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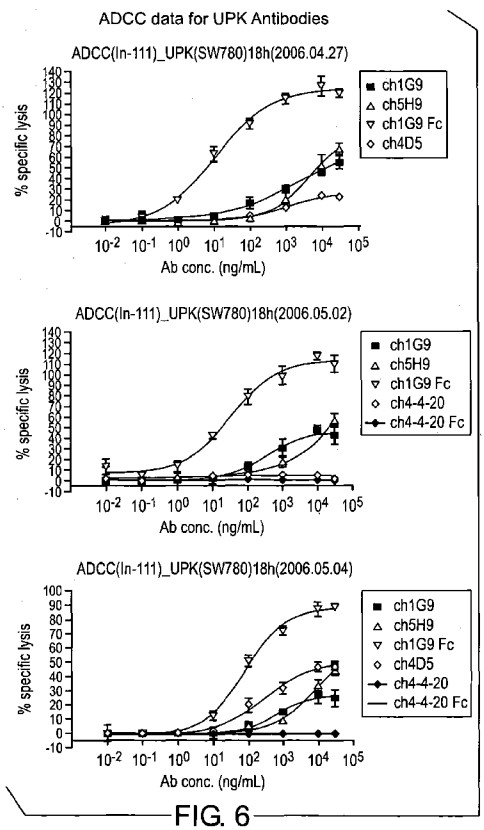

FIG. 6

(57) Abstract: This invention provides antibodies that specifically recognize and bind to UPK-Ib. Also included are diagnostic and therapeutic assays directed to diseases associated with unregulated levels of UPK-Ib using said antibodies. The antibodies and derivatives thereof can also be useful in the diagnisic and/or treatment of cancer associated with abnormal expression of UPK-Ib.

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COMPOSITION AND METHOD OF TREATING CANCER WITH AN ANTI-UROPLAKIN IB ANTIBODY

SEQUENCE LISTING

A sequence listing is attached hereto referring to SEQ ID NOS: 1-16, and is incorporated by reference herein.

FIELD OF THE INVENTION

Disclosed is the generation of novel monoclonal antibodies or fragments thereof to an extracellular domain of uroplakin and hybridoma cell-lines expressing such antibodies, as well as methods for using antibodies or fragments thereof for diagnostic, analytical, and therapeutic purposes.

BACKGROUND

Uroplakins (*Uro*thelial *Plaq*ue-related proteins) are structural proteins present on the luminal surface of the bladder, covering 90% of the surface of the bladder wall. Four uroplakins (*i.e.*, UPK-Ia, UPK-Ib, UPK-II, and UPK-III) form a 16 nm particle present on the apical surface of the bladder (Wu et al., 1990, *J. Biol. Chem.* 265: 19170-19779). These particles strengthen and stabilize the bladder wall though interactions with the cytoskeleton. Mice deficient in UPK-III expression fail to form these 16 nm particles, and were observed to exhibit a phenotype that included hyperpermeability of the bladder and hydronephrosis.

In 2005, there were an estimated 63,000 new cases of bladder cancer and 13,000 deaths in the United States (Jemal et al., 2005, *Cancer J. Clin.* 55: 10-30). The majority of these cases were initially diagnosed in patients over the age of sixty. The most common treatment at this time is surgical removal of affected tissue and local administration of Bacille Calmette-Guerin (BCG), a vaccine more commonly used for the prevention of tuberculosis in many countries. Use of the BCG vaccine reduces recurrence of transitional cell carcinoma (TCC) of the bladder from approximately 70% to as low as 10%. However, recurrent TCC typically has extensive tumor invasion into the bladder wall and metastases. While the efficacy of platinum-based chemotherapeutic agents are under active investigation, the typical TCC patient is less tolerant to the side effects of such drugs due for example advanced patient age. Clearly, a more specific therapy with fewer potential side effects would be of great value in the treatment of this common malignancy.

SUMMARY

The present invention relates to novel antibodies capable of specifically binding to UPK-1b. The UPK-Ib antibodies are capable of specifically binding to the native form of UPK-Ib expressed on a human cell. The antibodies and the therapeutically active fragments thereof of the invention include murine, chimeric, primatized, humanized, and human antibodies, as well as the amino acid and nucleic acid sequences coding for these antibodies.

In one embodiment, the antibody or fragment thereof specifically binds to a fusion protein comprising an extracellular region of human UPK-Ib and a human IgG Fc region, wherein the antibody or fragment thereof does not recognize the Fc region of the fusion protein. In some instances, the extracellular region of human UPK-Ib comprises the amino acid sequence set forth in SEQ ID NO: 13 and the Fc region comprises the amino acid sequence set forth in SEQ ID NO: 14.

In another embodiment, the antibody or fragment thereof of comprises a heavy chain variable region of SEQ ID NO: 1 and a light chain variable region of SEQ ID NO: 3. In another embodiment, the antibody or fragment thereof comprises a heavy chain variable region of SEQ ID NO: 5 and a light chain variable region of SEQ ID NO: 7.

In certain aspects, the antibody or fragment thereof comprises a heavy chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO: 2 and a light chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO: 4. Also included in the invention is an antibody or fragment thereof comprising a heavy chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO: 6 and a light chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO: 8.

In another embodiment, the antibody or fragment thereof can specifically bind to UPK-Ib expressed on a cell. The binding of the UPK-Ib antibodies or fragments thereof can occur *in vitro*, *in vivo*, *in situ*, unfixed tissue, or any combination thereof.

In yet another embodiment, the UPK-Ib specific antibodies or fragments thereof are of murine origin. In addition, the UPK-Ib specific antibodies or fragments thereof can be primatized or humanized. The invention also includes variations of the UPK-Ib antibodies including, but not limited to, Fv, Fab, Fab', $F(ab')_2$, single chain Fv (scFv), and the like.

In one embodiment, the UPK-Ib specific antibodies or fragments thereof comprise a human IgG wild type Fc. Examples of IgG isotypes include but are not limited to IgG1, IgG2, IgG3, IgG4, and any allotype thereof. In certain aspects, the UPK-Ib specific antibodies can comprises an enhancing Fc mutant.

In another embodiment, the UPK-Ib specific antibodies or fragments thereof can further comprise an anti-tumor agent. An anti-tumor agent includes, but is not limited to, a chemotherapeutic agent and an anti-cell proliferation agent. Examples of a chemotherapeutic agent include, but are not limited to, alkylating agents nitrosoureas, antimetabolites, antitumor antibiotics, plant alkyloids, taxanes, hormonal agents. Examples of an anti-proliferation agent include but are not limited to apoptosis-inducing agents and cytotoxic agents.

The invention also includes a method of diagnosing a disease state in a mammal. The method comprises determining the level of UPK-Ib using a UPK-Ib specific antibody or fragment thereof of the invention. The disease state includes, but is not limited to, bladder cancer, transitional cell carcinoma of the bladder, ovarian cancer, Brenner ovarian cancer, lung cancer, and the like.

The UPK-Ib specific antibodies or fragments thereof can also be used to induce antibody-dependent cellular cytotoxicity against a cell expressing UPK-Ib. Antibody-dependent cellular cytotoxicity can be accomplished by contacting a cell expressing UPK-Ib with an antibody or fragment thereof of the invention. The cell can be a cancer cell including, but is not limited to, a bladder cell, an ovarian cell, and a lung cell.

The invention also includes a method of treating a cancer associated with abnormal expression level of UPK-Ib. The method comprises contacting a cell expressing UPK-Ib with a UPK-Ib specific antibody or fragment thereof of the invention. Such cancers include but are not limited to bladder cancer, transitional cell carcinoma of the bladder, ovarian cancer, Brenner ovarian cancer, and lung cancer.

In certain aspects, the UPK-Ib specific antibody or fragment thereof can be used in combination with other cancer therapy, such as surgery, radiation, gene therapy, hormone therapy, and immunotherapy. The combination therapy can involve administering a UPK-Ib specific antibody or fragment thereof concomitantly, in succession, prior to, or subsequent to the other types of cancer therapy.

The invention includes an isolated or purified humanized UPK-Ib specific antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1 or a glycosylation variant, fusion molecule or a chemical derivative thereof.

In another embodiment, the isolated or purified humanized UPK-Ib specific antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 3 or a glycosylation variant, fusion molecule or a chemical derivative thereof.

In yet another embodiment, the isolated or purified humanized UPK-Ib specific antibody

comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5 or a glycosylation variant, fusion molecule or a chemical derivative thereof.

In a further embodiment, the isolated or purified humanized UPK-Ib specific antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 7 or a glycosylation variant, fusion molecule or a chemical derivative thereof.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the disclosed compounds, compositions, and methods.

BRIEF DESCRIPTION OF DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 shows the sequence of the UPK-G1 fusion protein sequence. The region corresponding to the human IgG1 Fc region is underlined. The 121 amino acids representing UPK-Ib sequence are shaded.

Figure 2 is a schematic diagram illustrating the region of UPK-Ib extracellular domain used for generating the UPK-G1 fusion protein.

Figure 3A is a chart depicting the results from individual UPK-Ib specific monoclonal antibodies (MAbs). Plates were coated with 50 ng UPK-G1 fusion protein and blocked with bovine serum albumin. Hybridoma supernatants were added to plates and serial 3-fold dilution series were incubated overnight. Plates were washed the following morning and incubated with alkaline phosphatase conjugated Goat-anti-Mouse IgG (H+L) from Pierce Chemical at a dilution of 1:4000 for one hour. After several washes, the fluorescent substrate 5-methylumbelliferyl phosphate was added to each well. Fluorescence determinations are plotted against the log MAb concentrations.

Figure 3B is a chart depicting the results from a binding assay of chimeric and mouse antibody to UPK1-Fc as determined by ELISA. The antibodies tested include monoclonal antibodies 1GP and 5H9 and their corresponding chimeric antibody.

Figure 4A is a series of charts that depict the binding of 1G9 and 5H9 monoclonal antibodies (MAb) to UPK-Ib transfected cells. Each supernatant was incubated with either UPK-Ib negative HEK293 cells or with UPK-Ib transfected HEK293 cells. Cells were washed and incubated with an anti-mouse IgG antibody conjugated to phycoerythrin. Cells were washed

again and analyzed for fluorescence on a Becton Dickinson FACsCalibur. The top two curves demonstrate minimal staining of the 5H9 and 1G9 monoclonal antibodies to a UPK-Ib negative 293 cell line. The bottom two curves represent good staining of UPK-Ib transfected 293 cells.

Figure 4B is a series of charts depicting the binding of 1G9 and 5H9 monoclonal antibodies to the human transitional cell carcinoma line, SW780. SW780 cells were stained as described elsewhere herein. Cells were stained with either the 1G9 or 5H9 culture supernatants (wide curves) or with a negative control of hybridoma supernatant (thin curves).

Figure 5A shows the amino acid sequences of 1G9 and 5H9 heavy and light chain CDRs.

Figure 5B shows the nucleic acid sequences of 1G9 and 5H9 heavy and light chain CDRs.

Figure 6 is a series of charts showing results from Antibody-Dependent Cellular Cytotoxicity (ADCC) assays. The ADCC studies were performed using SW780 cells as tumor targets and human peripheral blood mononuclear cells (PBMCs) as effector cells. These assays have been performed using several independent sources of human donor cells at an effector:target ratio of 70:1. The wild type chimeric forms of 1G9 and 5H9 were observed to mediate ADCC. The introduction of the enhancing Fc mutant into the human Fc region enhanced killing by 1G9 dramatically.

Figure 7 shows an immunohistochemical analysis of four independent tissue samples representing normal bladder tissue and tissue obtained from patients with tumors Grade I-III. Staining of normal bladder is limited to the apical surface of the bladder wall. Staining of tumor tissue demonstrates the presence of UPK-Ib on tumor cells after the disease has become more advanced. The UPK-Ib specific MAb, 1A10, was used to determine target expression levels.

Figure 8 is a chart summarizing the immunohistochemical data of UPK-Ib expression. The UPK-Ib specific MAb, 1A10, was used to determine target expression levels on a normal/tumor bladder array (Biomax). The staining of normal bladder was limited to the luminal area of the bladder wall.

Figure 9 shows the amino acid sequence of the extra cellular domain of UPK-Ib (upper case) fused with human G1 Fc (lower case) by overlapping PCR. A proteinase digestion site and enterokinase site (underlined) (SEQ ID NO: 16) were inserted between the UPK-Ib extra cellular domain and human G1 Fc. Artificial signal sequence was added at the amino acid terminal.

Figure 10 is a chart depicting the binding of chimeric 1G9-enhanced Fc mutant antibody to human lung tumor cell lines A549 and EKVX. The CD32B antibody (2B6-enhancing Fc mutant) was used as negative control.

DETAILED DESCRIPTION

Uroplakins (*Uro*thelial *Plaq*ue-related proteins) include UPK-1a, UPK-1b, UPKII and UPKIII are structural proteins present on the luminal surface of the bladder, covering 90% of the surface of the bladder wall. Expression of uroplakins has been demonstrated in a large percentage of bladder transitional cell carcinomas (TCC) in humans, making it an attractive target candidate for monoclonal antibody-based therapy. Other diseases and disorders associated with unregulated expression of uroplakins include, but are not limited to, ovarian and lung cancers.

The present invention relates to novel antibodies capable of specifically binding to UPK-1b. In one aspect, the antibody is an antibody that is capable of specifically binding to the native form of UPK-Ib expressed on a human cell. The antibody of the invention includes monoclonal antibodies of murine, chimeric, primatized, humanized, and human, as well as the amino acid and nucleic acid sequences coding for these antibodies and therapeutically active fragments of these antibodies.

The invention also includes the use of these antibodies as a medicament for the therapeutic treatment of cancers overexpressing UPK-Ib or any pathology connected with the overexpression of UPK-Ib and in processes or kits for diagnosis of illnesses connected with the overexpression of UPK-Ib.

1. Acronyms and Definitions

The following acronyms and definitions are used throughout the application, unless indicated otherwise. In accordance with this detailed description, the following abbreviations and definitions apply. It must be noted that as used herein, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an antibody" includes a plurality of such antibodies, and reference to "the dosage" includes reference to one or more dosages and equivalents thereof known to those skilled in the art, and so forth.

1.1 Acronyms

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ADCC	Antibody-Dependent Cellular Cytotoxicity
ADCP	antibody dependent cell mediated phagocytosis
BCG	Bacille Calmette-Guerin
bp	base pair

CCDS	charge coupled devices
CDC	complement dependent cytotoxicity
CDR	complementarity determining regions
CHO	Chinese hamster ovary
Chol	cholesterol
CMV	cytomegalovirus
CPM	counts per minute
DCP	dicetyl phosphate
DMPC	dimyristyl phosphatidylcholine
DMPG	dimyristyl phosphatidylglycerol
ELISA	enzyme-linked immunosorbant assay
FcR	Fc receptor
FR	framework
HPLC	high performance liquid chromatography
HIV	human immunodeficiency virus
huMAb	human monoclonal antibody
IgH	immunoglobulin heavy chain
IgL	immunoglobulin light chain.
IgG	immunoglobulin G
LCR	ligase chain reaction
LTR	long terminal repeat
MAb	monoclonal antibody
MMTV	mouse mammary tumor virus
MoMuLV	Moloney murine leukemia virus
MSCV	murine stem cell virus
NK	natural killer
PBMCs	human peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCR	polymerase chain reaction
rhuMAb	recombinant human monoclonal antibody
RIA	radioimmunoassays
RT-PCR	reverse transcription polymerase chain reaction
scFv	single chain variable fragment

SV40	simian virus 40
TCC	transitional cell carcinoma
TLC	thin layer chromatography
tk	thymidine kinase
UPK	uroplakin
VH	variable heavy
VL	variable light

1.2 Definitions

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. The following terms are provided below.

