_H of 2E9; and

b) a light chain comprising a V_L CDR1, a V_L CDR2, and a V_L CDR3, wherein the amino acid sequences of the V_L CDR1, the V_L CDR2, and the V_L CDR3 are each independently selected from

(i) the amino acid sequences of the V_L CDR1, the V_L CDR2, and the V_L CDR3 of 2E9l; and

(ii) the amino acid sequences of the V_L CDR1, the V_L CDR2, and the V_L CDR3 of 2G10.

10. The isolated antibody of claim 8, wherein said antibody comprises:

a) a heavy chain comprising an amino acid sequence of at least 85% amino acid sequence identity to the full length V_H of 2E9; and

b) a light chain comprising an amino acid sequence of at least 85% amino acid sequence identity to the full length V_L of 2E9.

11. The antibody of claim 8, wherein said antibody competes for binding to uPAR with an antibody from clone 2E9.

12. An isolated antibody that specifically binds urokinase-type plasminogen activator receptor, wherein said antibody comprise:

a) a V_HCDR1 comprising an amino acid sequence of a 2G10 V_HCDR1 as set forth in Figure 1;

b) a V_HCDR2 comprising an amino acid sequence of a 2G10 V_HCDR2 as set forth in Figure 1; and

c) a V_HCDR3 comprising an amino acid sequence of a 2G10 V_HCDR3 as set forth in Figure 1.

13. The isolated antibody of claim 12, wherein said antibody comprises:

b) a heavy chain comprising an amino acid sequence of at least 85% amino acid sequence identity to the full length V_H of 2G10; and

c) a light chain comprising a V_L CDR1, a V_L CDR2, and a V_L CDR3, wherein the amino acid sequences of the V_L CDR1, the V_L CDR2, and the V_L CDR3 are each independently selected from

(i) the amino acid sequences of the V_L CDR1, the V_L CDR2, and the V_L CDR3 of 2G10;
(ii) the amino acid sequences of the V_L CDR1, the V_L CDR2, and the V_L CDR3 of 1F6;
(iii) the amino acid sequences of the V_L CDR1, the V_L CDR2, and the V_L CDR3 of 1D5;
(iv) the amino acid sequences of the V_L CDR1, the V_L CDR2, and the V_L CDR3 of 3H7;
(v) the amino acid sequences of the V_L CDR1, the V_L CDR2, and the V_L CDR3 of 2B8; and
(vi) the amino acid sequences of the V_L CDR1, the V_L CDR2, and the V_L CDR3 of 4B6.

14. The isolated antibody of claim 12 comprising:

a) a heavy chain comprising an amino acid sequence of at least 85% amino acid sequence identity to the full length V_H of 2G10; and

b) a light chain comprising an amino acid sequence of at least 85% amino acid sequence identity to the full length V_L of 2G10.

15. The antibody of claim 12, wherein said antibody competes for binding to uPAR with an antibody from clone 2G10.

16. The antibody of claim 1, 8, or 12, wherein said antibody is radiolabeled.

17. A pharmaceutical composition comprising the binding agent of claim 1 or the antibody of claim 8 or 12 and a pharmaceutical acceptable excipient.

18. The pharmaceutical composition of claim 17, wherein said composition comprises the binding agent of claim 1 and a second binding agent comprising an antibody, wherein said second binding agent comprising an antibody competes with urokinase for binding to uPAR.

19. The pharmaceutical composition of claim 18, wherein said second antibody is an antibody of Claim 8 or 12.

20. A pharmaceutical composition comprising the antibody of claim 8 and the antibody of claim 12.

21. A method of detecting a cancer cell comprising:

contacting the binding agent of claim 1 or the antibody of claim 8 or 12 with a cell suspected of being cancerous;

detecting said binding agent or said antibody bound to said cell.

22. The method of claim 21, wherein said cell is in a subject suspected of having cancer.

23. The method of claim 21, wherein said cancer cell is a breast cancer cell.

24. The method of claim 21 or 22, wherein said binding agent or said antibody is detectably labeled.

25. The method of claim 21, wherein said detecting comprises imaging tissues of said subject.

26. The method of claim 21, wherein said antibody is radiolabeled.

27. The method of claim 21, wherein said detecting comprises single photon emission computed tomography.

28. A method of treating a subject having cancer comprising:
administering the composition of claim 17 to said subject, wherein said administering reduces proliferation of cancer cells or reduces migration or metastasis of cancer cells in said subject.

29. The method of Claim 28, wherein said method further comprises administering an anti-cancer agent.

30. A method for screening for a binding agent that binds urokinase-type plasminogen activator receptor (uPAR) comprising:
a) contacting uPAR with a candidate agent;
b) detecting binding of said candidate agent with uPAR, wherein said detecting comprises detecting a decrease in binding of uPAR to β1 integrin in the presence of said candidate agent relative to binding of uPAR to β1 integrin in the absence of said candidate agent.

31. The method of Claim 30, wherein said detecting binding comprises detecting a modulation of uPAR activity.

32. The method of Claim 30, wherein said detecting comprises employing an enzyme-linked immunosorbent assay.

33. The method of Claim 30, wherein said detecting comprises detecting protease activity.

34. The method of Claim 30, wherein said candidate agents comprise an antibody.

35. A kit for detecting cancer cells in a subject comprising:
the binding agent of claim 1 or the antibody of claim 8 or 12; and
reagents for detecting cells expressing uPAR.

36. A method of inhibiting uPAR signaling comprising:
contacting a cell comprising uPAR with a uPAR binding agent, wherein said uPAR binding agent inhibits a first uPAR signaling pathway; and
contacting said cell with an anti-uPAR antibody, wherein said anti-uPAR antibody inhibits a second uPAR signaling pathway.

37. The method of claim 36, wherein the uPAR binding agent inhibits signaling mediated by uPA binding to uPAR.

38. The method of claim 36, wherein the anti-uPAR antibody inhibits signaling mediated by integrin binding to uPAR.

39. The method of claim 36, wherein the integrin is a β1 integrin.

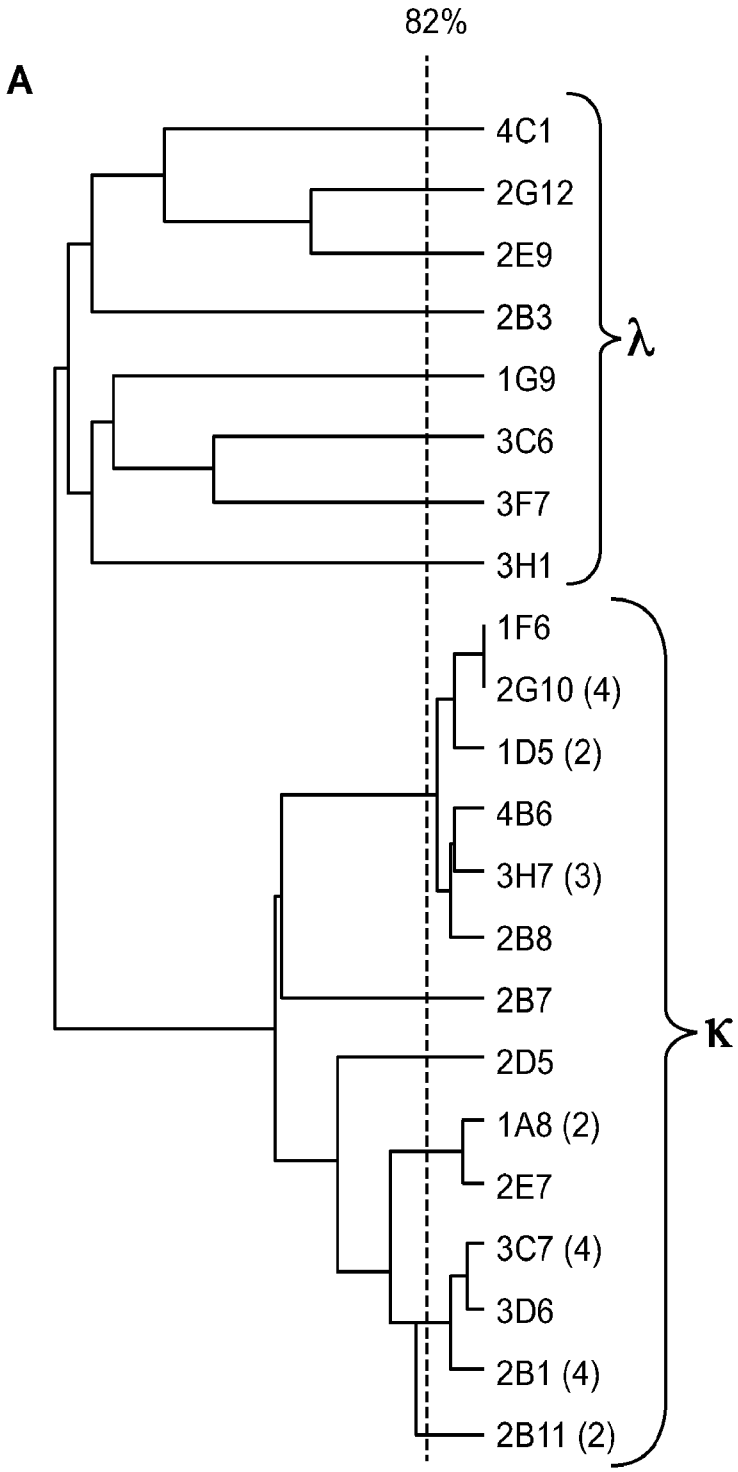

82%
A
4C1
2G12
2E9
2B3
1G9
3C6
3F7
3H1
λ
1F6
2G10 (4)
1D5 (2)
4B6
3H7 (3)
2B8
2B7
2D5
1A8 (2)
2E7
3C7 (4)
3D6
2B1 (4)
2B11 (2)
κ