An "amino acid" as used herein is meant to include both natural and synthetic amino acids, and both D and L amino acids. "Standard amino acid" means any of the twenty L-amino acids commonly found in naturally occurring peptides. "Nonstandard amino acid residues" means any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or derived from a natural source. As used herein, "synthetic amino acid" also encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and substitutions. Amino acids contained within the peptides, and particularly at the carboxy- or amino-terminus, can be modified by methylation, amidation, acetylation or substitution with other chemical groups, which can change a peptide's circulating half life without adversely affecting activity of the peptide. Additionally, a disulfide linkage may be present or absent in the peptides.

An "antigen" is any agent, *e.g.*, a protein (or immunogenic fragments of proteins such as a fragment of an adhesion protein), a peptide or peptide conjugate, immunogen, or a polysaccharide, that elicits an immune response. In this instance, the antigen is derived from a uroplakin, such as UPK-Ib. The immunogenic composition can comprise one or more uroplakin antigens or immunogens. For example, it can contain a UPK-Ia, UPK-Ib, UPK-II, or UPK-III antigen, or any combinations thereof. In one embodiment, the antigen is a recombinant protein comprising a 121 amino acid long extracellular region of the native human UPK-Ib protein having the sequence set forth in SEQ ID NO: 13. The antigen can further be conjugated to another protein such as an IgG1 Fc region. The amino acid sequence of a desired IgG1 Fc region is set forth in SEQ ID NO: 14. The fusion protein comprising comprising a 121 amino acid long

extracellular region of the native human UPK-Ib protein fused to the human IgG1 Fc region is designated as a UPK-G1 fusion protein. The amino acid sequence of the recombinant fusion protein UPK-G1 is set forth in SEQ ID NO: 15.

As used herein, "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, $F(ab')_2$, Fv fragments, and single chain variable fragments (scFv) which are capable of binding an epitopic determinant. "Antibody fragments" refer to antigen-binding immunoglobulin peptides which are at least about 5 to about 15 amino acids or more in length, and which retain the capacity to bind to the antigen. Antibody, as used herein, includes polyclonal and monoclonal antibodies, chimeric, hybrid, single chain, primatized, humanized antibodies, human, as well as Fab fragments, including the products of a Fab or other immunoglobulin expression library.

An "antibody heavy chain," as used herein, refers to the larger of the two types of polypeptide chains present in all antibody molecules in their naturally occurring conformations.

An "antibody light chain," as used herein, refers to the smaller of the two types of polypeptide chains present in all antibody molecules in their naturally occurring conformations.

"Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (*e.g.*, natural killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently lyse the target cell. To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. Nos. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC).

A "chimeric antibody" refers to a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a constant region derived from a human immunoglobulin.

The term "cancer" as used herein is defined as a disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers include, but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, bladder cancer, lung cancer and the like.

The term "cytotoxic" refers to an agent having a toxic or destructive effect on one or more cells, such as cancer cells, including in a tumor, such as a solid tumor.

"Derivative" in the context of proteins and peptides includes any purposefully generated amino acid sequence that in its entirety, or in part, comprises a substantially similar amino acid

sequence to a desired protein. For example, a recombinant protein derived from an uroplakin may be characterized by a protein having single or multiple amino acid substitutions, deletions, additions, or replacements, or any combinations thereof. The term derivative can also be applied to the antibodies described herein such that "derivative" includes any purposefully generated peptide, which in its entirety, or in part, comprises a substantially similar amino acid sequence to an antibody having UPK-Ib or a fragment thereof as the immunogen. Derivatives of the antibodies may be characterized by single or multiple amino acid substitutions, deletions, additions, or replacements. Derivatives may include: (a) derivatives in which one or more amino acid residues are substituted with conservative or non-conservative amino acids; (b) derivatives in which one or more amino acids are added; (c) derivatives in which one or more of the amino acids of the amino acid sequence includes a substituent group; (d) derivatives in which amino acid sequences or a portion thereof is fused to another peptide (*e.g.*, serum albumin or protein transduction domain); (e) derivatives in which one or more nonstandard amino acid residues (*e.g.*, those other than the 20 standard L-amino acids found in naturally occurring proteins) are incorporated or substituted into the amino acid sequences; (f) derivatives in which one or more non-amino acid linking groups are incorporated into or replace a portion of the amino acids; and (g) derivatives in which one or more amino acid is modified by glycosylation, acetylation, myristylation, and the like.

As used herein, the term "diabody molecule" refers to a complex of two or more polypeptide chains or proteins, each comprising at least one VL and one VH domain or fragment thereof, wherein both domains are comprised within a single polypeptide chain. "Diabody molecule" includes molecules comprising an Fc or a hinge-Fc domain. The polypeptide chains in the complex may be the same or different, *i.e.*, the diabody molecule may be a homo-multimer or a hetero-multimer. "Diabody molecule" includes dimers or tetramers or the polypeptide chains containing both a VL and VH domain. The individual polypeptide chains comprising the multimeric proteins may be covalently joined to at least one other peptide of the multimer by interchain disulfide bonds.

"Effector function" as used herein refers to a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, antibody dependent cell mediated phagocytosis (ADCP), and complement dependent cytotoxicity (CDC). Effector functions include both those that operate after the binding of an antigen and those that operate independent of antigen binding.

"Effector cell" as used herein refers to a cell of the immune system that expresses one or

more Fc receptors and mediates one or more effector functions. Effector cells include but are not limited to monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, NK cells, and may be from any organism including but not limited to humans, mice, rats, rabbits, and monkeys.

The term "effective amount" as used herein means an amount that is determined by such considerations as are known in the art of treating cancer (*e.g.*, bladder, ovarian cancer, lung, etc.), wherein it is effective to provide measurable relief in treated subjects, such as exhibiting improvements including, but not limited to, improved survival rate, more rapid recovery, improvement or elimination of symptoms, reduction of recurrence, reduction of tumor load, and where appropriate, antibody titer or increased titer against the uroplakin.

As used herein, the term "fragment," as applied to a nucleic acid, refers to a subsequence of a larger nucleic acid. A "fragment" of a nucleic acid can be at least about 15 nucleotides in length; for example, at least about 50 nucleotides to about 100 nucleotides; at least about 100 to about 500 nucleotides, at least about 500 to about 1000 nucleotides; at least about 1000 nucleotides to about 1500 nucleotides; about 1500 nucleotides to about 2500 nucleotides; or about 2500 nucleotides (and any integer value in between).

As used herein, the term "fragment," as applied to a protein or peptide, refers to a subsequence of a larger protein or peptide. A "fragment" of a protein or peptide can be at least about 20 amino acids in length; for example, at least about 50 amino acids in length; at least about 100 amino acids in length; at least about 200 amino acids in length; at least about 300 amino acids in length; or at least about 400 amino acids in length (and any integer value in between).

"Homologous" as used herein, refers to the subunit sequence identity between two polymeric molecules, *e.g.*, between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then they are homologous at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; *e.g.*, if half (*e.g.*, five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (*e.g.*, 9 of 10), are matched or homologous, the two sequences are 90% homologous. By way of example, the DNA sequences 3'ATTGCC5' and 3'TATGGC are 50% homologous.

The term "hybridoma," as used herein refers to a cell resulting from the fusion of a spleen cell

and a myeloma cell. A hybridoma can be cloned and maintained indefinitely in cell culture and is able to produce monoclonal antibodies. A hybridoma can also be considered to be a hybrid cell.

The term "hybrid antibody" refers to a molecule in which different portions of the antibody are derived from different genes. The genes can be of the same or different animal species. An example of a hybrid antibody is antibody having a variable region derived from one sequence and a constant region derived from another sequence.

An "immunogenic composition" is a preparation containing an immunogen, *e.g.*, a protein or peptide, administered to stimulate a recipient's humoral and cellular immune systems to one or more of the antigens present in the immunogenic composition.

"Immunization" is the process of administering an immunogenic composition and stimulating an immune response to an antigen in a host (*i.e.*, rodents and rabbits). Preferred hosts are mammals, such as primates (*e.g.*, humans) as well as veterinary animals and agriculatural animals. An "immunogen" is an immunogenic composition used to immunized the host. In some instances, the immunogen comprises UPK-Ib or any fragment thereof.

An "immune response" refers to the activities of the immune system, including activation and proliferation of specific cytotoxic T-cells and B-cells resulting in antigen-specific antibody production, after contact with an antigen.

"Isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

An "isolated nucleic acid" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, e.g., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, e.g., the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, e.g., RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding

additional polypeptide sequence.

"Mammal" as used herein refers to any warm-blooded vertebrate animal of the class Mammalia. Mammals include but are not limited to rodents, feline, cannines, caprines, camelids, equines, bovines, porcine, ovines, ungulates, cetaceans, and primates (e.g., monkeys, apes, and humans).

As used herein, the term "monoclonal antibody" includes homogeneous antibodies which display a binding specificity and affinity for a particular epitope. These antibodies are mammalian-derived antibodies, including murine, human, and humanized antibodies. The term "human monoclonal antibody" as used herein, refers to antibodies displaying a single binding specificity which have variable and constant regions derived from human germ-line immunoglobulin sequences.

A "mutation," as used herein, refers to a change in nucleic acid or polypeptide sequence relative to a reference sequence (which is preferably a naturally-occurring normal or "wild-type" sequence), and includes translocations, deletions, insertions, and substitutions/point mutations. A "mutant" as used herein, refers to either a nucleic acid or protein comprising a mutation.

A "nucleic acid" refers to a polynucleotide and includes poly-ribonucleotides and poly-deoxyribonucleotides.

The term "oligonucleotide" typically refers to short polynucleotides of about 50 nucleotides or less in length. It will be understood that when a nucleotide sequence is represented herein by a DNA sequence (*e.g.*, A, T, G, and C), this also includes the corresponding RNA sequence (*e.g.*, A, U, G, C) in which "U" replaces "T".

As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

As used herein, "polynucleotide" includes cDNA, RNA, DNA/RNA hybrid, antisense RNA, ribozyme, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified to contain non-natural or derivatized, synthetic, or semi-synthetic nucleotide bases. Also, contemplated are alterations of a wild type or synthetic gene, including but not limited to deletion, insertion, substitution of one or more nucleotides, or fusion to other polynucleotide sequences, provided that such changes in the primary sequence of the gene do not alter the expressed peptide ability to elicit passive immunity.

As used here, "primatized" antibodies are referred to antibodies which contain human constant and primate variable regions. Primatized antibodies are well tolerated in humans.

A "unit dose" is a defined and predetermined concentration or amount of the immunogenic composition that is safe and effective to elicit an immune response in the recipient of the composition.

The term "therapeutic" as used herein means a treatment and/or prophylaxis. A therapeutic effect is obtained by suppression, remission, or eradication of a disease state associated with abnormal expression of a uroplakin.

"Parenteral" administration of an immunogenic composition includes, *e.g.*, subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, or infusion techniques.

As used herein, "pharmaceutical compositions" include formulations for human and veterinary use.

"Pharmaceutically acceptable" means material that is not biologically or otherwise undesirable, *i.e.*, the material can be administered to a subject along with other composition 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.

As used herein, "substantially purified" refers to being essentially free of other components. For example, a substantially purified cell is a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers simply to a cell that have been separated from the cells with which they are naturally associated in their natural state.

"Uroplakins (*Uro*thelial *Plaq*ue-related proteins)" refer to a faimily of proteins that include at least UPK-1a, UPK-1b, UPK-II, and UPK-III.

"Variant" as the term is used herein, is a nucleic acid sequence or a peptide sequence that differs in sequence from a reference nucleic acid sequence or peptide sequence respectively, but

retains essential properties of the reference molecule. Changes in the sequence of a nucleic acid variant may not alter the amino acid sequence of a peptide encoded by the reference nucleic acid, or may result in amino acid substitutions, additions, deletions, fusions, and truncations, and combinations thereof. Changes in the sequence of peptide variants are typically limited or conservative, so that the sequences of the reference peptide and the variant are closely similar overall and, in many regions, identical. A variant and reference peptide can differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A variant of a nucleic acid or peptide can be a naturally occurring such as an allelic variant, or can be a variant that is not known to occur naturally. Non-naturally occurring variants of nucleic acids and peptides may be made by mutagenesis techniques or by direct synthesis.

The term "chemotherapeutic agent" as used herein refers to at least conventional chemotherapeutic agents of the following exemplary classes: alkylating agents; nitrosoureas; antimetabolites; antitumor antibiotics; plant alkyloids; taxanes; hormonal agents; and miscellaneous agents.

2.1 Preparation of Monoclonal Antibodies

The invention provides monoclonal antibodies that specifically bind to UPK-Ib. In another aspect, the monoclonal antibodies can specifically bind to the native form of UPK-Ib expressed on a human cell. The antibodies of the present invention include those having UPK-Ib or any fragment thereof as the immunogen. In one aspect, the immunogen comprises a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:13.

Antibodies of the invention are generated against the extracellular domain of UPK-Ib. The monoclonal antibodies may be produced by injecting a human or a non-human animal with an antigen, collecting the spleen lymphocytes, fusing collected lymphocytes with myeloma cells and culturing them to obtain colonies of hybridomas that produce a virtually endless supply of monoclonal antibodies directed against the antigen.

In one aspect, the antibodies of the invention are generated from a recombinant protein comprising an extracellular region of the native human UPK-Ib protein fused to the human IgG1 Fc region. The recombinant protein is designated as UPK-G1 fusion protein and comprises the amino acid sequence set forth in SEQ ID NO: 15.

The compounds, compositions, and methods described herein are based in part on monoclonal antibodies generated against the UPK-Ib protein. For example, the UPK-G1 fusion protein can be used as an immunogen to generate hybridomas that secrete antibodies specific for

the 121 amino acid domain of the UPK-Ib protein. In one embodiment, the antibodies do not cross-react with the Fc domain of the immunogen. A particular advantage of the present monoclonal antibodies is the ability of the antibodies to recognize native human UPK-Ib expressed on a cell.

In one aspect of the invention, the monoclonal antibody 1G9 comprises a heavy chain sequence as set forth in SEQ ID NO: 1 and a light chain sequence as set forth in SEQ ID NO: 3. In another aspect, the monoclonal antibody 1G9 comprises a heavy chain sequence and a light chain sequence encoded by the nucleic acid comprising the sequence as set forth in SEQ ID NO: 2 and SEQ ID NO: 4, respectively, or nucleic acid sequences that respectively encode SEQ ID NOs: 1 and 3.

In another aspect, the monoclonal antibody 5H9 comprises a heavy chain sequence as set forth in SEQ ID NO: 5 and a light chain sequence as set forth in SEQ ID NO: 7. In another aspect, the monoclonal antibody 5H9 comprises a heavy chain sequence and a light chain sequence encoded by the nucleic acid comprising the sequence as set forth in SEQ ID NO: 6 and SEQ ID NO: 8, respectively, or nucleic acid sequences that respectively encode SEQ ID NOs: 5 and 7.

UPK-Ib specific antibodies include individual, allelic, strain, or species variants, and fragments thereof, both in their naturally occurring (full-length) forms and in recombinant forms. In certain aspects, the antibodies are capable of recognizing and binding to native form of UPK-Ib expressed on the surface of a cell that is associated with cancer (*e.g.*, bladder cancer, ovarian cancer, lung cancer, etc.). Different anti-idiotypic antibodies can also be generated. Many methods of making antibodies that specifically bind to a particular epitope are known to those skilled in the art. The following discussion is presented as a general overview of the techniques available; however, a skilled artisan will recognize that many variations upon the following methods are known.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as rodents (*e.g.*, mice), primates (*e.g.*, humans), etc. Descriptions of techniques for preparing such monoclonal antibodies are well known and are described, for example, in Harlow et al., ANTIBODIES: A LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, Cold Spring Harbor, N.Y. (1988); Harlow et al., USING ANTIBODIES: A LABORATORY MANUAL, (Cold Spring Harbor Press, New York, 1998); Breitling et al., RECOMBINANT ANTIBODIES (Wiley-Spektrum, 1999); and Kohler et al., 1997 *Nature* 256: 495-497; U.S. Pat. No. 5,693,762; U.S. Pat. No. 5,693,761; U.S. Pat. No. 5,585,089; U.S. Pat. No.

6,180,370.

General techniques for producing monoclonal antibodies are outlined in Harlow et al., ANTIBODIES: A LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, Cold Spring Harbor, N.Y. (1988), which provides detailed guidance for the production of hybridomas and monoclonal antibodies which specifically bind to target proteins. Briefly, as a non-limiting example, a protein of interest, *e.g.*, without limitation, is a recombinant protein comprising a 121 amino acid long extracellular region of the native human UPK-Ib protein fused to the human IgG1 Fc region is injected into a mouse. The spleen of the mouse is removed and the spleen cells are isolated and fused with immortalized mouse cells. The hybrid cells, *i.e.*, hybridomas, are cultured and those cells which secrete antibodies are selected. The antibodies are analyzed and, if found to specifically bind to the protein of interest, the hybridoma which produces them is cultured to produce a continuous supply of antigen-specific antibodies.

For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, hybrid, primatized, humanized, or human antibodies. Methods for producing chimeric and hybrid antibodies are known in the art. *See e.g.*, Morrison, 1985 *Science* 229: 1202-1207; U.S. Pat. Nos. 6,965,024, 5,807,715; 4,816,567; and 4,816,397. Humanized antibodies are antibody molecules from non-human species that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions and constant domains from a human immunoglobulin molecule. Often, framework residues in the human framework regions are substituted with the corresponding residue from the CDR donor antibody to alter and in some instances improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. *See, e.g.*, Queen et al., U.S. Pat. No. 5,585,089. Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting, veneering or resurfacing, and chain shuffling. Humanized antibodies may be generated using any of the methods disclosed in U.S. Pat. No. 5,693,762, U.S. Pat. No. 5,693,761, U.S. Pat. No. 5,585,089, U.S. Pat. No. 6,180,370.

The invention also includes a humanized antibody that is capable of recognizing and binding UPK-Ib. The antibody can recognize and bind to the native form of UPK-Ib that is expressed on the surface of a cell, such as a cell associated with cancer (*e.g.*, bladder cancer, ovarian cancer, lung cancer, etc.). Portions of humanized antibodies are made up of human

polypeptide sequences. The humanized UPK-Ib specific antibodies can be produced in using a wide variety of methods (*see e.g.*, U.S. Pat. No. 5,001,065 and WO 03/052082). In addition, the invention also encompasses primatized antibodies which contain human constant and primate variable regions (*see e.g.*, U.S. Pat. No. 6,113,898).

In addition to obtaining UPK-Ib antibodies from a hybridoma cell, the antibodies can also be generated by cloning antibody genes from the hybridoma into one or more expression vectors, and transforming the vector into a cell line such as the cell lines typically used for expression of recombinant or humanized antibodies.

The genes encoding the heavy and light chains of immunoglobulins secreted by the cell lines are cloned according to methods, including but not limited to, the polymerase chain reaction (PCR), known in the art (*see, e.g.*, Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1-3 (3rd ed., Cold Spring Harbor Press, NY 2001). For example, genes encoding heavy and light chains can be cloned from the antibody secreting cell's genomic DNA, or cDNA can be produced by reverse transcription of a cell's RNA. Cloning is accomplished by conventional techniques including the use of PCR primers that hybridize to the sequences flanking or overlapping the genes, or segments of genes, to be cloned.

Typically, recombinant constructs comprise DNA segments encoding a complete human immunoglobulin heavy chain and/or a complete human immunoglobulin light chain of an immunoglobulin expressed by an antibody secreting cell line. Alternatively, DNA segments encoding only a portion of the primary antibody genes are produced, which portions possess binding and/or effector activities. Other recombinant constructs contain segments of the cell's immunoglobulin genes fused to segments of other immunoglobulin genes, particularly segments of other human constant region sequences (heavy and/or light chain).

In addition to the DNA segments encoding UPK-Ib specific antibody or fragments thereof, other substantially homologous modified antibodies can be readily designed and manufactured utilizing various recombinant DNA techniques such as site-directed mutagenesis. Such modified segments will usually retain antigen binding capacity and/or effector function. Moreover, the modified segments are typically not changed from the original genomic sequences of the antibody producing cell to prevent hybridization to these sequences under stringent conditions to the parent sequences from which they were derived. Because like many genes, immunoglobulin genes contain separate functional regions, each having one or more distinct biological activities, the genes may be fused to functional regions from other genes to produce fusion proteins having novel properties or novel combinations of properties.

Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the UPK-Ib antigen. The antibodies are used with or without modification, and include chimeric or otherwise hybrid antibodies such as antibodies having different antibody isotypes.

As more fully discussed elsewhere herein, the antibodies can be manipulated, for example, by way of mutating the sequences corresponding to the antibodies described herein to create antibodies with altered binding characteristics. For example, a cell expressing a specific antibody gene sequence can be subjected to a mutagenesis procedure such that a progeny of that cell produces antibodies with different characteristics than the original antibody. The antibodies produced from the progeny have an increased avidity for UPK-Ib compared to the original antibody.

2.2 Equivalents of Monoclonal Antibodies

The invention also includes functional equivalents of the antibodies described herein. Functional equivalents have binding characteristics comparable to those of the antibodies, and include, for example, hybridized and single chain antibodies, as well as fragments thereof. Methods of producing such functional equivalents are disclosed in PCT Application WO 93/21319 and PCT Application WO 89/09622.

Functional equivalents include polypeptides with amino acid sequences substantially the same as the amino acid sequence of the variable or hypervariable regions of the antibodies. "Substantially the same" amino acid sequence is defined herein as a sequence with at least 70%, preferably at least about 80%, more preferably at least about 90%, even more preferably at least about 95%, and most preferably at least 99% homology to another amino acid sequence (or any integer in between 70 and 99), as determined by the FASTA search method in accordance with Pearson and Lipman, 1988 *Proc. Nat'l. Acad. Sci. USA* 85: 2444-2448. Chimeric or other hybrid antibodies have constant regions derived substantially or exclusively from human antibody constant regions and variable regions derived substantially or exclusively from the sequence of the variable region of a monoclonal antibody from each stable hybridoma.

Single chain antibodies (scFv) or Fv fragments are polypeptides that consist of the variable region of the heavy chain of the antibody linked to the variable region of the light chain, with or without an interconnecting linker. Thus, the Fv comprises an antibody combining site.

Functional equivalents of the antibodies of the invention further include fragments of antibodies that have the same, or substantially the same, binding characteristics to those of the whole antibody. Such fragments may contain one or both Fab fragments or the $F(ab')_2$ fragment. The antibody fragments contain all six complement determining regions of the whole antibody, although fragments containing fewer than all of such regions, such as three, four or five complement determining regions, are also functional. The functional equivalents are members of the IgG immunoglobulin class and subclasses thereof, but may be or may combine with any one of the following immunoglobulin classes: IgM, IgA, IgD, or IgE, and subclasses thereof. Heavy chains of various subclasses, such as the IgG subclasses, are responsible for different effector functions and thus, by choosing the desired heavy chain constant region, hybrid antibodies with desired effector function are produced. Exemplary constant regions are gamma 1 (IgG1), gamma 2 (IgG2), gamma 3 (IgG3), and gamma 4 (IgG4). The light chain constant region can be of the kappa or lambda type.

The immunoglobulins of the present invention can be monovalent, divalent or polyvalent. Monovalent immunoglobulins are dimers (HL) formed of a hybrid heavy chain associated through disulfide bridges with a hybrid light chain. Divalent immunoglobulins are tetramers (H_2L_2) formed of two dimers associated through at least one disulfide bridge.

The UPK-Ib specific antibodies of the present invention can also be combined with any known modifications in the art with respect to generating a diabody molecule. The of covalent diabodies is based on the single chain Fv construct (scFv) (*see*, Holliger et al., 1993, Proc. Natl. Acad. Sci. USA 90: 6444-6448; herein incorporated by reference in its entirety). In an intact, unmodified IgG, the VL and VH domains are located on separate polypeptide chains, *i.e.*, the light chain and the heavy chain, respectively. Interaction of an antibody light chain and an antibody heavy chain and, in particular, interaction of VL and VH domains forms one of the epitope binding sites of the antibody. The diabody molecule of the present invention is a complex of two or more polypeptide chains or proteins, each comprising at least one VL and one VH domain or fragment thereof. In some instances, the VL domain and the VH domain are covalently linked such that the domains are constrained from self assembly. The diabody molecule can recognize the same or different epitopes on the same or differing antigens. Diabody molecules are extensively disclosed in U.S. Publication No. 20070004909, which is herein incorporated by reference in its entirety.

Interaction of two of the polypeptide chains of the diabody molecule produces two VL-VH pairings, forming two epitope binding sites, *i.e.*, a bivalent molecule. Neither the VH or VL

domain is constrained to any position within the polypeptide chain, *i.e.*, restricted to the amino (N) or carboxy (C) teminus, nor are the domains restricted in their relative positions to one another, *i.e.*, the VL domain may be N-terminal to the VH domain and vice-versa.

Where the VL and VH domains are derived from the same UPK-Ib specific antibody, the two complimentary polypeptide chains may be identical. For example, where the binding domains are derived from an antibody specific for epitope A (*i.e.*, the binding domain is formed from a VL_A-VH_A interaction), each polypeptide comprises a VH_A and a VL_A. Homodimerization of two polypeptide chains of the antibody results in the formation two VL_A-VH_A binding sites, resulting in a bivalent monospecific antibody.

Where the VL and VH domains are derived from different UPK-Ib specific antibodies, formation of a functional bispecific diabody involves the interaction of two different polypeptide chains, *i.e.*, formation of a heterodimer. For example, for a bispecific diabody, one polypeptide chain comprises a VL_A and a VL_B; homodimerization of this chain results in the formation of two VL_A-VH_B binding sites. In some instances where two differing polypeptide chains are free to interact, *e.g.*, in a recombinant expression system, one comprising a VL_A and a VH_B and the other comprising a VL_B and a VH_A, two differing binding sites form: VL_A-VH_A and VL_B-VH_B.

Diabody molecules of the invention encompass tetramers of polypeptide chains, each of which polypeptide chain comprises a VH and VL domain. In certain embodiments, two polypeptide chains of the tetramer further comprise an Fc domain. The tetramer is therefore comprised of two `heavier` polypeptide chains, each comprising a VL, VH and Fc domain, and two `lighter` polypeptide chains, comprising a VL and VH domain. Interaction of a heavier and lighter chain into a bivalent monomer coupled with dimerization of the monomers via the Fc domains of the heavier chains leads to formation of a tetravalent immunoglobulin-like molecule. In certain aspects the monomers are the same, and the tetravalent diabody molecule is monospecific or bispecific. In other aspects the monomers are different, and the tetra valent molecule is bispecific or tetraspecific.

At least two binding sites of the diabody molecule can recognize the same or different epitopes. Different epitopes can be from the same antigen or epitopes from different antigens. In one embodiment, the epitopes are from different cells. In another embodiment, the epitopes are cell surface antigens on the same cell. The epitopes binding sites can recognize any antigen to which an antibody can be generated. For example, proteins, nucleic acids, bacterial toxins, cell surface markers, autoimmune markers, viral proteins, drugs, etc. In particular aspects, at least one epitope binding site of the diabody is specific for UPK-Ib.

The invention also encompasses diabody molecules comprising variant Fc or variant hinge-Fc domains (or portion thereof), which variant Fc domain comprises at least one amino acid modification (e.g. substitution, insertion deletion) relative to a comparable wild-type Fc domain or hinge-Fc domain (or portion thereof). Molecules comprising variant Fc domains or hinge-Fc domains (or portion thereof) (*e.g.*, antibodies) normally have altered phenotypes relative to molecules comprising wild-type Fc domains or hinge-Fc domains or portions thereof. The variant phenotype may be expressed as altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function as assayed in an NK dependent or macrophage dependent assay. Fc domain variants identified as altering effector function are disclosed in U.S. Patent Application Publications US 2005/0037000, US 2005/0064514, US 2006/0134709, US 2007/0036799, and U.S. Provisional Applications 60/850,674 filed October 9, 2006 and 60/869,254 filed December 8, 2006, each of which is incorporated by reference in its entirety.

The bispecific diabodies of the invention can simultaneously bind two separate and distinct epitopes. In certain embodiments the epitopes are from the same antigen (*e.g.*, UPK-Ib). In other embodiments, the epitopes are from different antigens. Bispecific diabody molecules of the invention offer unique opportunities to target specific cell types. For example, the bispecific diabody or diabody molecule can be engineered to comprise a combination of epitope binding sites that recognize a set of antigens unique to a target cell or tissue type. Additionally, where either or both of the individual antigens is/are fairly common separately in other tissue and/or cell types, low affinity biding domains can be used to construct the diabody or diabody molecule. Such low affinity binding domains will be unable to bind to the individual epitope or antigen with sufficient avidity for therapeutic purposes. However, where both epitopes or antigens are present on a single target cell or tissue, the avidity of the diabody or diabody molecule for the cell or tissue, relative to a cell or tissue expressing only one of the antigens, will be increased such that the cell or tissue can be effectively targeted by the invention. Such a bispecific molecule can exhibit enhanced binding to one or both of its target antigens on cells expressing both of said antigens relative to a monospecific diabody or an antibody with a specificity to only one of the antigens.

2.3 Recombinant Expression of the Antibody

Using the information provided herein, the antibodies can be produced recombinantly using standard techniques well known to those of skill in the art. For example, the amino acid

sequences provided herein (*see, e.g.*, Figure 5) can be used to determine and clone appropriate nucleic acid sequences encoding the antibodies and the nucleic acids sequences can then be used to express one or more antibodies. The nucleic acid sequence may be optimized to reflect particular codon "preferences" for various expression systems according to methods known in the art.

Using the sequence information provided herein, the nucleic acids may be synthesized according to a number of standard methods known in the art. Oligonucleotide synthesis can be carried out on commercially available solid phase oligonucleotide synthesis machines or manually synthesized using the solid phase phosphoramidite triester method described by Beaucage et. al., 1981 *Tetrahedron Letters* 22: 1859-1862.

Once a nucleic acid encoding a UPK-Ib specific antibody is synthesized, it may be amplified and/or cloned according to standard methods in order to produce recombinant antibodies. Molecular cloning techniques to achieve these ends are known in the art. A wide variety of cloning and *in vitro* amplification methods suitable for the construction of recombinant nucleic acids are known to those skilled in the art. Examples of these techniques and instructions sufficient to direct skilled artisan are found in Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1-3 (3rd ed., Cold Spring Harbor Press, NY 2001) or any of the prior editions by Sambrook. Methods of producing recombinant immunoglobulins are also known in the art. *See, e.g.,* Cabilly, U.S. Pat. No. 4,816,567; and Queen et al., 1989 *Proc. Nat'l Acad. Sci. USA* 86: 10029-10033.

Examples of techniques sufficient to direct persons of skill through *in vitro* amplification methods, including the polymerase chain reaction (PCR), the ligase chain reaction (LCR), and other DNA or RNA polymerase-mediated techniques are found in Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1-3 (3rd ed., Cold Spring Harbor Press, NY 2001), as well as U.S. Pat. No. 4,683,202 and U.S. Pat. No. 5,426,039.