FIG. 1

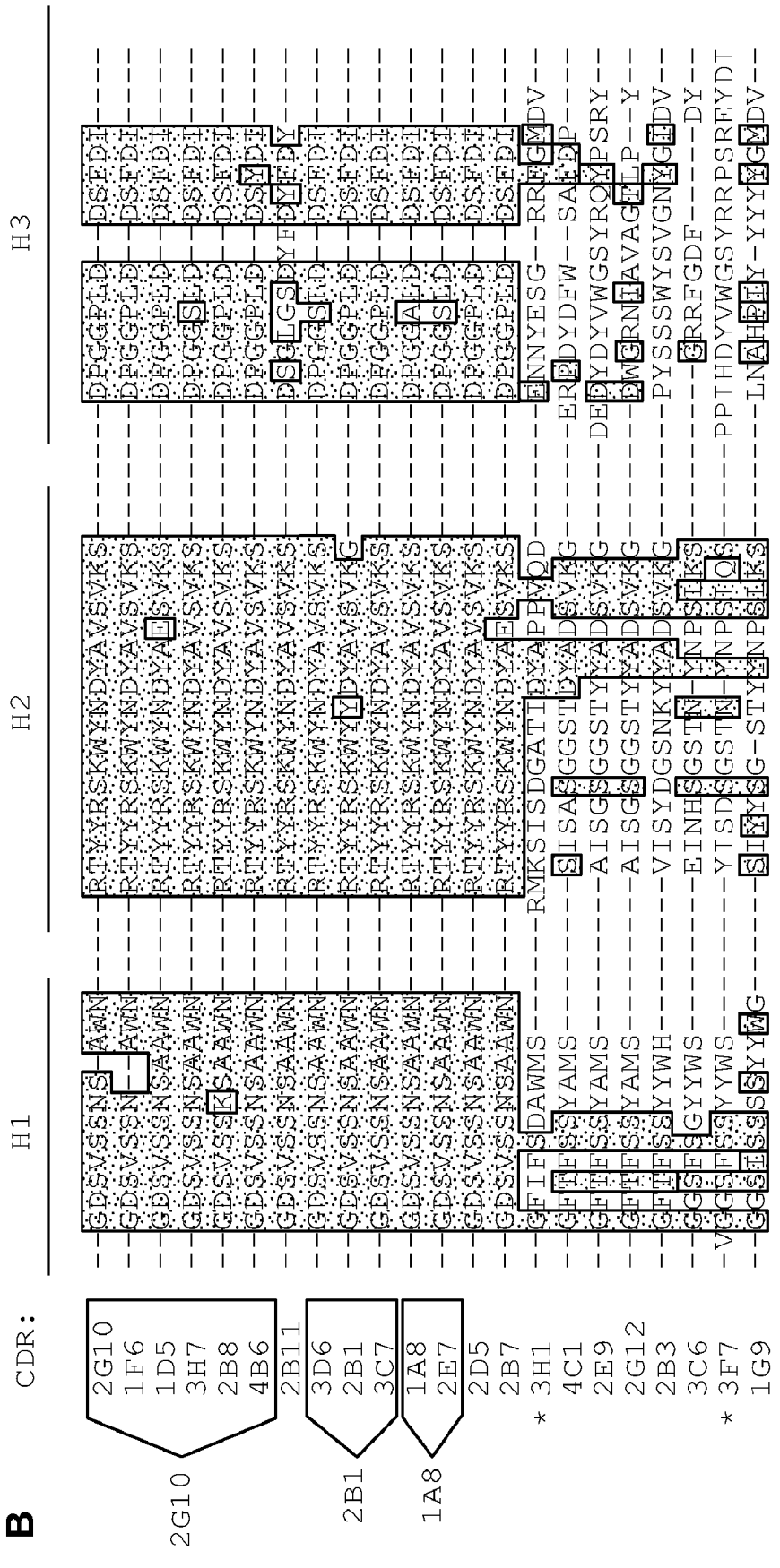

B
CDR:
H1
H2
H3
2G10
1F6
1D5
3H7
2B8
4B6
2B11
3D6
2B1
3C7
1A8
2E7
2D5
2B7
* 3H1
4C1
2E9
2G12
2B3
3C6
* 3F7
1G9
2G10
2B1
1A8

FIG. 1 (Cont. 1)

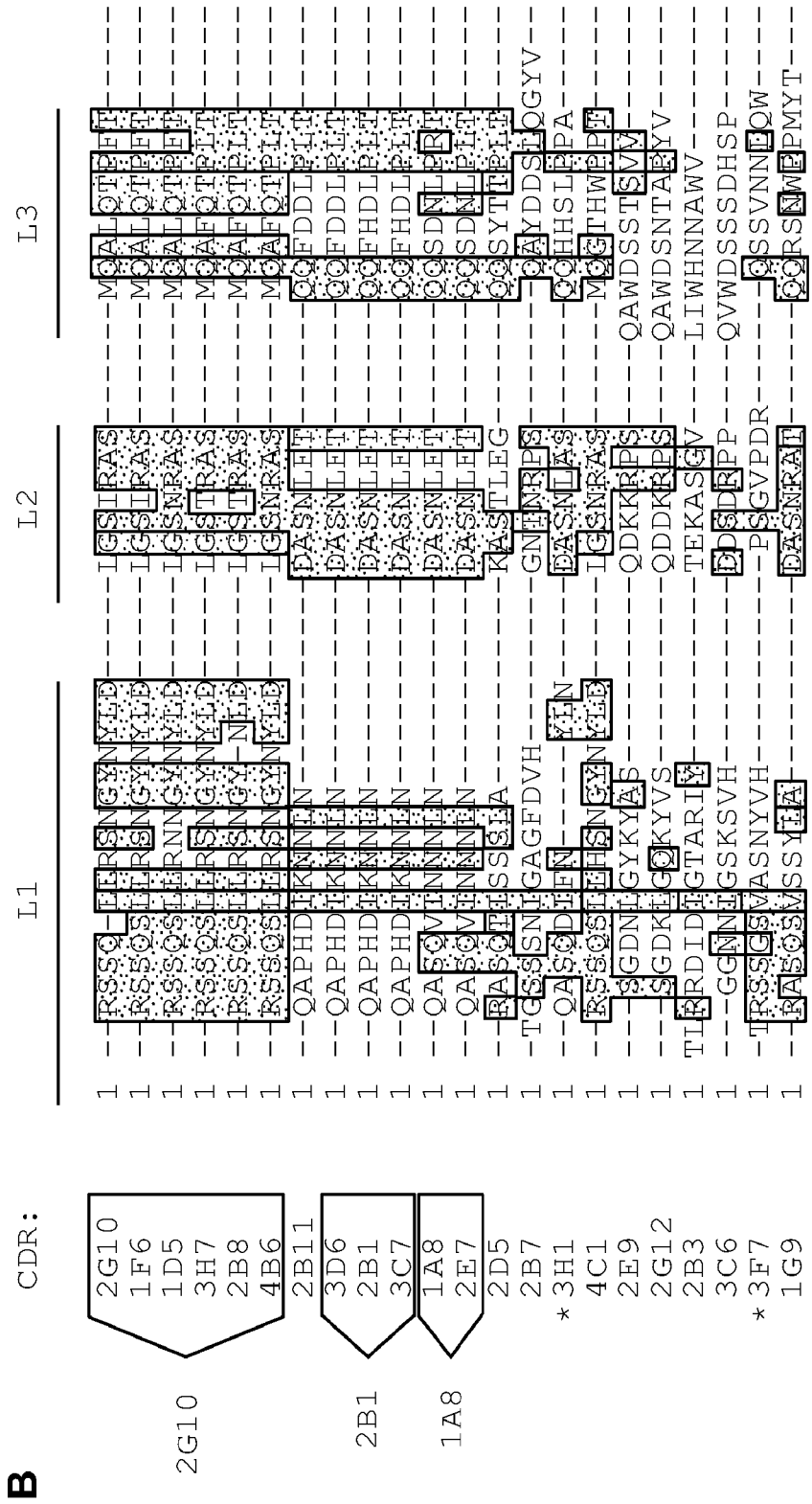

B
CDR:
L1
L2
L3
2G10
1F6
1D5
3H7
2B8
4B6
2B11
3D6
2B1
3C7
1A8
2E7
2D5
2B7
*3H1
4C1
2E9
2G12
2B3
3C6
*3F7
1G9
2G10
2B1
1A8