Once the nucleic acid for a UPK-Ib specific antibody is isolated and cloned, a skilled artisan may express the recombinant gene(s) in a variety of engineered cells. Examples of such cells include bacteria, yeast, filamentous fungi, insect (especially employing baculoviral vectors), and mammalian cells. It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of UPK-Ib specific antibodies.

2.3.1. Vectors

Nucleic acids encoding an antibody or antibody equivalents may be replicated in wide

variety of cloning vectors in a wide variety of host cells.

In brief summary, the expression of natural or synthetic nucleic acids encoding UPK-Ib specific antibodies will typically be achieved by operably linking a nucleic acid encoding the antibody or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

In some aspects, the expression vector is selected from the group consisting of a viral vector, a bacterial vector, and a mammalian cell vector. Numerous expression vector systems exist that comprise at least a part or all of the compositions discussed above. Prokaryote- and/or eukaryote-vector based systems can be employed to produce polynucleotides, or their cognate polypeptides. Many such systems are commercially and widely available.

Further, the expression vector may be provided to a cell, exemplary a Chinese hamster ovary (CHO) cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1-3 (3rd ed., Cold Spring Harbor Press, NY 2001), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. Exemplary, a murine stem cell virus (MSCV) vector is used to express a desired nucleic acid. MSCV vectors have been demonstrated to efficiently express desired nucleic acids in myeloma cells. However, the invention should not be limited to only using a MSCV vector, rather the immunoglobulin nucleic acids can be introduced into cells using retroviral or other viral expression methods. For example, a Moloney murine leukemia virus (MoMuLV) vector is used to express a desired nucleic acid in a cell such as CHO cells. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers. (*e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

For expression of the antibody or portions thereof, at least one module in each promoter functions to position the start site for RNA synthesis. The best known example of this is the

TATA box, but in some promoters lacking a TATA box, such as the promoter for the mammalian terminal deoxynucleotidyl transferase gene and the promoter for the SV40 genes, a discrete element overlying the start site itself helps to fix the place of initiation.

Additional promoter elements, *e.g.*, enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either co-operatively or independently to activate transcription.

A promoter may be one naturally associated with a gene or polynucleotide sequence, as may be obtained by isolating the 5′ non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as "endogenous." Similarly, an enhancer may be one naturally associated with a polynucleotide sequence, located either downstream or upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding polynucleotide segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a polynucleotide sequence in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a polynucleotide sequence in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not "naturally occurring," *e.g.*, containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR, in connection with the compositions disclosed herein (*e.g.*, U.S. Patent 4,683,202, U.S. Patent 5,928,906). Furthermore, it is contemplated the control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

Naturally, it will be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the cell type, organelle, and organism chosen for expression. The promoters employed may be constitutive, tissue-specific, inducible, and/or

useful under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins and/or peptides. The promoter may be heterologous or endogenous.

An example of a promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the muscle creatine promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence to which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

In order to assess the expression of an antibody or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a protein whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells.

Suitable reporter genes may include genes encoding luciferase, beta-galactosidase,

chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tei et al., 2000 *FEBS Letters* 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

2.3.2. Method for Expression

Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, *e.g.*, mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. *See*, for example, Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1-3 (3rd ed., Cold Spring Harbor Press, NY 2001).

Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, *e.g.*, human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. *See*, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*e.g.*, an artificial membrane vesicle).

In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (*in vitro*, *ex vivo* or *in vivo*). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the

oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates which are not uniform in either size or shape.

Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma, St. Louis, MO; dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, NY); cholesterol ("Chol") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, AL.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Chloroform is used as the only solvent since it is more readily evaporated than methanol.

"Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991 *Glycobiology* 5: 505-10). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the nucleic acid, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern

and Northern blotting, reverse transcription polymerase chain reaction (RT-PCR) and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, *e.g.*, by immunological means (ELISAs and Western blots).

Following the generation of the antibodies or portions thereof, the antibody or portions thereof can be used in a wide range of experimental and/or therapeutic purposes. For example, techniques for using and manipulating antibodies are found in Harlow et al., ANTIBODIES: A LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, Cold Spring Harbor, N.Y. (1988); Harlow et al., USING ANTIBODIES: A LABORATORY MANUAL, (Cold Spring Harbor Press, New York, 1998); and Breitling et al., RECOMBINANT ANTIBODIES (Wiley-Spektrum, 1999), and Kohler et al., 1975 *Nature* 256: 495-497.

2.4 Chimeric (hybrid) antibodies

"Chimeric" or "hybrid" antibodies include, but are not limited to, antibodies having diverse components. For example, several different effector functions have been achieved by linking new sequences to those encoding the antigen binding domain within the antibody. In general, chimeric antibodies are produced by preparing, for each of the light and heavy chain components of the antibody, a fused gene comprising a first DNA segment that encodes at least the functional portion of the UPK-Ib specific antibody, such as a murine variable region linked (*e.g.*, functionally rearranged variable region with joining segment) to a second DNA segment encoding at least a part of a human or primate constant region. Each fused gene is assembled in or inserted into an expression vector. Recipient cells capable of expressing the gene products are then transfected with the genes. The transfected recipient cells are cultured under conditions that permit expression of the incorporated genes and the expressed immunoglobulins or immunoglobulin chains are recovered.

Methods of producing chimeric antibodies include for example the following steps (the order of some steps may be interchanged): (a) identifying and cloning the correct gene segment encoding the antigen binding portion of the antibody molecule; this gene segment (known as the VDJ, variable, diversity and joining regions for heavy chains or VJ, variable, joining regions for light chains (or simply as the V or variable region) may be in either the cDNA or genomic form; (b) cloning the gene segments encoding the constant region or desired part thereof; (c) ligating the variable region to the constant region so that the complete chimeric antibody is encoded in a transcribable and translatable form; (d) ligating this construct into a vector containing a selectable marker and gene control regions such as promoters, enhancers and poly(A) addition signals; (e)

introducing the DNA into eukaryotic cells (transfection) such as mammalian lymphocytes; and culturing the host cell under conditions suitable for expression of the chimeric antibody.

Antibodies of several distinct antigen binding specificities have been manipulated by these protocols to produce chimeric proteins (*e.g.*, anti-TNP: Boulianne et al., 1984 *Nature* 312: 643-646; and anti-tumor antigens: Sahagan et al., 1986 *J. of Immunology* 137: 1066-1074). Likewise, several different effector functions have been achieved by linking new sequences to those encoding the antigen binding region. Some of these include enzymes (Neuberger et al., 1984 *Nature* 312: 604-608), immunoglobulin constant regions from another species, and constant regions of another immunoglobulin chain (Sharon et al., 1984 *Nature* 309: 364-367; and Tan et al., 1985 *J. of Immunology* 135: 3565-3567).

In one aspect, a recombinant DNA vector is used to transfect a cell line that produces a UPK-Ib specific antibody. The novel recombinant DNA vector contains a "replacement gene" to replace all or a portion of the gene encoding the immunoglobulin constant region in the cell line (*e.g.*, a replacement gene may encode all or a portion of a constant region of a human immunoglobulin, a specific immunoglobulin class, or an enzyme, a toxin, a biologically active peptide, a growth factor, inhibitor, or a linker peptide to facilitate conjugation to a drug, toxin, or other molecule, etc.), and a "target sequence" that allows for targeted homologous recombination with immunoglobulin sequences within the antibody producing cell.

In another aspect, a recombinant DNA vector is used to transfect a cell line that produces an antibody having a desired effector function, (*e.g.*, a constant region of a human immunoglobulin or a constant region of another immunoglobulin chain) in which case, the replacement gene contained in the recombinant vector may encode all or a portion of a region of a UPK-Ib specific antibody and the target sequence contained in the recombinant vector allows for homologous recombination and targeted gene modification within the antibody producing cell. In either aspect, when a portion of the variable or constant region is replaced, the resulting chimeric antibody may define the same antigen and/or have the same effector function yet be altered or improved so that the chimeric antibody may demonstrate a greater antigen specificity, greater affinity binding constant, increased effector function, or increased secretion and production by the transfected antibody producing cell line, etc.

Regardless of the embodiment practiced, the processes of selection for integrated DNA (via a selectable marker), screening for chimeric antibody production, and cell cloning, can be used to obtain a clone of cells producing the chimeric antibody.

Thus, a piece of DNA which encodes a modification for a monoclonal antibody can be

targeted directly to the site of the expressed immunoglobulin gene within a B-cell or hybridoma cell line. DNA constructs for any particular modification may be used to alter the protein product of any monoclonal cell line or hybridoma. Such a procedure circumvents the costly and time consuming task of cloning both heavy and light chain variable region genes from each B-cell clone expressing a useful antigen specificity. In addition to circumventing the process of cloning variable region genes, the level of expression of the hybrid antibody should be higher when the gene is at its natural chromosomal location rather than at a random position.

Without being bound by any particular theory, chimeric antibodies can also be generated using genes encoding the variable region of immunoglobulin heavy and light chains from cells that produce the monoclonal UPK-Ib specific antibody. For example, the hybridoma cell line that produces the desired monoclonal antibody against UPK-Ib provides a source of immunoglobulin variable region for the present chimeric antibodies. Constant regions are obtained from human or primate antibody-producing cells by standard cloning techniques. Alternatively, because genes representing the two classes of light chains and the five classes of heavy chains have been cloned, constant regions of human origin are readily available from these clones. Chimeric antibody binding fragments such as $F(ab')_2$ and Fab fragments are prepared by designing a hybrid heavy chain gene in truncated form. For example, a hybrid gene encoding a $F(ab')_2$ heavy chain portion would include DNA sequences encoding the CH_1 domain and hinge region of the heavy chain. Alternatively, such fragments can be obtained by enzymatic cleavage of a hybrid immunoglobulin. For instance, papain or pepsin cleavage can generate Fab or $F(ab')_2$ fragments, respectively.

The fused genes encoding the heavy and light hybrid chains, or portions thereof, are assembled in one or two different expression vectors that can be used to cotransfect a recipient cell. In the case of using two different expression vectors, each vector contains two selectable genes, one for selection in a bacterial system and one for selection in an eukaryotic system, each vector having a different pair of genes. These vectors allow production and amplification of the fused genes in bacterial systems, and subsequent co-transfection of eukaryotic cells and selection of the cotransfected cells. Examples of selectable genes for the bacterial system include, but are not limited to, the genes that confer ampicillin resistance and the gene that confers chloramphenicol resistance. Two selectable genes for selection of eukaryotic transfectants are preferred, but are not limited to: (i) the xanthine-guanine phosphoribosyltransferase gene (*gpt*), and (ii) the phosphotransferase gene from Tn5 (designated neo). Selection with *gpt* is based on the ability of the enzyme encoded by this gene to use xanthine as a substrate for purine nucleotide

synthesis; the analogous endogenous enzyme cannot. In a medium containing xanthine and mycophenolic acid, which blocks the conversion of inosine monophosphate to xanthine monophosphate, only cells expressing the gpt gene can survive. The product of the neo blocks the inhibition of protein synthesis in eukaryotic cells caused by the antibiotic G418 and other antibiotics of its class. The two selection procedures can be used simultaneously or sequentially to select for the expression of immunoglobulin chain genes introduced on two different DNA vectors into a eukaryotic cell.

In accordance with the present invention, nucleic acid sequences coding for heavy and light chains of the monoclonal UPK-Ib specific antibody, or a fragment or homolog thereof, are inserted into an appropriate expression vector. This vector contains the necessary elements for transcription and translation of the inserted protein-coding sequence so as to generate recombinant DNA molecules that direct the expression of heavy and light chain immunoglobulins for the formation of monoclonal UPK-Ib specific antibody.

An exemplary recipient cell line is a myeloma cell. Myeloma cells can synthesize, assemble and secrete immunoglobulins encoded by transfected immunoglobulin genes. Further, they possess the mechanism for glycosylation of the immunoglobulin. Myeloma cells can be grown in culture or in the peritoneum of mice where secreted immunoglobulin can be obtained from ascites fluid. Other lymphoid cells such as B lymphocytes or hybridoma cells can serve as suitable recipient cells.

Several methods exist for transfecting lymphoid cells with vectors containing immunoglobulin encoding genes. An exemplary way of introducing DNA into lymphoid cells is by electroporation. In this procedure, recipient cells are subjected to an electric pulse in the presence of the DNA to be incorporated. Another way to introduce DNA is by protoplast fusion. In this method, lysozyme is used to strip cell walls from bacteria harboring the recombinant plasmid containing the immunoglobulin gene. The resulting spheroplasts are fused with myeloma cells with polyethylene glycol. After protoplast fusion, the transfectants are selected and isolated. Another technique that can be used to introduce DNA into many cell types is calcium phosphate precipitation.

The immunoglobulin genes can also be expressed in nonlymphoid cells, such as CHO cells, bacteria or yeast. When expressed in bacteria, the immunoglobulin heavy chains and light chains become part of inclusion bodies. Thus, the chains must be isolated and purified and then assembled into functional immunoglobulin molecules. Other strategies for expression in *E. coli* are available (*e.g.*, Pluckthun, 1991 *BioTechnology* 9: 545-551; Skerra et al., 1991

BioTechnology 9: 273-278), including secretion from *E. coli* as fusion proteins comprising a signal sequence.

2.4.1 Enhancing mutant #1 (Fc enhancing mutant)

The present invention relates partly to the discovery that the Fc region of an antibody can be mutated to have one or more amino acid modifications (*e.g.*, substitutions, but also including insertions or deletions) in one or more regions, which modifications alter, *e.g.*, increase or decrease, the affinity of the variant Fc region for an Fcγ receptor (*e.g.*, FcγR1, FcγRII-A, FcγII-B1, FcγIIB2, FcγRIII). As such, the UPK-Ib specific antibodies can be engineered to contain a variant Fc region whereby modification enhances the efficacy of the antibody for therapeutic use (otherwise referred herein as Fc enhancing mutant). For example, the modification of the Fc region can enhance the effector function of the therapeutic antibodies (*e.g.*, enhancing ADCC).

The invention also encompasses UPK-Ib specific antibodies comprising variant Fc or variant hinge-Fc domains (or portion thereof), which variant Fc domain comprises at least one amino acid modification (*e.g.* substitution, insertion deletion) relative to a comparable wild-type Fc domain or hinge-Fc domain (or portion thereof). Molecules comprising variant Fc domains or hinge-Fc domains (or portion thereof) (*e.g.*, antibodies) normally have altered phenotypes relative to molecules comprising wild-type Fc domains or hinge-Fc domains or portions thereof. The variant phenotype may be expressed as altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function as assayed in an NK dependent or macrophage dependent assay. Fc domain variants identified as altering effector function are disclosed in U.S. Patent Application Publications US 2005/0037000, US 2005/0064514, US 2006/0134709, US 2007/0036799, and U.S. Provisional Applications 60/850,674 filed October 9, 2006 and 60/869,254 filed December 8, 2006, each of which is incorporated by reference in its entirety.

Accordingly, the UPK-Ib specific antibodies or fragments thereof can be combined with any of the known Fc modifications in the art such as those disclosed in Tables 1 through 3 set forth below. The numbering of the residues is that of the EU index as set forth in Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, (5th ed., Public Health Service, MD 1991).