FIG.1 (Cont. 2)

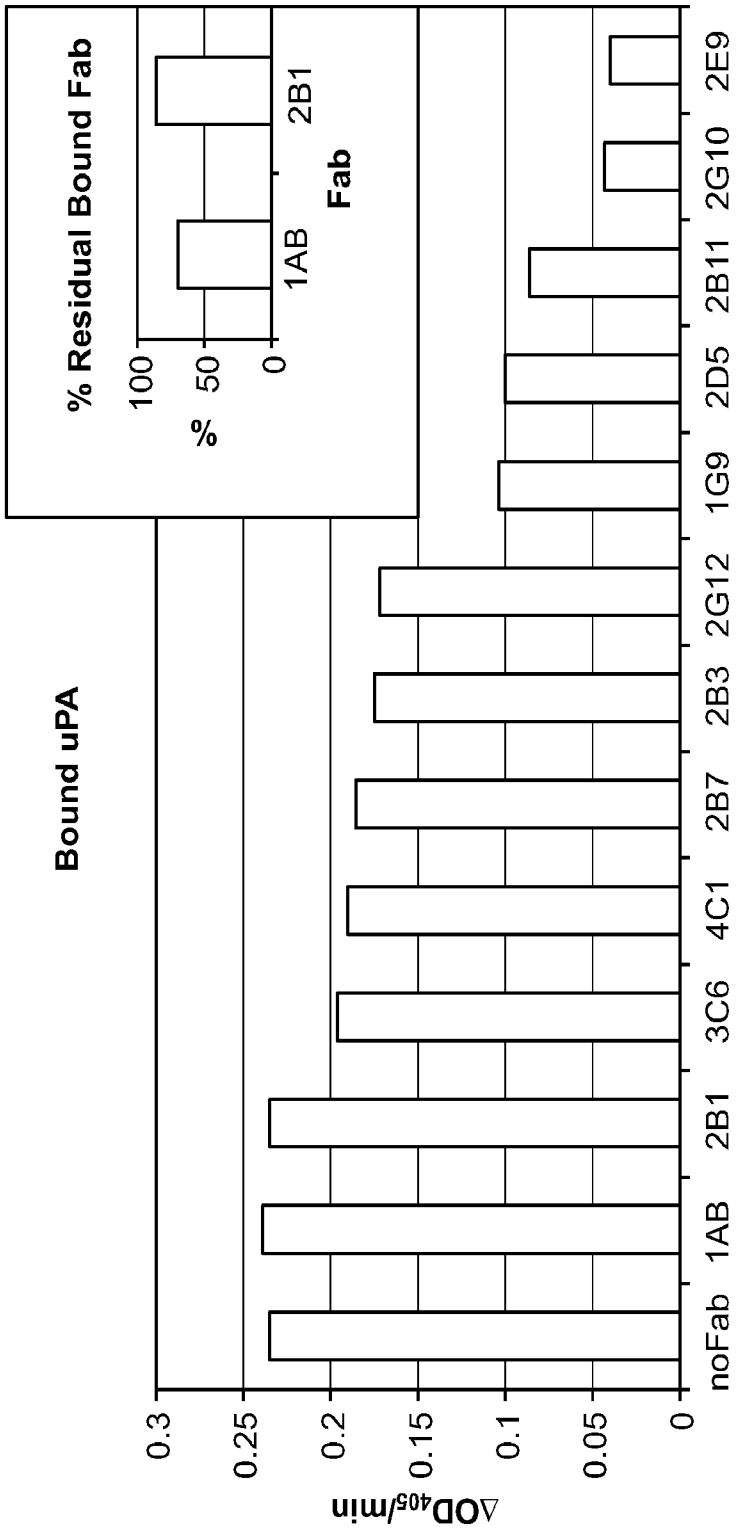

Bound uPA
ΔOD405/min
0.3
0.25
0.2
0.15
0.1
0.05
0
noFab
1AB
2B1
3C6
4C1
2B7
2B3
2G12
1G9
2D5
2B11
2G10
2E9
Fab
% Residual Bound Fab
100
50
0
%
1AB
2B1
Fab

FIG. 2

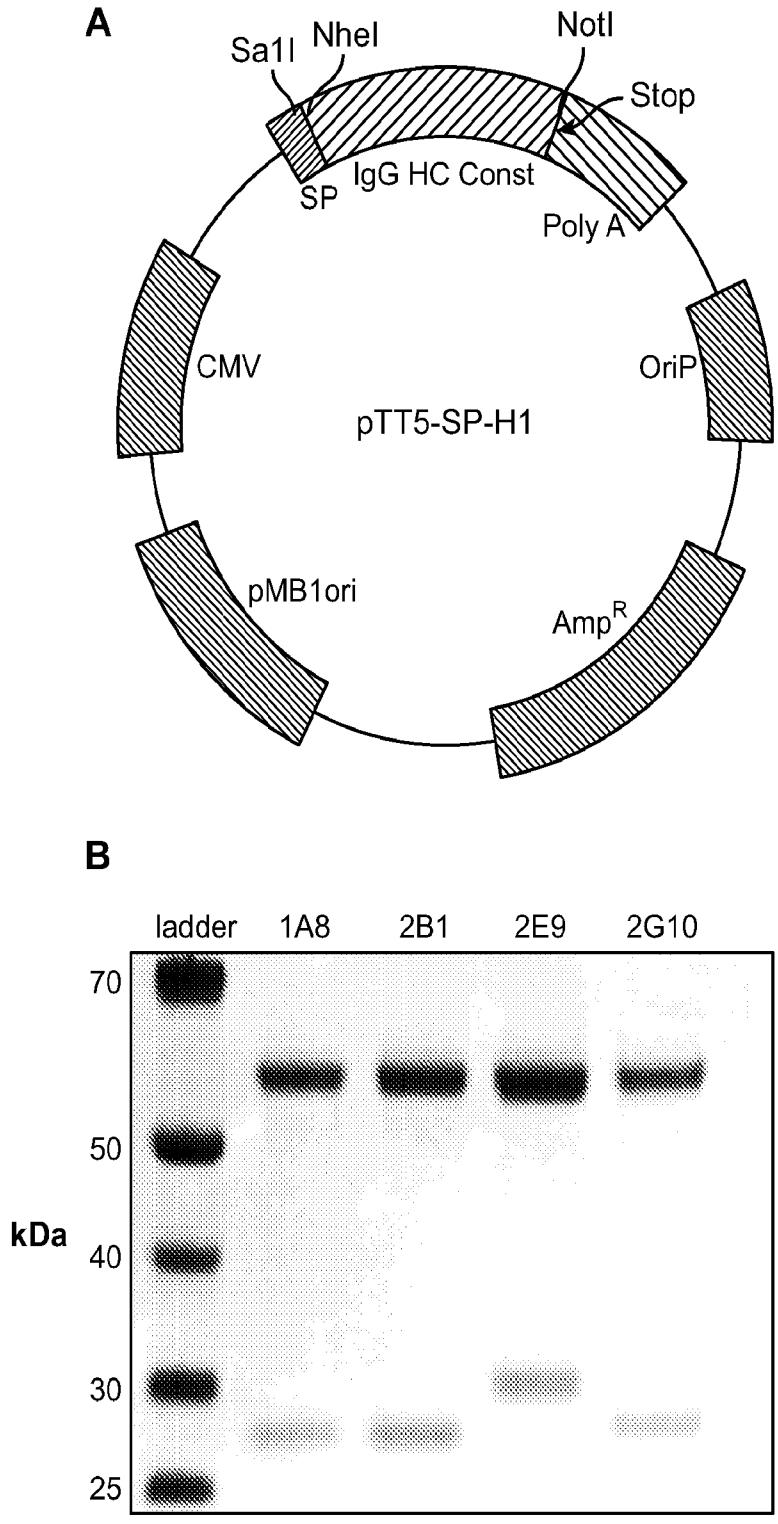
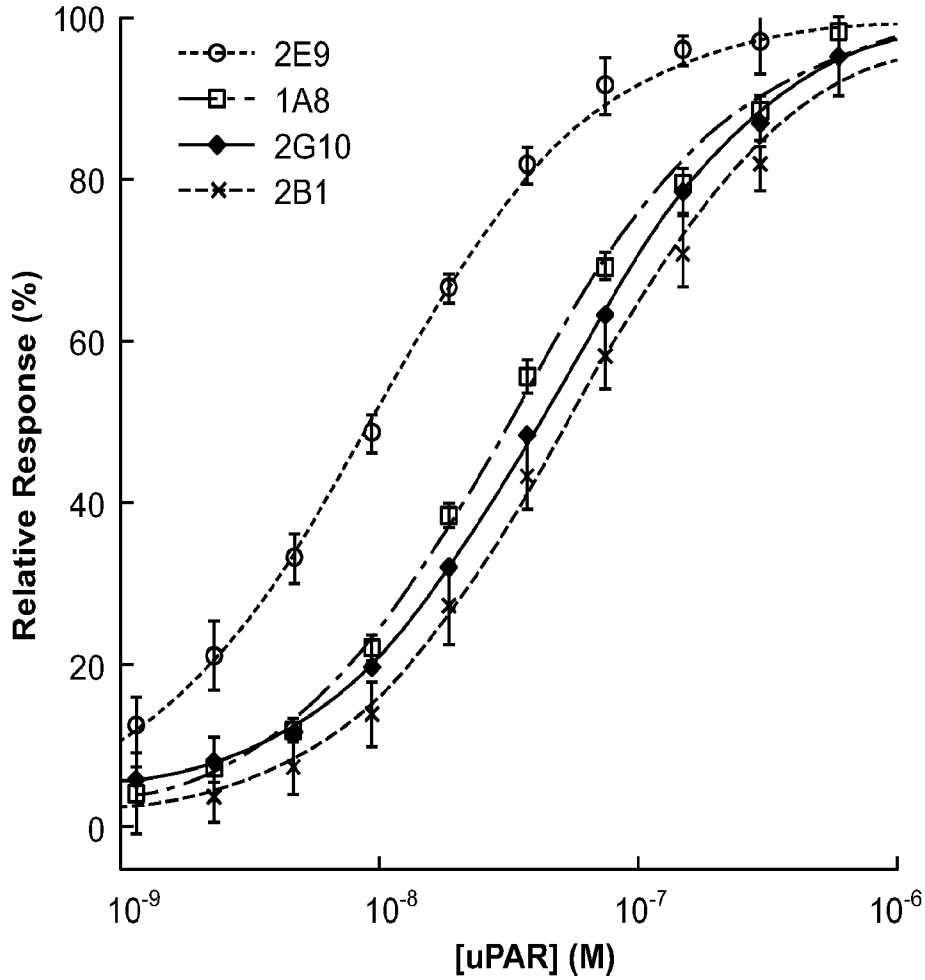