TABLE 1: SINGLE SITE Fc MUTANTS

Modification(s)
S132I
A162V
P217S
S219Y
K222N
H224L
T225S
P232G
E233G, N, P, or Y
P238A, or R
F243L
P247L
K248M
T250A
S267A, G, or T
D270E
V273I
F275V, or Y
V282K, or M
K288N, or W
R292G, L, or P
Y296F, G, or N
S298A, N, or T
Y300A, or L
R301M
V305A
N315I, or R
G316D
E318K
K326E
A330S, or V
K334E, I, or N
M352L
R355W
T359N
T366N, or S
F372Y
I377F, or N
V379L, or M
K392P, R, or T
P396H, or L
L398V
D399E
S400P
D401V

S407I
L410H
K414N
R416T
Q419H

TABLE 2: DOUBLE SITE Fc MUTANTS

Modification(s)
Q347H, A339V
S415I, L251F
K290E, L142P
G285E, P247H
K409R, S166N
E333A, K334A
R292L, K334E
K288N, A330S
R255L, E318K
F243L, E318K
V279L, P395S
K246T, Y319F
F243I, V379L
K288M, K334E
K334E, E308D
E233D, K334E
K246T, P396H
H268D, E318D
K246I, K334N
K320E, K326E
S375C, P396L
K288N, K326N
P247L, N421K
S298N, W381R
R255Q, K326E
V284A, F372L
T394M, V397M
P247L, E389G
K290T, G371D
P247L, L398Q
P247L, I377F
K326E, G385E
S298N, S407R
E258D, N384K
F241L, E258G
K370N, S440N
K317N, F423-DELETED
P227S, K290E
K334E, E380D

P291S, P353Q
V240I, V281M
P232S, S304G
P247L, L406F
D399E, M428L
L251F, F372L
D399E, G402D
D399E, M428L
K392T, P396L
H268N, P396L
K326I, P396L
H268D, P396L
K210M, P396L
L358P, P396L
K334N, P396L
V379M, P396L
P227S, P396L
P217S, P396L
Q419H, P396L
K370E, P396L
L242F, P396L
R255L, P396L
V240A, P396L
T250A, P396L
P247S, P396L
L410H, P396L
Q419L, P396L
V427A, P396L
E258D, P396L
N384K, P396L
V323I, P396L
P244H, P396L
V305L, P396L
S400F, P396L
V303I, P396L
A330V, Q419H
V263Q, E272D
K326E, A330T
F243L, R292P
F243L, P396L
R292P, V305I
G385E, P247H

TABLE 3: THREE OR MORE Fc MUTANTS

Modification(s)
D399E, R292L, V185M
R301C, M252L, S192T

P291S, K288E, H268L, A141V
S383N, N384K, T256N, V262L, K218E, R214I, K205E, F149Y, K133M
S408I, V215I, V125L
V348M, K334N, F275I, Y202M, K147T
H310Y, T289A, Y407V, E258D
R292L, P396L, T359N
F275I, K334N, V348M
F243L. R255L, E318K
K334E, T359N, T366S
T256S, V305I, K334E, N390S
T335N, K370E, A378V, T394M, S424L
K334E, T359N, T366S, Q386R
K288N, A330S, P396L
P244H, L358M, V379M, N384K, V397M
P217S, A378V, S408R
P247L, I253N, K334N
D312E, K327N, I378S
D280E, S354F, A431D, L441I
K218R, G281D, G385R
P247L, A330T, S440G
T355N, P387S, H435Q
P247L, A431V, S442F
P343S, P353L, S375I, S383N
E216D, E345K, S375I
K288N, A330S, P396L
K222N, T335N, K370E, A378V, T394M
G316D, A378V, D399E
N315I, V379M, T394M
K326Q, K334E, T359N, T366S
A378V, N390I, V422I
V282E, V369I, L406F
V397M, T411A, S415N
T223I, T256S, L406F
L235P, V382M, S304G, V305I, V323I
P247L, W313R, E388G
D221Y, M252I, A330G, A339T, T359N, V422I, H433L
F243I, V379L, G420V
A231V, Q386H, V412M
T215P, K274N, A287G, K334N, L365V, P396L
P244A, K326I, C367R, S375I, K447T
R301H, K340E, D399E
C229Y, A287T, V379M, P396L, L443V
E269K, K290N, Q311R, H433Y
E216D, K334R, S375I
T335N, P387S, H435Q
K246I, Q362H, K370E
K334E, E380D, G446V
V303I, V369F, M428L

K246E, V284M, V308A
E293V, Q295E, A327T
Y319F, P352L, P396L
D221E, D270E, V308A, Q311H, P396L, G402D
K290T, N390I, P396L
K288R, T307A, K344E,P396L
V273I, K326E, L328I, P396L
K326I, S408N, P396L
K261N, K210M, P396L
F243L, V305I, A378D, F404S, P396L
K290E, V369A, T393A, P396L
K210N, K222I, K320M, P396L
P217S, V305I, I309L, N390H, P396L
K246N, Q419R, P396L
P217A, T359A, P396L
V215I, K290V, P396L
F275L, Q362H, N384K, P396L
A330V, H433Q, V427M
V263Q, E272D, Q419H
N276Y, T393N, W417R
V282L, A330V, H433Y, T436R
V284M, S298N, K334E, R355W
A330V, G427M, K438R
S219T, T225K, D270E, K360R
K222E, V263Q, S298N
E233G, P247S, L306P
S219T, T225K, D270E
S254T, A330V, N361D, P243L
V284M, S298N, K334E, R355W R416T
D270E, G316D, R416G
K392T, P396L, D270E
R255L, P396L, D270E
V240A, P396L, D270E
Q419H, P396L, D270E
K370E, P396L, D270E
P247L, N421K, D270E
R292P, V305I, F243L
V284M, R292L, K370N
R292P, V305I, P396L
F243L, R292P, Y300L
F243L, R292P, P396L
F243L, R292P, V305L
F243L, Y300L, V305I, P396L
F243L, R292P, V305I, P396L
F243L, R292P, Y300L, P396L
F243I, R292P, Y300L, V305I, P396L

The modified or otherwise chimeric antibody includes any immunoglobulin molecule that binds, preferably, immunospecifically, *i.e.*, competes off non-specific binding as determined by immunoassays well known in the art for assaying specific antigen-antibody binding. Such antibodies include, but are not limited to, polyclonal, monoclonal, bi-specific, multi-specific, human, humanized, primatized, chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, disulfide-linked Fvs, and fragments containing either a VL or VH domain or even a CDR that specifically binds an antigen, in certain cases, engineered to contain or fused to an FcγR binding region.

The Fc enhancing mutant encompasses modification of an Fc region of the IgG1 wild type heavy chain. Example of the amino acid sequence of the Fc enhancing mutant is set forth in Tables 1-3, relative to the amino acid sequence of the wild type IgG1 heavy chain. Any Fc mutant can be applied to the UPK-1b specific antibodies described elsewhere herein. For example, different Fc variants are described in U.S. Patent Application No. 10/524,134, which is published as Publication No. US 2005/0215767. In addition, the Fc variants discussed herein may be combined with any of the known Fc modifications in the art.

Throughout the present specification, the numbering of the residues in an IgG heavy chain is that of the EU index as set forth in Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, (5th ed., Public Health Service, MD 1991). The "EU index as in Kabat" refers to the numbering of the human IgG1 EU antibody. Therefore, the term "Fc region" is used to define a C-terminal region of an IgG heavy chain. In some instances, although the boundaries may vary slightly, the human IgG heavy chain Fc region is defined to stretch from Cys226 to the carboxy terminus. The Fc region of an IgG comprises two constant domains, CH2 and CH3. The CH2 domain of a human IgG Fc region usually extends from amino acids 231 to amino acid 341. The CH3 domain of a human IgG Fc region usually extends from amino acids 342 to 447.

In some aspects, the Fc region of the UPK-Ib antibodies can be modified to have one or more amino acid modifications, wherein one or more amino acid modification is a substitution at position 243 with leucine, at position 292 with proline, at position 300 with leucine, at position 305 with isoleucine, and position 396 with leucine with respect to the wild type Fc region according to the EU index as set forth in Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, (5th ed., Public Health Service, MD 1991).

The Fc mutant of the invention is based, in part, on the identification of mutant human IgG1 heavy chain Fc regions, with altered affinities for different Fcγ receptors (FcγR), using a yeast display system. Briefly, the Fc enhancing mutant comprising the amino acid sequence set

forth in Tables 1-3 and other variants were identified from a yeast library of mutants and assayed using an ELISA assay for determining binding to an FcγR. The Fc mutants were also tested in an ADCC assay, by cloning the Fc variants into a desired antibody. Accordingly, the invention relates to molecules including, but are not limited to polypeptides, immunoglobulins, and antibodies, comprising a variant Fc region, having one or more amino acid modifications (*e.g.*, substitutions, but also including insertions or deletions) in one or more regions, which modifications alter the affinity of the variant Fc region for an FcγR.

It is also contemplated that the variant Fc region can include unnatural amino acids. Such methods are known to those skilled in the art such as those using the natural biosynthetic machinery to allow incorporation of unnatural amino acids into proteins (*e.g.*, Wang et al., 2002 *Chem. Comm.* 1: 1-11; Wang et al., 2001 *Science* 292: 498-500; van Hest et al., 2001 *Chem. Comm.* 19: 1897-1904).

In one aspect, the UPK-Ib specific antibodies can be engineered in a manner that the Fc regions of the antibody can be either homodimers or heterodimers with respect to comprising at least one Fc region from any one of the Fc enhancing mutants disclosed herein. Heterodimers comprising Fc regions refer to molecules where the two Fc chains have the same or different sequences. In some aspects, in the heterodimeric molecules comprising variant Fc regions, each chain has one or more different modifications from the other chain. In other aspects, in the heterodimeric molecules comprising variant Fc regions, one chain contains the wild-type Fc region and the other chain comprises one or more modifications. Methods of engineering heterodimeric Fc containing molecules are known in the art.

The UPK-Ib specific antibodies engineered to comprise a variant Fc region binds with a greater affinity to one or more FcγRs compared to an otherwise identical UPK-Ib antibody having a wild Fc region. Such molecules can mediate effector function, such as ADCC, more effectively as discussed elsewhere herein. For example, increased effector function would be directed to tumor and foreign cells or otherwise a cell that expresses a relative higher level of UPK-Ib compared with an otherwise identical healthy cell.

In another aspect, the UPK-Ib specific antibody comprising the Fc enhancing mutant may be combined with other Fc modifications known in the art to provide additive, synergistic, or novel properties to the UPK-Ib specific antibodies. For example, the Fc variants can be combined with other known Fc variants such as those disclosed in Duncan et al., 1988 *Nature* 332: 563-564; Lund et al., 1991 *J. Immunology* 147: 2657-2662; Lund et al., 1992 *Molecular Immunology* 29: 53-59; Alegre et al., 1994 *Transplantation* 57: 1537-1543; Hutchins et al., 1995

Proc. Natl. Acad. Sci. U.S.A. 92: 11980-11984; Jefferis et al., 1995 *Immunology Letters* 44: 111-117; Lund et al., 1995 *FASEB J.* 9: 115-119; Jefferis et al., 1996 *Immunology Letter* 54: 101-104; Lund et al., 1996 *J. Immunology* 157: 49634969; Armour et al., 1999 *Eur. J. Immunology* 29: 2613-2624; Idusogie et al., 2000 *J. Immunology* 164: 41784184; Reddy et al., 2000 *J. Immunology* 164: 1925-1933; Xu et al., 2000 *Cell Immunology* 200: 16-26; Idusogie et al., 2001 *J. Immunology* 166: 2571-2575; Shields et al., 2001 *J. Biol. Chem.* 276: 6591-6604; Jefferis et al., 2002 *Immunology Letters* 82: 57-65; Presta et al., 2002 *Biochem. Soc. Trans.* 30:487-490; U.S. Patent No. 5,624,821; U.S. Patent No. 5,885,573; U.S. Patent. No. 6,194,551; WO 00/42072; and WO 99/58572.

The Fc variant designated as the Fc enhancing mutant described herein may be subjected to further modifications, often times depending on the intended use of the variant. Such modifications may involve further alteration of the amino acid sequence (substitution, insertion and/or deletion of amino acid residues), fusion to heterologous polypeptide(s) and/or covalent modifications. Such further modifications may be made prior to, simultaneously with, or following, the amino acid modification(s) disclosed herein which results in altered properties such as an alteration of Fc receptor binding and/or ADCC activity.

The UPK-Ib specific antibody comprising the Fc enhancing mutant can exhibit enhanced affinity for an FcγR including, but is not limited to, FcγRI, FcγRII-A, FcγRII-B FcγRII-C, FcγRIIIA, and the like as determined using ADCC activity assays disclosed herein. Examples of effector functions that could be mediated by the antibodies of the invention include, but are not limited to, complement-dependent cytotoxicity (CDC), ADCC, phagocytosis, and the like. Also contemplated is the multifunctionality of the Fc variant with respect to the effector function. For example, the Fc enhancing mutant can alter only one of the effector function or can alter any combination of the effector functions. The effector functions of the antibodies can be assayed using standard methods known in the art.

In one aspect, the UPK-Ib specific antibodies comprising the Fc enhancing mutant exhibits enhanced affinity for an FcγR mediate ADCC 2-fold more effectively, than an otherwise identical antibody comprising a wild-type Fc region. In other aspects, the UPK-Ib specific antibodies comprising the Fc enhancing mutant exhibit enhanced affinity for an FcγR with respect to mediating ADCC at least 4-fold, at least 8-fold, at least 10-fold, at least 100-fold, at least 1000-fold, at least 10^4-fold, at least 10^5-fold more effectively, or any integer in between, than an UPK-Ib antibody comprising a wild-type Fc region.

In another aspect, the UPK-Ib specific antibodies comprising the Fc enhancing mutant

exhibits enhanced affinity for an FcγR and therefore can alter CDC. The UPK-Ib specific antibodies comprising the Fc enhancing mutant can exhibit enhanced affinity for an Fcγ with respect to mediating CDC 2-fold more effectively, than an otherwise identical antibody comprising a wild-type Fc region. In other aspects, the UPK-Ib specific antibodies comprising the Fc enhancing mutant exhibit enhanced affinity for an Fcγ with respect to mediating CDC at least 4-fold, at least 8-fold, at least 10-fold, at least 100-fold, at least 1000-fold, at least 10^4-fold, at least 10^5-fold higher CDC, or any integer in between, than an UPK-Ib antibody comprising a wild-type Fc region.

In another aspect, the UPK-Ib specific antibodies comprising the Fc enhancing mutant exhibits enhanced affinity for an FcγR and therefore can altered effector function with respect to phagocytosis activity as determined by standard assays known to one skilled in the art or disclosed herein. The UPK-Ib specific antibodies comprising the Fc enhancing mutant can exhibit enhanced affinity for an Fcγ with respect to mediating phagocytosis 2-fold more effectively, than an otherwise identical antibody comprising a wild-type Fc region. In other aspects, the UPK-Ib specific antibodies comprising the Fc enhancing mutant exhibit enhanced affinity for an Fcγ with respect to mediating phagocytosis at least 4-fold, at least 8-fold, at least 10-fold, at least 100-fold, at least 1000-fold, at least 10^4-fold, at least 10^5-fold higher phagocytosis activity, or any integer in between, than an UPK-Ib antibody comprising a wild-type Fc region.

2.4.2 Anti-tumor moitey

In some aspects, the antibody can further be engineered to comprise an anti-tumor agent including but is not limited to a chemotherapeutic agent, an anti-cell proliferation agent or any combination thereof. The chimeric antibody comprising an anti-tumor agent may act additively or synergistically against the cancer. The term "synergistically" as used herein refers to the combined effect of the antibody and anti-tumor agent being greater than the sum of their individual effects.

U. S. Pat. No. 4,680,338, describes bifunctional linkers useful for producing conjugates of ligands with amine-containing polymers and/or proteins, especially for forming antibody conjugates with chelators, drugs, enzymes, detectable labels and the like. U.S. Pat. Nos. 5,141,648 and 5,563,250 disclose cleavable conjugates containing a labile bond that is cleavable under a variety of mild conditions. This linker is particularly useful in that the agent of interest may be bonded directly to the linker, with cleavage resulting in release of the active agent.

Exemplary uses include adding a free amino or free sulfhydryl group to a protein, such as an antibody, or a drug.