A
Sa1I
NheI
NotI
Stop
SP
IgG HC Const
Poly A
CMV
OriP
pTT5-SP-H1
pMB1ori
Amp^R
B
ladder
1A8
2B1
2E9
2G10
70
50
40
30
25
kDa

FIG. 3



Antibody	1A8	2B1	2E9	2G10
Kd (nM)	23.8+/-0.2	53.5+/-0.2	9.2+/-0.02	40.5+/-0.1

FIG. 4

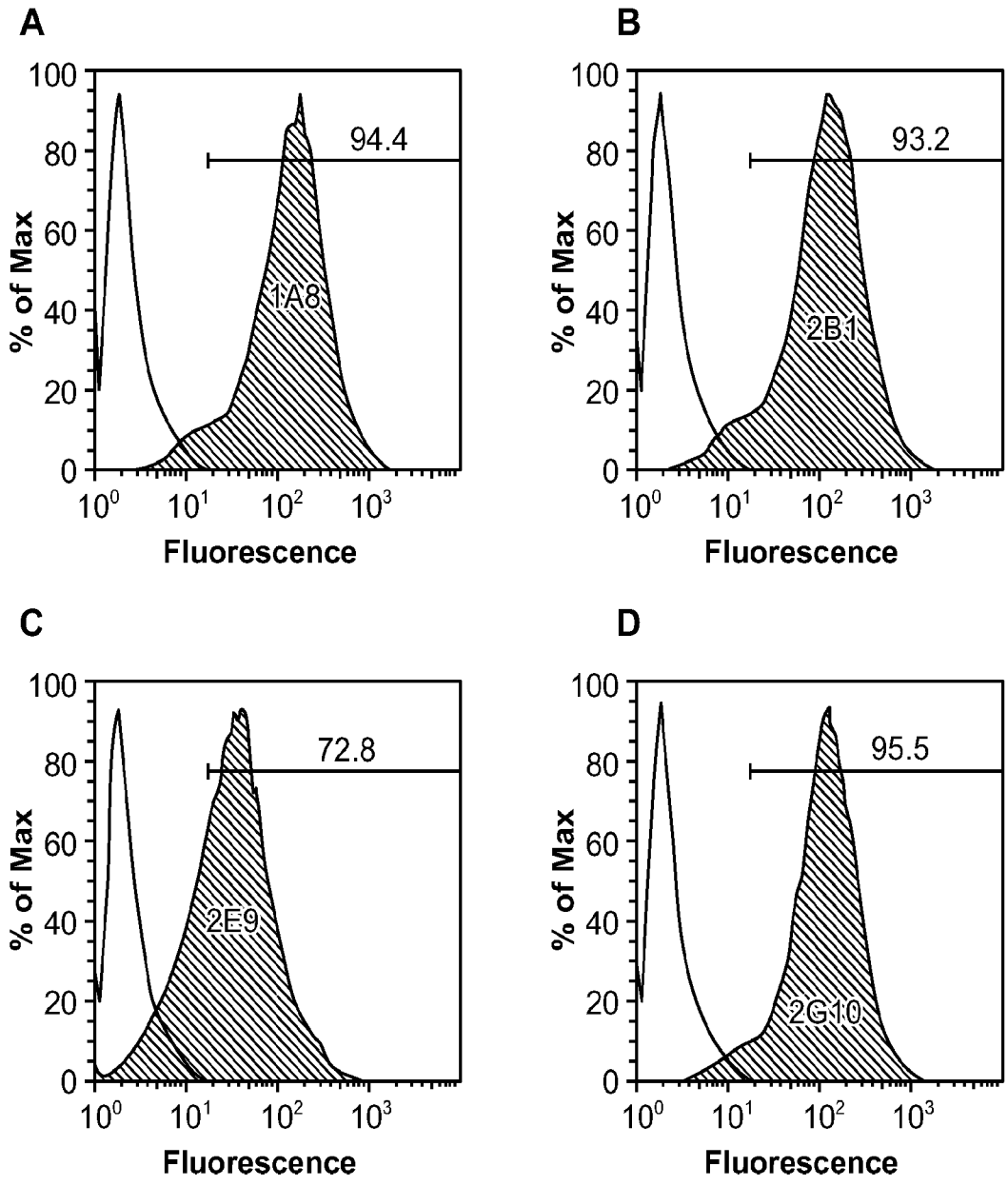

A
B
C
D
% of Max
Fluorescence
94.4
1A8
93.2
2B1
72.8
2E9
95.5
2G10
0
20
40
60
80
100
10^0
10^1
10^2
10^3

FIG. 5

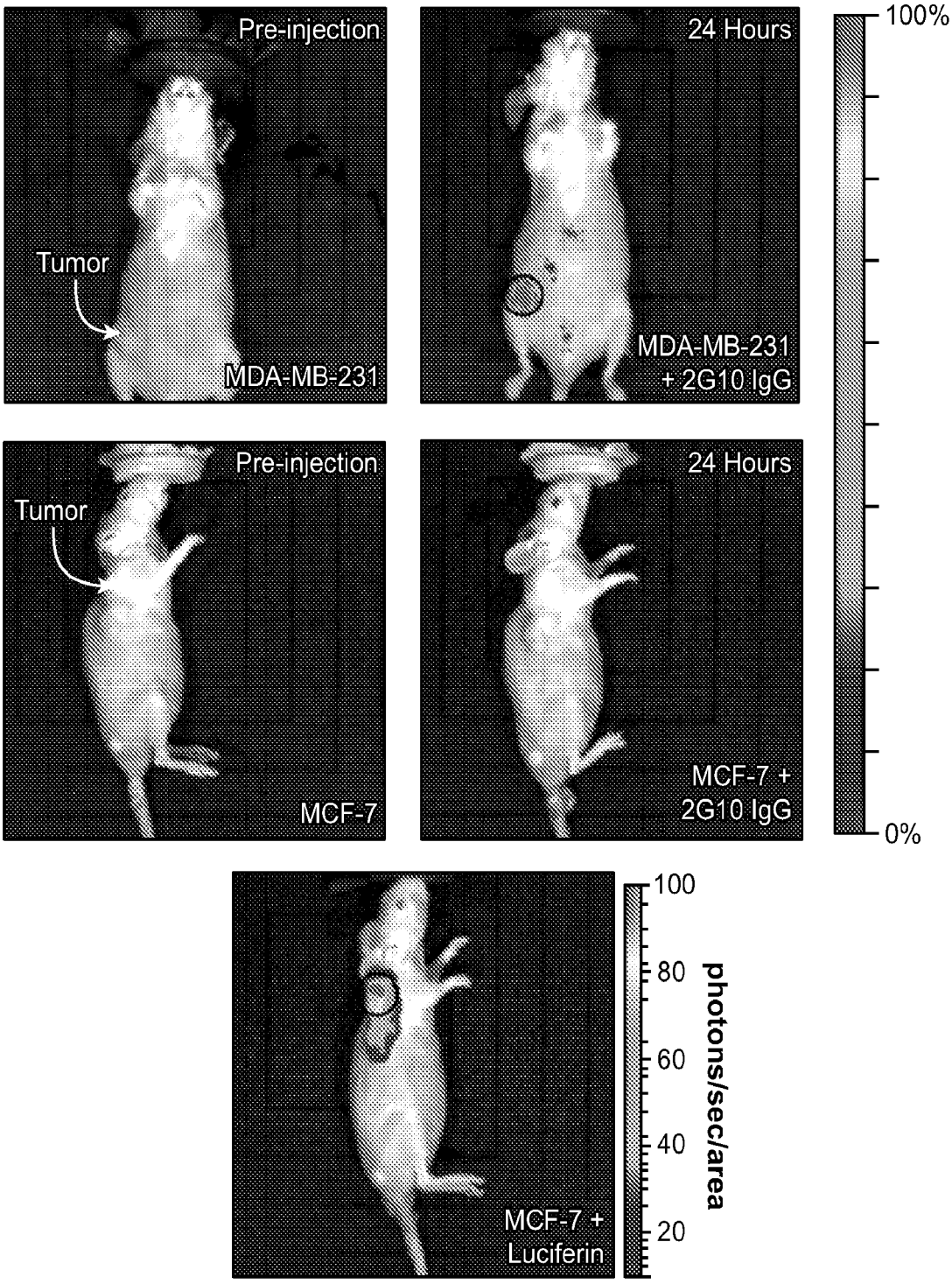

Pre-injection
Tumor
MDA-MB-231
24 Hours
MDA-MB-231
+ 2G10 IgG
100%
0%
Pre-injection
Tumor
MCF-7
24 Hours
MCF-7 +
2G10 IgG
MCF-7 +
Luciferin
100
80
60
40
20
photons/sec/area

FIG. 6

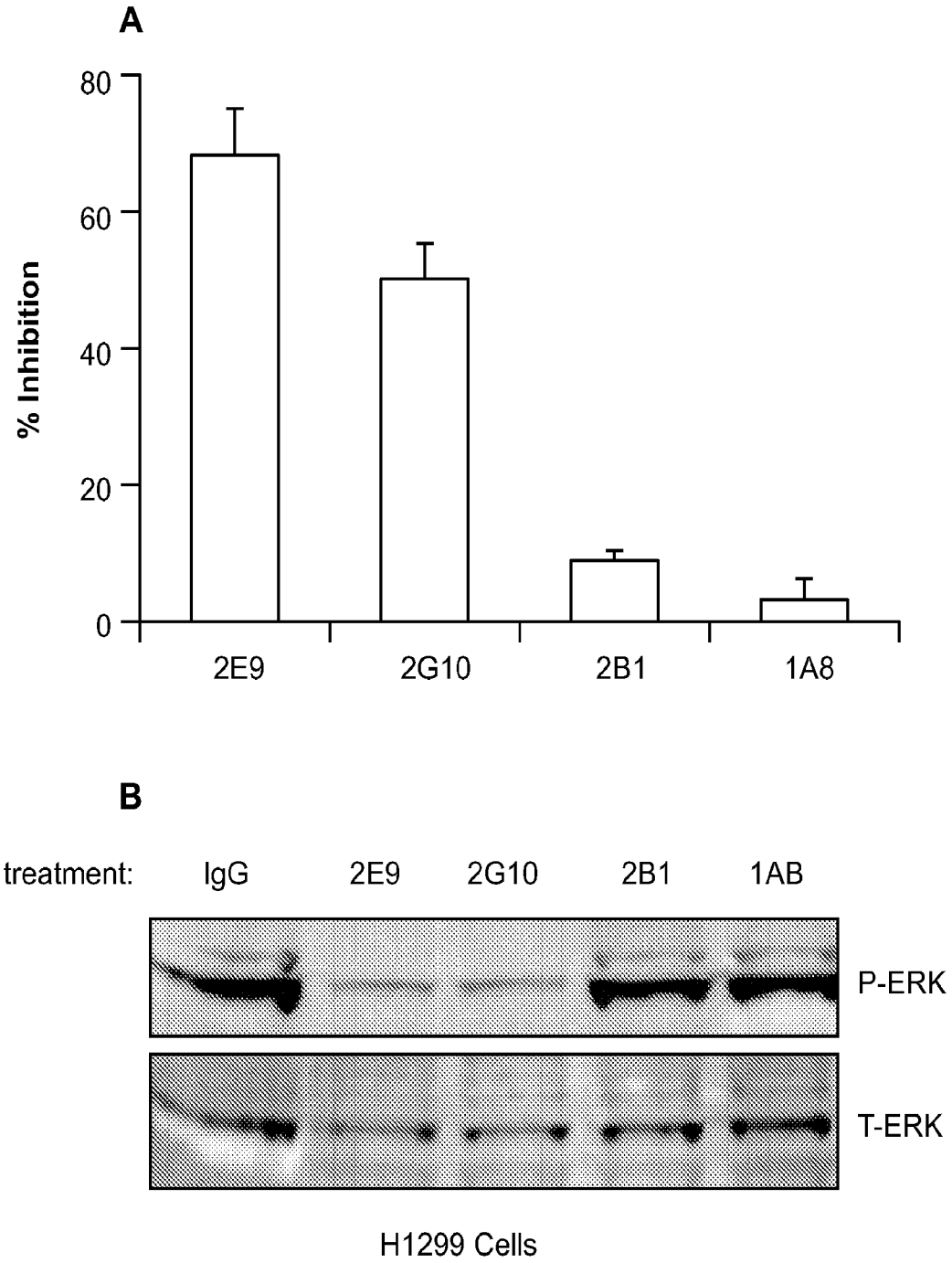

A
80
60
40
20
0
% Inhibition
2E9
2G10
2B1
1A8
B
treatment:
IgG
2E9
2G10
2B1
1AB
P-ERK
T-ERK
H1299 Cells