U.S. Pat. No. 5,856,456 provides peptide linkers for use in connecting polypeptide constituents to make fusion proteins, *e.g.*, single chain antibodies. The linker can be up to about 50 amino acids in length, can contain at least one occurrence of a charged amino acid (*e.g.*, arginine or lysine) followed by a proline, and can be characterized by greater stability and reduced aggregation. U.S. Pat. No. 5,880,270 discloses aminooxy-containing linkers useful in a variety of immunodiagnostic and separative techniques.

Another aspect contemplates linking a chemotherapeutic agent to a UPK-Ib specific antibody. For example, any conventional chemotherapeutic agents of the following exemplary classes are included: alkylating agents; nitrosoureas; antimetabolites; antitumor antibiotics; plant alkyloids; taxanes; hormonal agents; and miscellaneous agents.

Alkylating agents are so named because of their ability to add alkyl groups to many electronegative groups under conditions present in cells, thereby interfering with DNA replication to prevent cancer cells from reproducing. Most alkylating agents are cell cycle non-specific. In specific aspects, they stop tumor growth by cross-linking guanine bases in DNA double-helix strands. Examples include busulfan, carboplatin, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, ifosfamide, mechlorethamine hydrochloride, melphalan, procarbazine, thiotepa, and uracil mustard.

Nitrosoureas are referred to as alkylating agents because they act by the process of alkylation to inhibit DNA repair. Most nitrosourea drugs are cell cycle-nonspecific. Examples include carmustine, lumustine, and streptozocin.

Anti-metabolites prevent incorporation of bases into DNA during the synthesis (S) phase of the cell cycle, prohibiting normal development and division. Exemplary antimetabolites include drugs such as 5-fluorouracil, 6-mercaptopurine, capecitabine, cytosine arabinoside, floxuridine, fludarabine, gemcitabine, methotrexate, and thioguanine.

There are a variety of antitumor antibiotics that generally prevent cell division by interfering with enzymes needed for cell division or by altering the membranes that surround cells. Included in this class are the anthracyclines, such as doxorubicin, which act to prevent cell division by disrupting the structure of the DNA and terminate its function. These agents are cell cycle non-specific. Antitumor antibiotics include dactinomycin, daunorubicin, doxorubicin, idarubicin, mitomycin-C, and mitoxantrone.

Plant alkaloids inhibit or stop mitosis or inhibit enzymes that prevent cells from making

proteins needed for cell growth. Frequently used plant alkaloids include vinblastine, vincristine, vindesine, and vinorelbine.

The taxanes affect cell structures called microtubules that are important in cellular functions. In normal cell growth, microtubules are formed when a cell starts dividing, but once the cell stops dividing, the microtubules are disassembled or destroyed. Taxanes prohibit the microtubules from breaking down such that the cancer cells become so clogged with microtubules that they cannot grow and divide. Exemplary taxanes include paclitaxel and docetaxel.

Hormonal agents and hormone-like drugs are utilized for certain types of cancer, including, for example, leukemia, lymphoma, and multiple myeloma. They are often employed with other types of chemotherapy drugs to enhance their effectiveness. Sex hormones are used to alter the action or production of female or male hormones and are used to slow the growth of breast, prostate, and endometrial cancers. Inhibiting the production (aromatase inhibitors) or action (tamoxifen) of these hormones can often be used as an adjunct to therapy. Some other tumors are also hormone dependent. Tamoxifen is an example of a hormonal agent that interferes with the activity of estrogen, which promotes the growth of breast cancer cells.

Miscellaneous agents include chemotherapeutics such as bleomycin, hydroxyurea, L-asparaginase, and procarbazine.

An anti-cell proliferation agent can further be defined as an apoptosis-inducing agent or a cytotoxic agent. The apoptosis-inducing agent may be a granzyme, a Bcl-2 family member, cytochrome C, a caspase, or a combination thereof. Exemplary granzymes include granzyme A, granzyme B, granzyme C, granzyme D, granzyme E, granzyme F, granzyme G, granzyme H, granzyme I, granzyme J, granzyme K, granzyme L, granzyme M, granzyme N, or a combination thereof. In other specific aspects, the Bcl-2 family member is, for example, Bax, Bak, Bcl-Xs, Bad, Bid, Bik, Hrk, Bok, or a combination thereof.

In additional aspects, the caspase is caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10, caspase-11, caspase-12, caspase-13, caspase-14, or a combination thereof. Other exemplary cytotoxic agents include TNF-α, gelonin, Prodigiosin, a ribosome-inhibiting protein (RIP), *Pseudomonas* exotoxin, *Clostridium difficile* Toxin B, *Helicobacter pylori* VacA, *Yersinia enterocolitica* YopT, Violacein, diethylenetriaminepentaacetic acid, irofulven, diptheria toxin, mitogillin, ricin, botulinum toxin, cholera toxin, saporin 6, or a combination thereof.

The chimeric antibody (*e.g.*, UPK-Ib specific antibody linked to a anti-tumor agent) can

be administered concomitantly or in succession with other forms of therapy, such as chemotherapy, surgery, radiation, gene therapy, hormone therapy, immunotherapy, or a combination thereof. Therapeutic methods are discussed elsewhere herein.

2.4.3 Multispecific

The UPK-Ib antibodies may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide or may be specific for heterologous epitopes, such as a heterologous polypeptide or solid support material. *See, e.g.*, PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt et al., 1991 *J. Immunology* 147: 60-69; U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., 1992 *J. Immunology* 148: 1547-1553.

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., 1983 *Nature* 305: 537-539). Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., 1991 *EMBO J.* 10: 3655-3659.

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism.

In another approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This approach is disclosed in WO 94/04690. According to another approach described in WO96/27011, a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. An exemplary interface comprises at least a part of the CH3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side

chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine).

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360 and WO 92/200373). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

3.1 Immunological Binding Assays

A UPK-Ib polypeptide can be detected in an immunoassay utilizing a UPK-Ib specific antibody as a capture agent that specifically binds to the UPK-Ib polypeptide. As used herein, an immunoassay is an assay that utilizes an antibody (*e.g.* a UPK-Ib antibody) to specifically bind an analyte (*e.g.*, UPK-Ib). The immunoassay is characterized by the use of specific antibody binding to a UPK-Ib antibody as opposed to other physical or chemical properties to isolate, target, and quantify the UPK-Ib analyte. The UPK-Ib marker may be detected and quantified using any of a number of well recognized immunological binding assays (*See*, for example, U.S. Pat. Nos. 4,366,241; 4,376,110; 4,517,288; and 4,837,168).

Immunoassays are performed in any of several configurations, *e.g.*, those reviewed in Tijssen, PRACTICE AND THEORY OF ENZYME IMMUNOASSAYS: LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, (Elsevier Science Publishers B.V., Amsterdam, 1985); Harlow et al., USING ANTIBODIES: A LABORATORY MANUAL, (Cold Spring Harbor Press, New York, 1998); Breitling et al., RECOMBINANT ANTIBODIES (Wiley-Spektrum, 1999); and Price and Newman, PRINCIPLES AND PRACTICE OF IMMUNOASSAYS, (Stockton Press, New York, 1991). Immunoassays often utilize a labeling agent to specifically bind to and label the binding complex formed by the capture agent and the analyte (*e.g.*, a UPK-Ib antibody/UPK-Ib complex). The labeling agent may itself be one of the moieties comprising the antibody/analyte complex. Thus, the labeling agent may be a labeled UPK-Ib specific antibody. Alternatively, the labeling agent is optionally a third moiety, such as another antibody, that specifically binds to the UPK-Ib specific antibody, the UPK-Ib peptide, the anti-body/polypeptide complex, or to a modified capture group (*e.g.*, biotin) which is covalently linked to UPK-Ib or to the UPK-Ib specific antibody.

In one aspect, the labeling agent is an antibody that specifically binds to the UPK-I

specific antibody. Thus, for example, where the capture agent is a human derived UPK-Ib specific antibody, the label agent may be a mouse anti-human IgG, *e.g.*, an antibody specific to the constant region of the human antibody.

Other proteins capable of specifically binding immunoglobulin constant regions, such as streptococcal protein A or protein G are also used as the labeling agent. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non immunogenic reactivity with immunoglobulin constant regions from a variety of species.

Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, analyte, volume of solution, concentrations, and the like. Usually, the assays are carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 5°C to 45°C.

Immunoassays for detecting UPK-Ib can either be competitive or noncompetitive assays. Noncompetitive immunoassays are assays in which the amount of captured analyte (*e.g.*, UPK-Ib) is directly measured. An exemplary assay is the "sandwich" assay, for example, the capture agent (*e.g.*, UPK-Ib specific antibody) is bound directly or indirectly to a solid substrate where it is immobilized. These immobilized UPK-Ib specific antibodies capture UPK-Ib present in a test sample (*e.g.*, a tissue sample). The UPK-Ib thus immobilized is then bound by a labeling agent, such as a UPK-Ib specific antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. Free labeled antibody is washed away and the remaining bound labeled antibody is detected (*e.g.*, using a gamma detector where the label is radioactive).

In competitive assays, the amount of analyte (*e.g.*, UPK-Ib) present in the sample is measured indirectly by measuring the amount of an added (exogenous) analyte displaced (or competed away) from a capture agent (*e.g.*, UPK-Ib specific antibody) by the analyte present in the sample. In one competitive assay, a known amount of UPK-Ib is added to a test sample with an unquantified amount of UPK-Ib, and the sample is contacted with a capture agent, *e.g.*, a UPK-Ib antibody that specifically binds UPK-Ib. The amount of added UPK-Ib that binds to the UPK-Ib specific antibody is inversely proportional to the concentration of UPK-Ib present in the test sample.

The UPK-Ib specific antibody can be immobilized on a solid substrate. The amount of

UPK-Ib bound to the UPK-Ib specific antibody is determined either by measuring the amount of UPK-Ib present in a UPK-Ib/UPK-Ib specific antibody complex, or alternatively by measuring the amount of remaining uncomplexed UPK-Ib.

In another aspect, UPK-Ib polypeptides or UPK-Ib specific antibodies can also be detected and quantified by any of a number of other means well known to those of skill in the art. These include analytic biochemical methods such as spectrophotometry, radiography, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like. Immunological methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, and the like can be used to detect and quantify the antibodies.

Western blot analysis and related methods can also be used to detect and quantify the presence of UPK-Ib polypeptides in a sample. The technique generally comprises separating sample products by gel electrophoresis on the basis of molecular weight, transferring the separated products to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the antibodies that specifically bind to UPK-Ib polypeptide. The antibodies specifically bind to the biological agent of interest on the solid support. These antibodies are directly labeled or alternatively are subsequently detected using labeled antibodies (*e.g.*, labeled sheep anti-human antibodies where the antibody to a marker gene is a human antibody) which specifically bind to the antibody which binds UPK-Ib.

3.2 Selection/Measurement of Antibodies

Selection of UPK-Ib specific antibodies (whether produced by phage display, immunization methods, hybridoma technology, etc.) involves screening the resulting antibodies for specific binding to an appropriate antigen. Antibodies are selected to bind one or more epitopes bound by antibodies including but not limited to 1G9, 5H9 and IA10. Selection can be by any of a number of methods well known to those of skill in the art or disclosed elsewhere herein.

Selection for increased avidity involves measuring the affinity of a UPK-Ib specific antibody (or a modified UPK-Ib specific antibody) for UPK-Ib (or a UPK-Ib fragment, or an epitope on UPK-Ib, etc.). Methods of making such measurements are described elsewhere herein. In addition, the K_d of a UPK-Ib specific antibody and the kinetics of binding to UPK-Ib

can be determined in a BIAcore, a biosensor based on surface plasmon resonance. For this technique, antigen is coupled to a derivatized sensor chip capable of detecting changes in mass. When antibody is passed over the sensor chip, antibody binds to the antigen resulting in an increase in mass that is quantifiable. Measurement of the rate of association as a function of antibody concentration can be used to calculate the association rate constant (k_{on}). After the association phase, buffer is passed over the chip and the rate of dissociation of antibody (k_{off}) determined. K_{on} is typically measured in the range 1.0×10^2 to 5.0×10^6 and k_{off} in the range 1.0×10^{-1} to 1.0×10^{-6}. The equilibrium constant K_d is then calculated as k_{off}/k_{on} and thus is typically measured in the range 10^{-5} to 10^{-12}. Affinities measured in this manner correlate well with affinities measured in solution by fluorescence quench titration.

3.3 Assaying for Cross-reactivity

In one aspect, the antibodies specifically bind to one or more epitopes recognized by antibodies including but are not limited to 1G9, 5H9 and 1A10. Two exemplary antibodies are cross-reactive with one of more of these antibodies. Means of assaying for cross-reactivity are well known to those of skill in the art (*e.g.*, Dowbenko et al., 1998 *J. of Virology* 62: 4703-4711).

This can be ascertained by providing an isolated UPK-Ib polypeptide attached to a solid support and assaying the ability of a test antibody to compete with antibodies including but are not limited to 1G9, 5H9 and 1A10 for UPK-Ib binding. Thus, immunoassays in a competitive binding format are used for cross-reactivity determinations. For example, in one aspect, a UPK-Ib polypeptide is immobilized to a solid support. Antibodies to be tested are added to the assay and compete with antibodies including but are not limited to 1G9, 5H9 and 1A10 for the binding to the immobilized UPK-Ib polypeptide. The ability of test antibodies to compete with the binding of the antibodies including but are not limited to 1G9, 5H9 and 1A10 to the immobilized protein are compared. The percent cross-reactivity above proteins is then calculated, using standard calculations.

Cross-reactivity to at least 1G9, 5H9 or 1A10 antibody can be ascertained by a number of other standard techniques (*e.g.*, Geysen et al., 1987 *J. Immunol. Methods* 102: 259-274). This technique involves the synthesis of large numbers of overlapping UPK-Ib peptides. The synthesized peptides are then screened against at least 1G9, 5H9 or 1A10 antibody and the characteristic epitopes specifically bound by these antibodies can be identified by binding specificity and affinity. The epitopes thus identified can be conveniently used for competitive assays as described herein to identify other cross-reacting antibodies.

Epitope mapping can be conveniently prepared using "Multipin" peptide synthesis techniques (*e.g.*, Geysen et al., 1987 *Science* 235: 1184-1190). Using the known sequence of UPK-Ib, overlapping UPK-Ib peptide sequences can be synthesized individually in a sequential manner on plastic pins in an array of one or more 96-well microtiter plate(s).

The procedure for epitope mapping using this multipin peptide system is described in for example U.S. Pat. No. 5,739,306. Briefly, the pins are first treated with a pre-coat buffer containing 2% bovine serum albumin and 0.1% Tween® 20 in PBS for 1 hour at room temperature. Then the pins are then inserted into the individual wells of 96-well microtest plate containing an antibody selected from the group consisting of for example 1G9, 5H9, 1A10 antibody or any combination thereof. The incubation is for about 1 hour at room temperature. The pins are washed in PBST (*e.g.*, 3 rinses for every 10 minutes), and then incubated in the wells of a 96-well microtest plate containing 100 μL of HRP-conjugated goat anti-mouse IgG (Fc) (Jackson ImmunoResearch Laboratories) at a 1:4,000 dilution for 1 hour at room temperature. After the pins are washed as before, the pins are placed into wells containing peroxidase substrate solution of diammonium 2,2'-azino-bis [3-ethylbenzthiazoline-b-sulfonate] (ABTS) and H_2O_2 (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Md.) for 30 minutes at room temperature for color reaction. The plate is read at 405 nm by a plate reader (*e.g.*, BioTek ELISA plate reader) against a background absorption wavelength of 492 nm. Wells showing color development indicated reactivity of the UPK-Ib peptides in such wells with at least 1G9, 5H9, 1A10 antibody.