FIG. 7

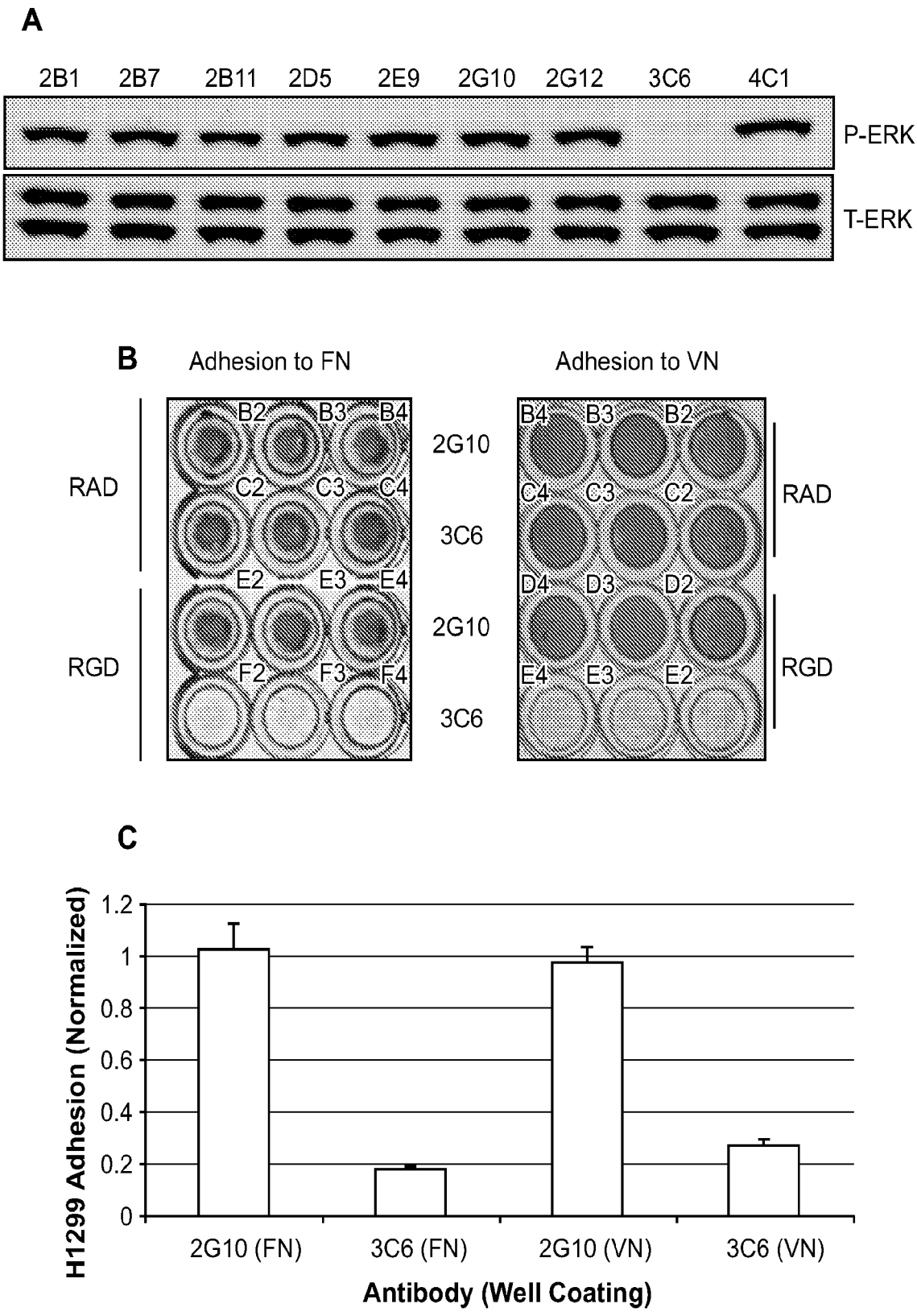

A
2B1
2B7
2B11
2D5
2E9
2G10
2G12
3C6
4C1
P-ERK
T-ERK
B
Adhesion to FN
Adhesion to VN
RAD
RGD
2G10
3C6
B2
B3
B4
C2
C3
C4
E2
E3
E4
F2
F3
F4
D4
D2
C
H1299 Adhesion (Normalized)
1.2
1
0.8
0.6
0.4
0.2
0
2G10 (FN)
3C6 (FN)
2G10 (VN)
3C6 (VN)
Antibody (Well Coating)

FIG. 8

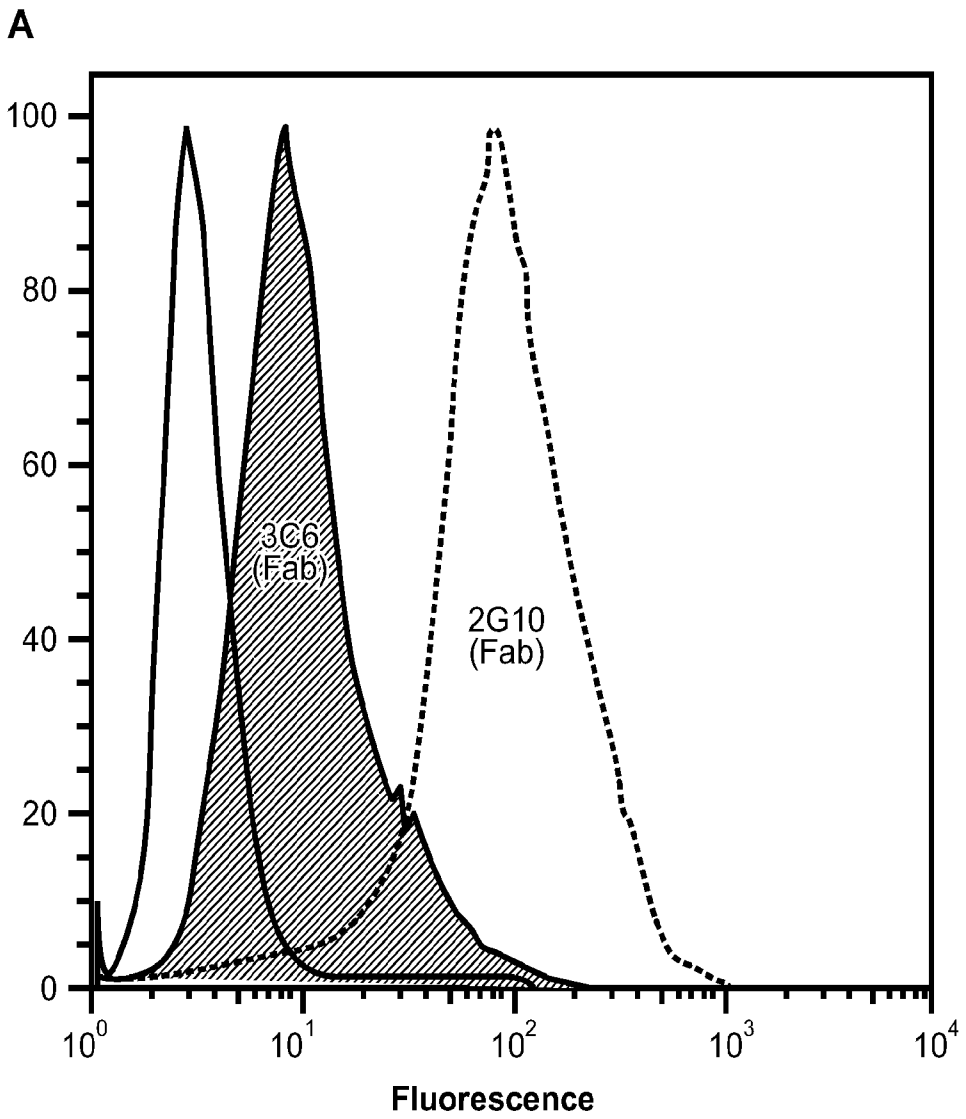

A
100
80
60
40
20
0
3C6
(Fab)
2G10
(Fab)
10^0
10^1
10^2
10^3
10^4
Fluorescence
B
IP
lysate
ctl
2G10
3C6
α5
uPAR
H1299 Cells

FIG. 9

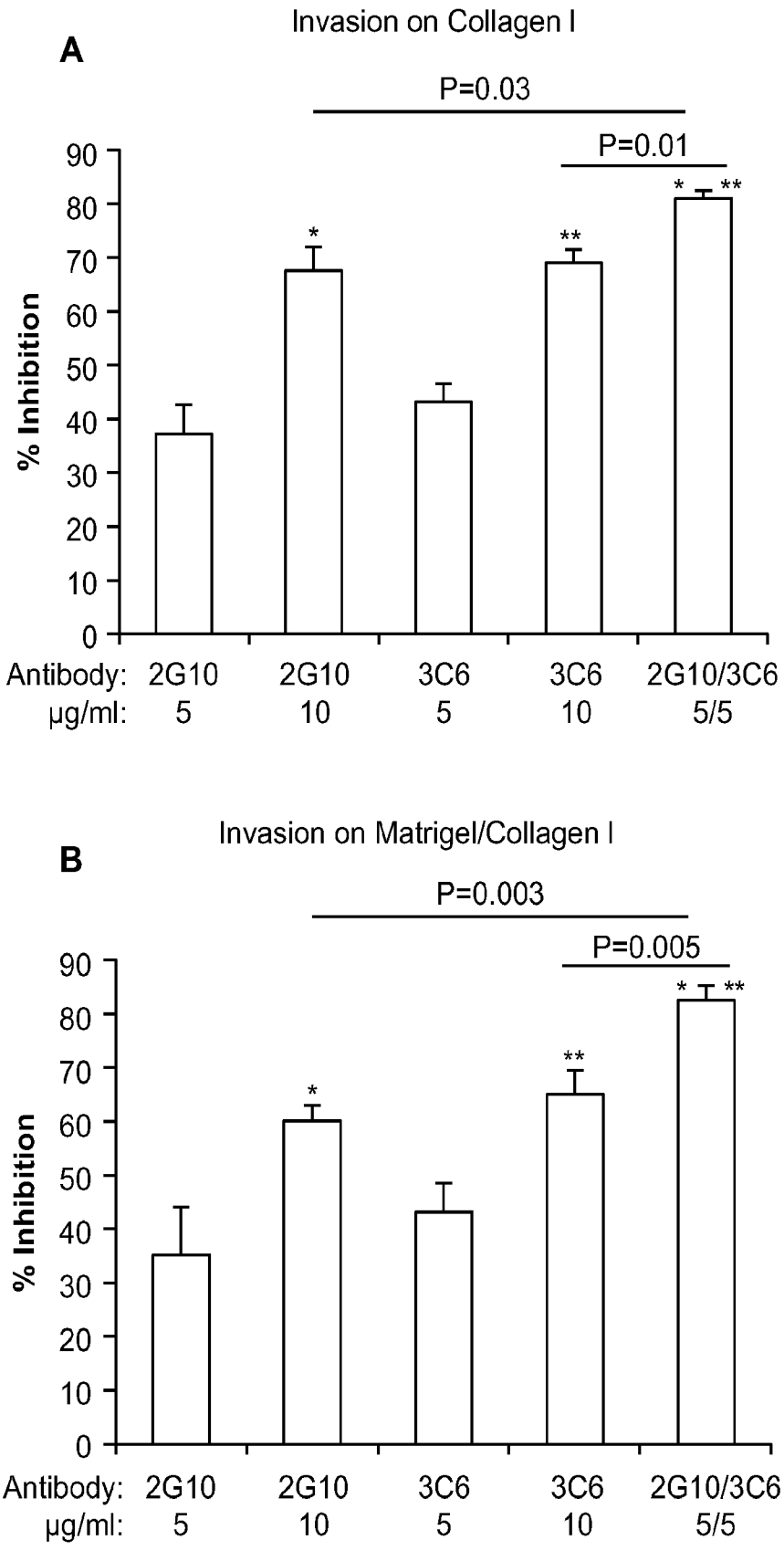

A
Invasion on Collagen I
P=0.03
P=0.01
% Inhibition
0
10
20
30
40
50
60
70
80
90
*
**
*
**
Antibody: 2G10 2G10 3C6 3C6 2G10/3C6
µg/ml: 5 10 5 10 5/5
B
Invasion on Matrigel/Collagen I
P=0.003
P=0.005
% Inhibition
0
10
20
30
40
50
60
70
80
90
*
**
*
**
Antibody: 2G10 2G10 3C6 3C6 2G10/3C6
µg/ml: 5 10 5 10 5/5

FIG. 10

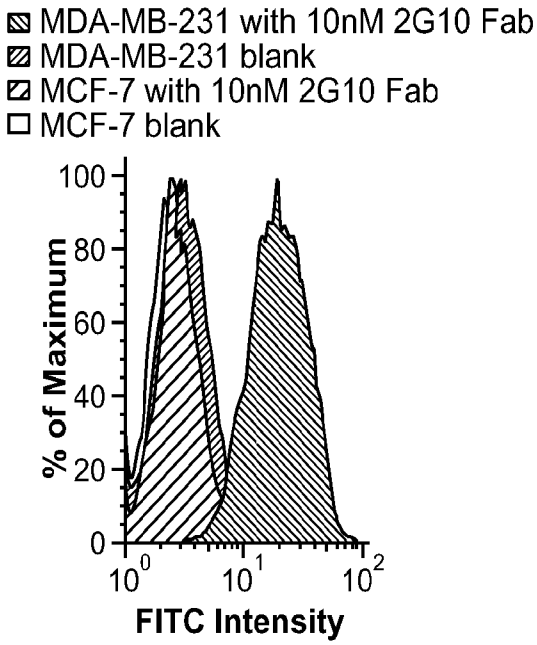

MDA-MB-231 with 10nM 2G10 Fab
MDA-MB-231 blank
MCF-7 with 10nM 2G10 Fab
MCF-7 blank
% of Maximum
100
80
60
40
20
0
10^0
10^1
10^2
FITC Intensity

FIG. 11

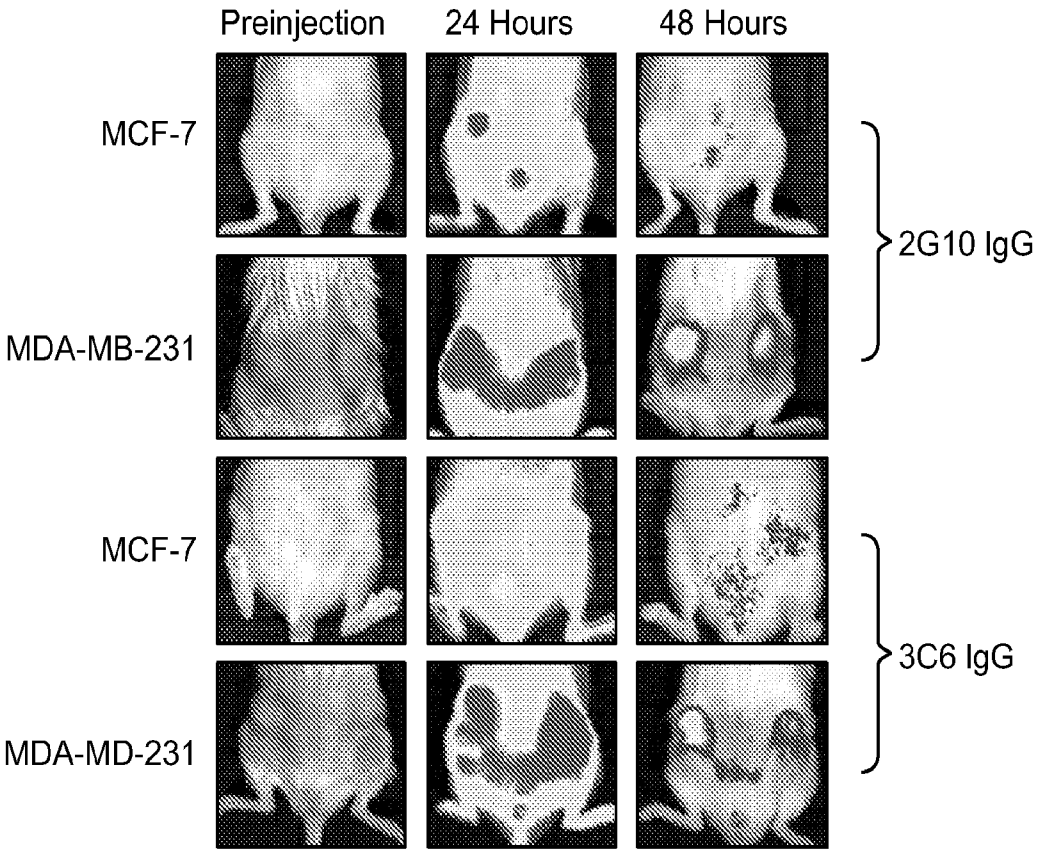

Preinjection
24 Hours
48 Hours
MCF-7
MDA-MB-231
2G10 IgG
MCF-7
MDA-MD-231
3C6 IgG