4. Detection of Antibodies

A labeling agent can be, *e.g.*, a monoclonal antibody, a polyclonal antibody, a protein or complex such as those described herein, or a polymer such as an affinity matrix, carbohydrate or lipid. Detection proceeds by any known method, including immunoblotting, western analysis, gel-mobility shift assays, tracking of radioactive or bioluminescent markers, nuclear magnetic resonance, electron paramagnetic resonance, stopped-flow spectroscopy, column chromatography, capillary electrophoresis, immunohistochemistry, or other methods which track a molecule based upon an alteration in size and/or charge. The particular detectable label or group used in the assay is not a critical aspect. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, any detectable label useful in such methods can be applied to the present invention. Thus, a detectable label for the purpose of detecting the antibody is any

composition that is detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful detectable labels include magnetic beads (*e.g.*, Dynabeads™), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (*e.g.*, ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (*e.g.*, LacZ, CAT, horse radish peroxidase, alkaline phosphatase and others, commonly used as detectable enzymes, either as marker gene products or in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, etc.) beads.

The detectable label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of detectable labels may be used, with the choice of detectable label depending on the sensitivity required, ease of conjugation of the compound, stability requirements, available instrumentation, and disposal provisions.

Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (*e.g.*, biotin) is covalently bound to the molecule. The ligand then binds to an anti-ligand (*e.g.*, streptavidin) molecule which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, for example, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody.

The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, *e.g.*, luminol. For a review of various labeling or signal producing systems which may be used, *see*, *e.g.* U.S. Pat. No. 4,391,904, which is incorporated herein by reference.

Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence, *e.g.*, by microscopy, visual inspection, via photographic film, by the use of

electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing appropriate substrates for the enzyme and detecting the resulting reaction product. Finally, simple colorimetric labels may be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of UPK-Ib. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

5. Modification of UPK-Ib Specific Antibodies

5.1 Phage Display

A phage display can be used to increase antibody affinity. To create antibodies of higher affinity for a uroplakin (*e.g.* UPK-Ib), mutant single chain variable fragment (scFv) gene repertories, based on the sequences disclosed herein, can be created and expressed on the surface of phage. Display of antibody fragments on the surface of viruses which infect bacteria (bacteriophage or phage) makes it possible to produce human or other mammalian antibodies with a wide range of affinities and kinetic characteristics. To display antibody fragments on the surface of phage (phage display), an antibody fragment gene is inserted into the gene encoding a phage surface protein and the antibody fragment-fusion protein is expressed on the phage surface (McCafferty et al., 1990 *Nature* 348: 552-554; and Hoogenboom et al., 1991 *Nucleic Acids Research* 19: 4133-4137).

Since the antibody fragments on the surface of the phage are functional, those phage bearing antigen binding antibody fragments can be separated from non-binding or lower affinity phage by antigen affinity chromatography (McCafferty et al., 1990 *Nature* 348: 552-554). Mixtures of phage are allowed to bind to the affinity matrix, non-binding or lower affinity phage are removed by washing, and bound phage are eluted by treatment with acid or alkali. Depending on the affinity of the antibody fragment, enrichment factors of 20 fold to 1,000,000 fold are obtained by single round of affinity selection.

One approach for creating mutant scFv gene repertoires involves replacing either the V_H or V_L gene from a binding scFv with a repertoire of V_H or V_L genes (otherwise known as chain shuffling) (Clackson et al., 1991 *Nature* 352: 624-628). Such gene repertoires contain numerous

variable genes derived from the same germline gene as the binding scFv, but with point mutations (Marks et al., 1992 *Biotechnology* 10: 779-783). Using light or heavy chain shuffling and phage display, the binding avidities of UPK-1b antibody fragment can be dramatically increased.

In order to generate an antibody having an increased affinity, during the screening for the antibody, the antigen concentration is decreased in each round of selection, reaching a concentration less than the desired K_d by the final rounds of selection. This results in the selection of a desired antibody on the basis of affinity (Hawkins et al., 2002 *J. Molecular Biology* 226: 889-896).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. *See* U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645; WO 98/50433; WO 98/24893; WO 98/16654; WO 96/34096; WO 96/33735; and WO 91/10741.

5.2) Site Directed Mutagenesis

The majority of antigen contacting amino acid side chains are located in the complementarity determining regions (CDRs). Three of the CDRs occur in the V_H (CDR1, CDR2, and CDR3) and three in the V_L (CDR1, CDR2, and CDR3) (Chothia et al., 1987 *J. of Molecular Biology* 196: 901-917; Chothia et al., 1986 *Science* 233: 755-8; Nhan et al., 1991 *J. of Molecular Biology* 217: 133-151). These residues contribute the majority of binding energetics responsible for antibody affinity for antigen. In other molecules, mutating amino acids that contact ligand has been shown to be an effective means of increasing the affinity of one protein molecule for its binding partner (Lowman et al., 1993 *J. of Molecular Biology* 234: 564-578; Wells, 1990 *Biochemistry* 29: 8509-8516). Thus, mutation of the CDRs and screening against UPK-Ib or the epitopes thereof identified herein, may be used to generate UPK-Ib specific antibodies having improved binding affinity.

In one aspect, each CDR is randomized in a separate library. To simplify affinity measurements, existing antibodies or other lower affinity UPK-Ib specific antibodies, are used as a template, rather than a higher affinity scFv. The CDR sequences of the highest affinity mutants from each CDR library are combined to obtain an additive increase in affinity. A similar approach has been used to increase the affinity of human growth hormone (hGH) for the growth

hormone receptor over 1500 fold from 3.4 X 10^{-10} to 9.0 X 10^{-13} M (Lowman et al., 1993, J. Mol. Biol., 234: 564-578).

To increase the affinity of UPK-Ib specific antibodies, amino acid residues located in one or more CDRs (*e.g.*, 9 amino acid residues located in V_L CDR3) are partially randomized by synthesizing a "doped" oligonucleotide in which the wild type nucleotide occurred with a frequency of about for example 49%. The oligonucleotide is used to amplify the remainder of the UPK-Ib specific scFv gene(s) using PCR.

For example in one aspect, to create a library in which V_H CDR3 is randomized, an oligonucleotide is synthesized which anneals to the UPK-Ib specific antibody V_H framework 3 and encodes V_H CDR3 and a portion of framework 4. At the four positions to be randomized, the sequence "NNS" can be used, where N is any of the 4 nucleotides, and S is "C" or "T". The oligonucleotide is used to amplify the UPK-Ib specific antibody V_H gene using PCR, creating a mutant UPK-Ib specific antibody V_H gene repertoire. PCR is used to splice the V_H gene repertoire with the UPK-Ib specific antibody light chain gene, and the resulting scFv gene repertoire is cloned into a phage display vector. Ligated vector DNA is used to transform electrocompetent *E. coli* to produce a phage antibody library.

To select higher affinity mutant scFv, each round of selection of the phage antibody libraries is conducted on decreasing amounts of UPK-Ib, as described elsewhere herein. Typically, clones from the third and fourth round of selection are screened for binding to the UPK-Ib antigen by ELISA.

5.3 Creation of $(scFv')_2$ Homodimers

To create UPK-Ib specific $(scFv')_2$ antibodies, two UPK-Ib specific scFvs are joined, either through a linker (*e.g.*, a carbon linker, a peptide, etc.) or through a disulfide bond between, for example, two cysteines. Thus, for example, to create disulfide linked UPK-Ib specific scFv, a cysteine residue can be introduced by site directed mutagenesis.

In one aspect, the $(scFv')_2$ dimer is created by joining the scFv fragments through a linker, for example through a peptide linker. This can be accomplished by a wide variety of means well known to those of skill in the art. For example, one approach is described by Holliger et al., 1993 *Proc. Nat'l. Acad. Sci. USA* 90: 6444-6448 (*See* also WO 94/13804).

Typically, linkers are introduced by PCR cloning. For example, synthetic oligonucleotides encoding the linker can be used to PCR amplify the UPK-Ib specific antibody V_H and V_L genes which are then spliced together to create the UPK-Ib specific diabody gene.

The gene is then cloned into an appropriate vector, expressed, and purified according to standard methods well known to those of skill in the art.

5.4 Preparation of $(scFv)_2$, Fab, and $(Fab')_2$ molecules

UPK-Ib specific antibodies such as UPK-Ib specific scFv, or variant(s) with higher affinity, are suitable templates for creating size and valency variants. For example, a UPK-Ib specific $(scFv')_2$ is created from the parent scFv as described above. An scFv gene can be excised using appropriate restriction enzymes and cloned into another vector.

A UPK-Ib specific Fab is expressed in *E. coli* using an expression vector similar to the one described by for example Better et. al., 1988 *Science* 240: 1041-1043. To create a UPK-Ib specific Fab, the V_H and V_L genes are amplified from the scFv using PCR. The V_H gene is cloned into an expression vector that provides an IgG C_{H1} domain downstream from, and in frame with, the V_H gene. The vector also contains a leader sequence to direct expressed V_H-C_{H1} domain into the periplasm, a leader sequence to direct expressed light chain into the periplasm, and cloning sites for the light chain gene. Clones containing the correct VH gene are identified, *e.g.*, by PCR fingerprinting. The V_L gene is spliced to the C_L gene using PCR and cloned into the vector containing the V_HC_{H1} gene.

6. Diagnostic and Screening Assays

The UPK-Ib specific antibodies may be used for the *in vivo* or *in vitro* detection of UPK-Ib and thus, are useful in the diagnosis (*e.g.*, confirmatory diagnosis) and/or screening of diseases associated with unregulated levels of UPK-Ib. The detection and/or quantification of an increased level of UPK-Ib in subject or a biological sample obtained from that subject, compared with the level of UPK-Ib in another otherwise identical healthy subject is indicative of a disease state associated with increased levels of UPK-Ib. Such a disease can include but is not limited to bladder cancer, ovarian cancer, lung cancer, etc.

For example, a UPK-Ib antigen may be quantified in a biological sample derived from a subject such as a cell, or a tissue sample derived from a subject. As used herein, a biological sample is a sample of biological tissue or fluid that contains an increased level of UPK-Ib may be correlated with and indicative of cancer.

Although the sample is typically taken from a human subject, the assays can be used to detect UPK-Ib antigen in cells from mammals in general, such as dogs, cats, sheep, cattle and pigs, and most particularly primates such as humans, chimpanzees, gorillas, macaques, and

baboons, and rodents such as mice, rats, and guinea pigs.

Tissue or fluid samples are isolated from a subject according to standard methods well known to those of skill in the art, most typically by biopsy. The sample is optionally pretreated as necessary by dilution in an appropriate buffer solution or concentrated, if desired. Any of a number of standard aqueous buffer solutions, employing one of a variety of buffers, such as phosphate, Tris, or the like, at physiological pH can be used.

7. Carriers and Excipients

The compositions disclosed can be administered in a variety of ways. It should be noted that the pharmaceutical composition containing the antibody(s) can be administered alone or in combination with one or more pharmaceutically acceptable carriers, stabilizers, preservatives, colorants, flavorants, and excipients and combinations thereof.

The compounds and compositions disclosed can be formulated with conventional carriers and excipients, which are selected in accord with ordinary practice. Aqueous formulations are prepared in sterile form, and when intended for delivery by routes other than oral administration, generally are isotonic. All formulations optionally contain excipients such as those provided for example in the HANDBOOK OF PHARMACEUTICAL EXCIPIENTS (5^{th} ed., Raymond C. Rowe et al., eds., 2006). Excipients include ascorbic acid and other antioxidants, chelating agents (*e.g.*, EGTA and EDTA), carbohydrates (*e.g.*, dextrin), hydroxyalkylcellulose, hydroxyalkyl-methylcellulose, stearic acid, and the like. The pH of the formulations ranges from about 3.0 to about 11.0, but is ordinarily about 7.0 to about 7.8.

Examples of physiologically acceptable carriers for routes of administration other than oral administration include but are not limited to saline solutions (*e.g.*, normal saline, Ringer's solution, PBS (phosphate-buffered saline); polysorbate 80; L-arginine; polyvinylpyrrolidone; α-D-glucopyranosyl; α-D-glucopyranoside (trehalose); and combinations, thereof. For example, trehalose can be present in the composition in an amount from about 2 to about 10% weight/volume of the composition. In another example, when trehalose and polysorbate 80 are both present in the composition, trehalose can be present in the amount of about 4 to about 6% wt./vol. and the polysorbate 80 can be present in the amount of about 0.001 to 0.01% (wt./vol.) and generally mixtures of various physiologically compatible salts including potassium and phosphate salts with or without sugar additives (*e.g.*, glucose).

Suitable excipients for use in the immunogenic formulations are, for example, water, saline, dextrose, glycerol, and ethanol. Non-toxic auxiliary substances, such as wetting agents,

buffers, stabilizers, or emulsifiers can also be added to the composition.

Parenteral administration, if used, is generally characterized by injection. Sterile injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions.

8. Diagnosis/Treatment Kits

The antibodies are useful for the diagnosis, assessment and treatment of diseases associated with unregulated expression of UPK-Ib. Moreover, the antibodies can be used to detect and or measure the amount of UPK-Ib present in a biological sample using well-known methods such as, but not limited to, Western blotting, enzyme-linked immunosorbent assay (ELISA), and immunohistochemistry. Further, the antibodies can be used to immunoprecipitate and/or immuno-affinity purify their cognate antigen as described elsewhere herein.

Another aspect contemplates a method of assessing the presence or degree of UPK-Ib present in a mammal. This method is useful to diagnose the levels of UPK-Ib and is also useful for assessing the progress of the efficacy of treatment in a subject having a disease associated with unregulated levels of UPK-Ib such as cancer (*e.g.,* bladder cancer, ovarian cancer, lung cancer, etc.). For example, the method can comprise obtaining a biological sample from a mammal at risk of or exhibiting characteristics of bladder cancer and assessing the amount of UPK-Ib present in the biological sample, wherein a larger amount of UPK-Ib, compared with the amount of UPK-Ib from an otherwise identical subject not at risk or having bladder cancer, is an indication that UPK-Ib is present in the subject, and further wherein the presence of UPK-Ib in the subject is related to the severity of the condition. Such a method is useful in the diagnosis of bladder cancer and in providing an assay for following up on the efficacy of bladder cancer treatments. That is, the amount of UPK-Ib present in a sick subject can be evaluated before, during and after treatment and the efficacy of the treatment could thus be assessed. Further, such a method allows a determination of the presence and/or the severity of the disease in a subject.

In another aspect, kits are provided for the treatment cancer (*e.g.*, bladder cancer, ovarian cancer, lung cancer, etc.) or for the detection/confirmation of cancer. Kits will typically comprise one or more UPK-Ib specific antibodies. For diagnostic purposes, the antibody(s) can be labeled. In addition the kits can include instructional materials disclosing means of using UPK-Ib specific antibodies in the treatment of symptoms of the desired cancer. The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, where a kit contains a UPK-Ib specific antibody wherein the antibody is labeled, the

kit may additionally contain means of detecting the label (*e.g.*, enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a sheep anti-human antibodies, or the like). The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

Conveniently, the antibodies may be conjugated with markers, such as magnetic beads, which allow for direct separation, biotin, which can be removed with avidin or streptavidin bound to a support, fluorochromes, which can be used with a fluorescence activated cell sorter, or the like, to allow for ease of separation of the particular sample.

9. Methods of Treatment and Administration

The invention further encompasses administering the antibodies of the invention in combination with other therapies known to those skilled in the art for the treatment or prevention of cancer, including but not limited to, current standard and experimental chemotherapies, hormonal therapies, biological therapies, immunotherapies, radiation therapies, or surgery. The antibodies of the invention may be administered in combination with a therapeutically or prophylactically effective amount of one or more anti-cancer agents, therapeutic antibodies, or other agents known to those skilled in the art for the treatment and/or prevention of cancer.