FIG. 12

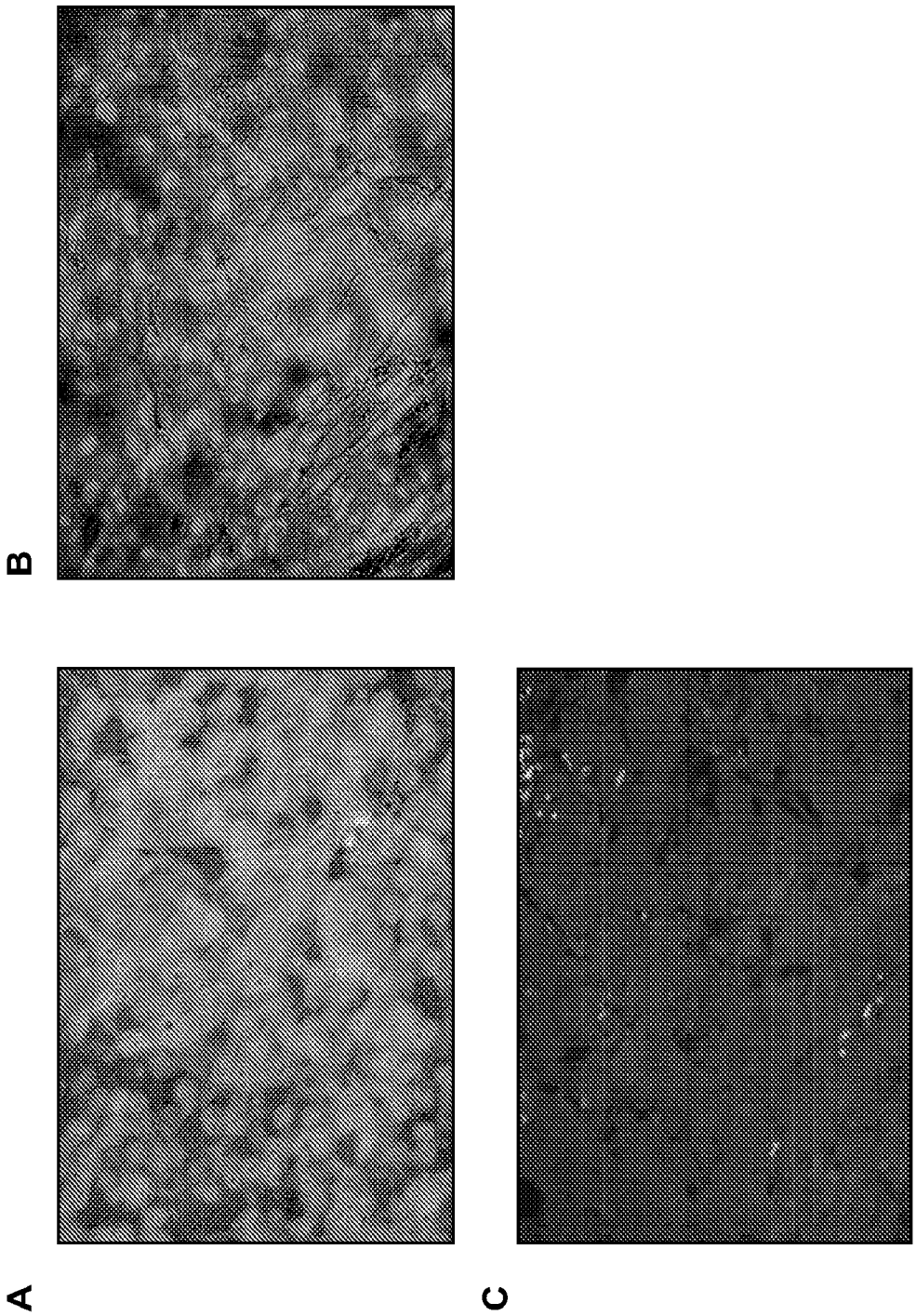


FIG. 13

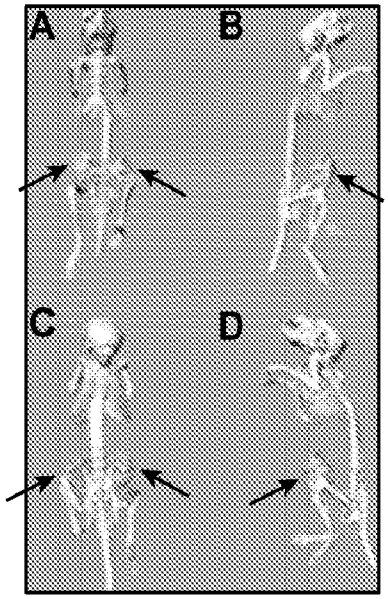

A
B
C
D

FIG. 14

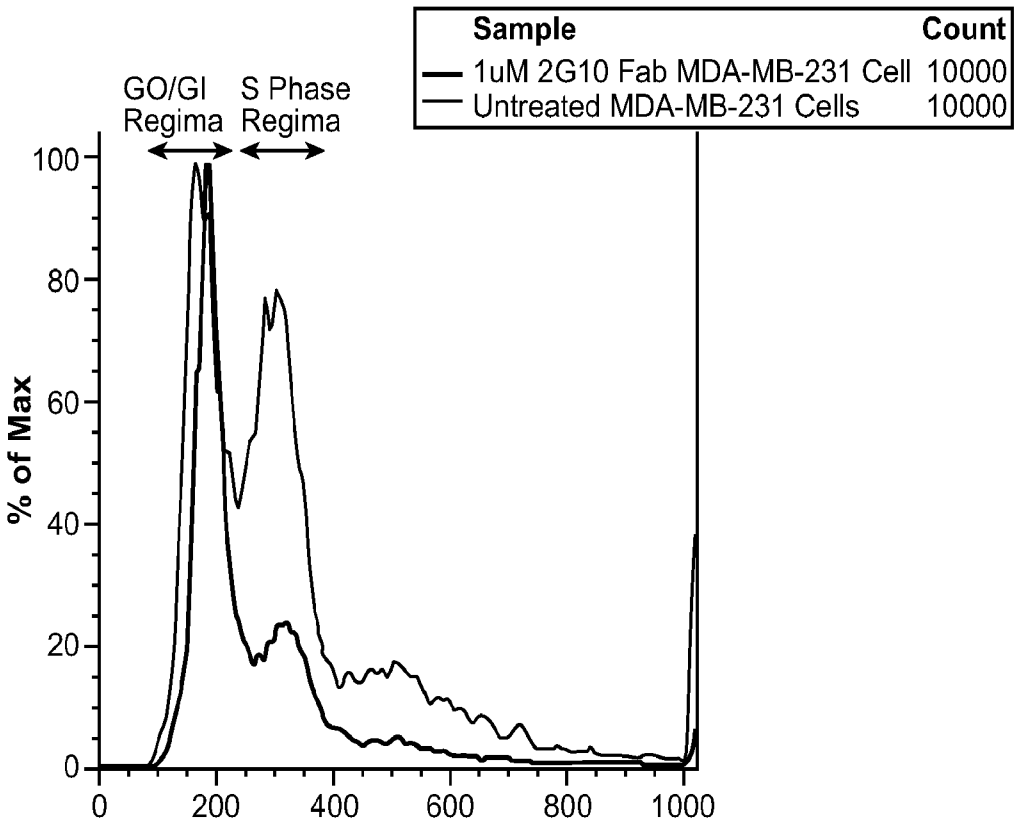

Sample
Count
1uM 2G10 Fab MDA-MB-231 Cell 10000
Untreated MDA-MB-231 Cells 10000
GO/GI Regima
S Phase Regima
% of Max
100
80
60
40
20
0
0
200
400
600
800
1000

FIG. 15

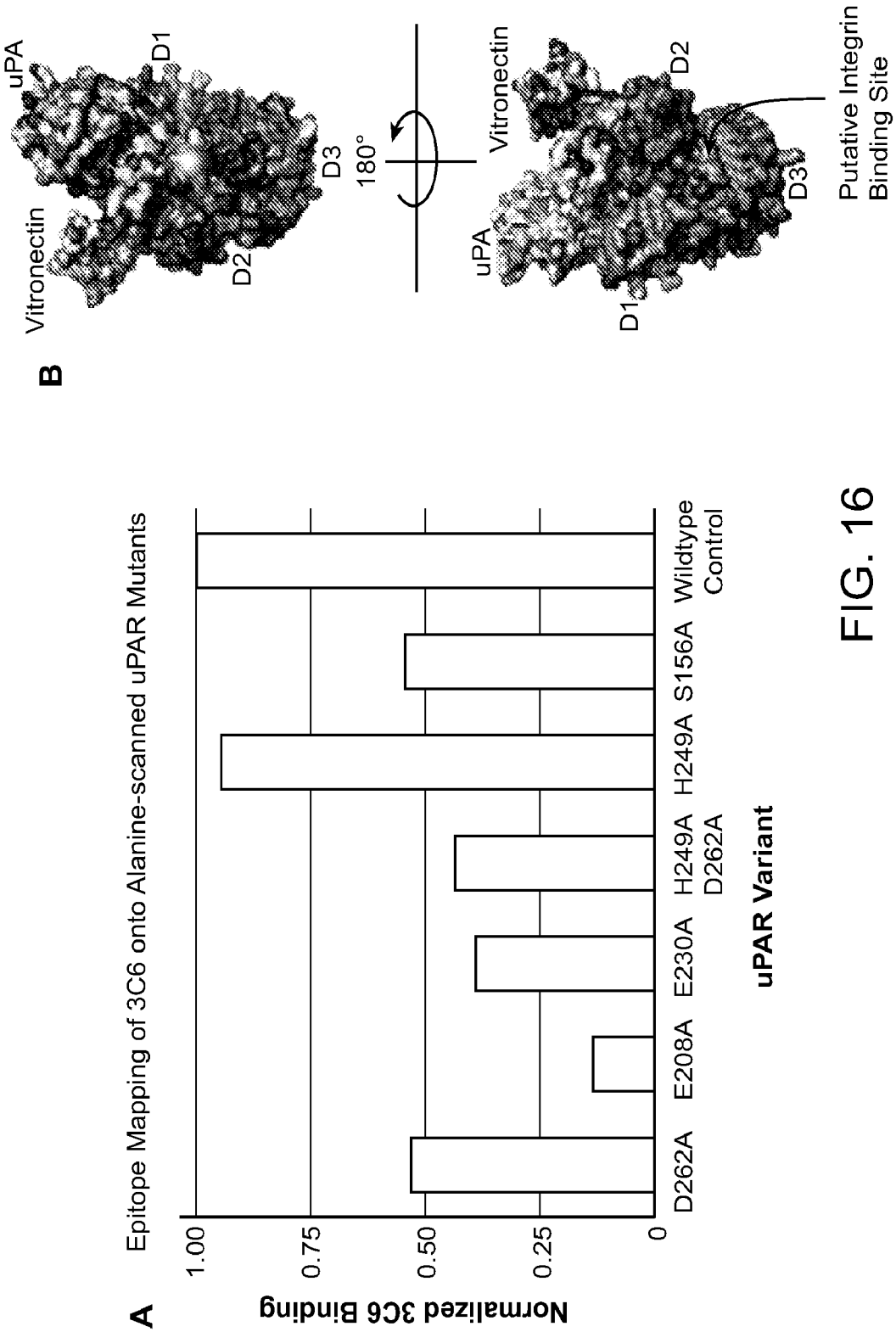

A
Epitope Mapping of 3C6 onto Alanine-scanned uPAR Mutants
Normalized 3C6 Binding
1.00
0.75
0.50
0.25
0
D262A
E208A
E230A
H249A D262A
H249A S156A
Wildtype Control
uPAR Variant
B
uPA
D1
Vitronectin
D2
D3
180°
Vitronectin
uPA
D2
D1
D3
Putative Integrin Binding Site

FIG. 16