In certain embodiments, one or more antibodies of the invention are administered to a mammal, preferably a human, concurrently with one or more other therapeutic agents useful for the treatment of cancer. The term "concurrently" is not limited to the administration of prophylactic or therapeutic agents at exactly the same time, but rather it is meant that an antibody of the invention and the other agent are administered to a mammal in a sequence and within a time interval such that the antibody of the invention can act together with the other agent to provide an increased benefit than if they were administered otherwise. For example, each prophylactic or therapeutic agent (*e.g.*, chemotherapy, radiation therapy, hormonal therapy or biological therapy) may be administered at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapeutic agent can be administered separately, in any appropriate form and by any suitable route. In various embodiments, the prophylactic or therapeutic agents are administered less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours

apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, no more than 24 hours apart or no more than 48 hours apart.

The prophylactic or therapeutic agents can also be administered at about 2 to 4 days apart, at about 4 to 6 days apart, at about 1 week part, at about 1 to 2 weeks apart, or more than 2 weeks apart. The prophylactic or therapeutic agents are administered in a time frame where both agents are still active. One skilled in the art would be able to determine such a time frame by determining the half life of the administered agents.

When used in combination with other prophylactic and/or therapeutic agents, the antibodies of the invention and the prophylactic and/or therapeutic agent can act additively or synergistically.

One aspect contemplates the administration of a pharmaceutical composition to a subject identified as exhibiting characterisitics of cancer (*e.g.*, bladder cancer, ovarian cancer, lung cancer, etc.). For each recipient, the total amount of the composition necessary can be derived from protocols for immunization. The exact amount of such immunogenic compositions required may vary from subject to subject, depending on the species, age, weight, and general condition of the subject, its mode of administration, whether it is administered with another antibody, and the like. Generally, dosage will approximate that which is typical for the administration of other immunogenic compositions.

The recipient is a mammal (*e.g.*, a cat, dog, horse, cow, pig, sheep, goat, primate, or human). Although human use is preferred, veterinary use of is also contemplated.

The immunogenic composition is typically administered as a sterile composition. The immunogenic composition can be administered by any suitable means, *e.g.*, parenteral (including subcutaneous, intramuscular, intravenous, perilymphatic, intranasal, intraplenic, intrapulmonary, intrathecal, and epidural) or orally. Other routes include nasal, intranasal, and topical (including buccal and sublingual).

The formulations include those suitable for the foregoing administration routes. The formulations can conveniently be presented in unit dosage form and can be prepared by any of the methods well known in the art of pharmacy. Techniques and formulations generally are found in REMINGTON'S PHARMACEUTICAL SCIENCES (Mack Publishing Co., Easton, PA). Such methods include the step of bringing into association the active ingredient with the carrier, which constitutes one or more accessory ingredients. In general, the formulations are prepared by

uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The oil phase of the emulsions can be constituted from known ingredients in a known manner. While the phase can comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. A hydrophilic emulsifier is included together with a lipophilic emulsifier, which acts as a stabilizer. Both an oil and a fat can be used.

Emulients and emulsion stabilizers suitable for use in the formulation include but are not limited to Tween® 60 (as well as other polyoxyethylene sorbitan ester surfactants), Span® 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate, and sodium lauryl sulfate.

Formulations suitable for parenteral administration include aqueous and nonaqueous, isotonic, sterile injection solutions that can contain antioxidants, buffers, bacteriostats, and solutes, which render the formulation isotonic with the blood of the intended recipient, and aqueous and nonaqueous sterile suspensions that can include suspending agents and thickening agents.

The formulations are presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water for injection, immediately prior to use. Injection solutions and suspensions are prepared from sterile powders, and granules of the kind previously described.

The compounds and compositions described herein further provide for veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier excipient, adjuvant, and/or stabilizer.

Veterinary carriers are materials useful for the purpose of administering the composition and can be solid, liquid or gaseous materials, which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These veterinary compositions can be administered orally, parenterally, or by any other desired route.

A formulation of the present invention can be administered to the patient in an injectable formulation containing any compatible carrier, such as various vehicles, adjuvants, additives, and diluents; or the compounds utilized in the present invention can be administered parenterally to the patient in the form of slow-release subcutaneous implants or targeted delivery systems such as polymer matrices, liposomes, and microspheres. An suitable implant can take the form of a

pellet, which slowly dissolves after being implanted, or a biocompatible delivery module well known to those skilled in the art. Such well-known dosage forms and modules are designed such that the active ingredients are slowly released over a period of several days to several weeks.

The compounds and compositions can also be used in combination with other active ingredients. Such combinations are selected based on the condition to be treated, cross-reactivities of ingredients, and pharmacological properties of the combination. Additionally, the compositions and compounds described herein can also be administered in conjunction with other conventional agents used to treat cancer. These compounds and compositions can be administered together with, or in the same course of, therapy with the compounds and compositions described herein. The individual components of the combination can be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

Treatment with the compositions and compounds disclosed herein can be given once or given daily over several days (*e.g.*, daily for one to two weeks). While it is possible that, for use in therapy, a compound can be administered as the raw chemical, it is preferable to present the active ingredient as a pharmaceutical formulation.

10. Effective Dosages

The antibodies of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the antibodies, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. A therapeutically effective amount is an amount effective to ameliorate or prevent the symptoms, or prolong the survival of, the patient being treated. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_5 as determined in cell culture. Such information can be used to more accurately determine useful doses in humans.

Initial dosages can also be estimated from *in vivo* data, *e.g.*, animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

Dosage amount and interval may be adjusted individually to provide plasma levels of the

molecules which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 5 mg/kg/day, preferably from about 0.5 to 1 mg/kg/day. Therapeutically effective serum levels may be achieved by administering multiple doses each day.

In cases of local administration or selective uptake, the effective local concentration of the proteins may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

The amount of antibody administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

The therapy may be repeated intermittently while symptoms detectable or even when they are not detectable. The therapy may be provided alone or in combination with other types of therapy. In the case of cancer, the antibodies may be used in combination with other types of therapies including, but are not limited to, surgery, radiation, gene therapy, hormone therapy, immunotherapy, or any combinations thereof

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out the preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLE 1

Preparation and Biochemical Properties of Antigen

The following experiments relate to amplifying human UPK-Ib for subsequent cloning into genetic immunization constructs for the purpose of raising UPK-Ib specific monoclonal antibodies. The antigen used was a recombinant protein comprised of a 121 amino acid long extracellular region of the native human UPK-Ib protein fused to the human IgG1 Fc region. The fusion protein is designated as UPK-G1. The sequence of this antigen and a diagram indicating the region of the UPK-Ib molecule are shown in Figures 1 and 2, respectively.

For the purpose of plasmid construction, the extracellular domain of UPK1 (upper case, *see* Figure 9) was fused with human G1 Fc (lower case) by overlapping PCR. Proteinase digestion site and enterokinase site (underlined), was inserted between the UPK1 extra cellular

domain and human G1 Fc. An artificial signal sequence was added at the amino acid terminal. The expression cassette was then cloned into the pCIneo vector (Promega, Inc.) at the NheI and EcoRI sites. Figure 9 depicts the amino acid sequence of the UPK1-Fc fusion protein expressed from the vector.

The pCIneo UPK1-Fc plasmid was then transiently transfected into 293H cells using LIPOFECTAMINE® 2000 reagent (Invitrogen, Inc.). The supernatant was harvested every three days for total three harvesting. Protein was then purified through Protein A column (Invitrogen, Inc.).

EXAMPLE 2

Preparation of Monoclonal Antibodies

The UPK-G1 fusion protein of Example 1 was sent to A&G Pharmaceuticals, Inc. for generation of monoclonal antibodies. Briefly, BALB/c mice were hyperimmunized with the UPK-G1 fusion protein and once antigen-specific serum titers were deemed sufficient for generation of UPK-Ib specific MAb production, lymphocytes were isolated and fused to an established hybridoma fusion partner.

The method of generating MAbs is a well known technique. Generally, an immunogen was administered (*e.g.*, intravenously) to a BALB/c mouse of 6 weeks of age. After a period of time, the spleen of the immunized mouse was aseptically excised to prepare about $2.5X10^7$ of spleen cells. After hemolysis of contaminated erythrocytes with erythrocyte lysis buffer (*e.g.*, Geyis solution), the cells were washed twice with RPMI 1640 medium.

Antibody non-producing mouse myeloma cells (*e.g.*, SP2/0) were used in the subsequent cell fusion. The SP2/0 cells were cultured and maintained in RPMI 1640 medium containing 10% fetal calf serum contained in a culture flask, and the cells at logarithmic growth phase were used in the cell fusion. The myeloma cells were recovered, adjusted to a density of $5X10^6$ cells and then washed twice with RPMI 1640 medium. The washed spleen cells and myeloma cells were thoroughly mixed in a fusion buffer and subjected to 5 minutes of centrifugation at 1,000 r.p.m. to remove the culture solution. The resulting pellet was again suspended in the fusion buffer. The cell fusion was carried out by an electrofusion method using a cell fusion apparatus. After the cell fusion, the cells were recovered in a container, which contained about 30 mL of RPMI medium supplemented with 10% FCS and then cultured for 30 minutes under conditions of 37°C and 5% CO_2/95% air. After 5 minutes of centrifugation at 1,000 rpm, the cells were re-suspended in RPMI 1640 medium containing 10% FCS and 10% HCF and dispensed in 0.1 mL

portions into 96 well plates. This was cultured under conditions of 37°C and 5% CO_2/95% air, and a HAT selection was carried out on the next day by adding 0.1 mL of HAT medium (RPMI 1640 medium containing 10% FCS, hypoxanthine, aminopterin and thymidine). After the fusion, half the volume of the medium was exchanged with HAT medium on the fourth day, and the culture supernatant fluids were collected on the tenth day to measure their antibody activity using an enzyme immunoassay method.

EXAMPLE 3

Testing for UPK-Ib Specific Monoclonal Antibodies

Supernatants from the cloned hybridoma lines were sent from A&G Pharmaceuticals, Inc. The supernatants from 25 hybridomas secreted antibodies specific for the 121 amino acid domain of UPK-Ib used for immunization, but did not cross-react with the Fc domain of the immunizing antigen. These supernatants were retested using standard immunoassay techniques.

Briefly, plates were coated with 50 ng UPK-Ib protein and blocked with bovine serum albumin. Hybridoma supernatants were added to plates and serial 3-fold dilution series were incubated overnight. Plates were washed the following morning and incubated with alkaline phosphatase conjugated goat-anti-mouse IgG (H+L) from Pierce Chemical at a dilution of 1:4000 for one hour. After several washes, the fluorescent substrate 5-methylumbelliferyl phosphate was added to each well. Fluorescence determinations were plotted against the log MAb concentrations. Of the 25 hybridomas, 24 of them were observed to be UPK-Ib positive in an ELISA assay. These data are shown in Figure 3A.

The *in vitro* testing of the individual antibodies was based on standard ELISA protocol. A standard ELISA protocol is a follows. Preliminarily, about 50 ng antigen (*e.g.*, UPK-Ib) is dispensed into 96 well plates. After removing the supernatant fluid by suction, the plates were washed with 1 X PBS/0.1% Tween® 20 (PBST) and blocking of the bound residues was carried out by adding 300 µl of 1% BSA/ PBST. The plates were incubated for 30 minutes at room temperature. After removing the supernatant fluid by suction, the plate was washed with PBST. A hybridoma culture supernatant fluid was dispensed in 50 µl portions into wells of the plate and allowed to undergo 1 hour of reaction at 37°C. After removing the supernatant fluid by suction, the plate was again washed with PBS-Tween® 20. After the washing, an anti-mouse immunoglobulin antibody-peroxidase conjugate was dispensed in 100 µl portions into the wells to carry out 1 hour of reaction at 37°C. After washing, 0.1 M citrate buffer (pH 4.2) containing O-phenylenediamine dihydrochloride or tetramethylbenzidine and hydrogen peroxide was

dispensed in 100 µl/well portions and then allowed to stand for 30 minutes at room temperature. After stopping the reaction by dispensing 2 N sulfuric acid in 50 µl/well portions, the OD at 492 nm was measured (OD 450 nm in the case of TMBZ). A hybridoma having higher reactivity with the antigen than with the control antigen was selected.

EXAMPLE 4

Binding of Monoclonal Antibodies to UPK-Ib Transfected Cells

In order to determine which monoclonal antibodies were capable of recognizing UPK-Ib when expressed on the surface of cells, each hybridoma culture supernatant was tested for the ability to bind to the surface of HEK293 cells transfected with a UPK-Ib expression construct but not to the surface of the HEK293 parental line.

Hybridoma culture supernatants were assayed to determine concentration of monoclonal antibody. Supernatants were diluted in FACS staining buffer (1% BSA in PBS containing 0.01% sodium azide as a preservative) to a final concentration of 1 µg/ml monoclonal antibody. Parental 293 cells and 293F cells expressing UPK1b (UPK-Ib transfected HEK293 cells) were spun down and pellets were resuspended in 1 ml of diluted culture supernatant. Incubation was on ice for 30 minutes. Cells were washed 3x in FACS staining buffer and cells were resuspended in goat anti-mouse IgG (Fab fragment) conjugated to phycoerythrin. Following a second 30 minute incubation on ice, cells were washed 2x in ice-cold PBS and analyzed by flow cytometry using a Becton Dickensen FACsCalibrator Flow Cytometer. Of the 24 UPK-Ib specific MAbs generated, 20 exhibited desirable binding to HEK293-UPK-Ib transfectants as determined by flow cytometric analysis. It was observed that two of these antibodies recognized UPK-Ib on the surface of a transitional cell carcinoma line. These were the 1G9 and 5H9 MAbs. The flow cytometric profiles are shown in Figure 4 (cell count on the y-axis and the measurement parameter on x-axis). The top two curves demonstrate minimal staining of the 5H9 and 1G9 monoclonal antibodies to a UPK-Ib negative 293 cell line. The bottom two curves represent good staining of UPK-Ib transfected 293 cells.

Both the 1G9 and 5H9 hybridomas were expanded and cDNA was prepared for immunoglobulin heavy and light chain sequence determination. The amino acid sequences are shown in Figure 5.

The cDNA cloning of 1G9 and 5H9 is as follows. RNA was converted to cDNA and the VH and VL segments were PCR amplified using the 5' RACE kit (Invitrogen, Inc.). Gene specific primers for the VH were SJ15R (5' GGTCACTGTCACTGGCTCAGGG 3'; SEQ ID

NO: 9) and SJ16R (5' aggcgGATCCAGGGGCCAGTGGATAGAC 3'; SEQ ID NO: 10). Gene specific primers for the VL were SJ17R (5'GCACACGACTGAGGCACCTCCAGATG 3'; SEQ ID NO: 11) and SJ18R (5' cggcggatccGATGGATACAGTTGGTGCAGCATC 3'; SEQ ID NO: 12). The RACE product was inserted into the plasmid pCR2.1-TOPO using a TOPO TA Cloning kit (Invitrogen, Inc.). The resulting plasmids were then subjected to DNA sequencing to determine the VH and VL sequences for 1G9 and 5H9. The resulting sequences were then translated and the predicted amino acid sequence determined for each. From these sequences the framework (FR) and complementarity determining (CDR) regions were identified as defined by Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, (5th ed., Public Health Service, MD 1991).

EXAMPLE 5

Modification of Monoclonal Antibodies

Once the sequences of genes encoding the 1G9 and 5H9 antibodies were determined, primers were designed to amplify the complementarity determinant regions (CDRs) and to generate chimeric constructs encoding the CDRs of the murine antibodies and fusing them to the human IgG1 wild type Fc and to the enhancing Fc mutant. For example, the mouse VH was joined to a human C-Gamma 1 constant region and an Ig leader sequence. The expression cassette was then inserted into pCI-neo at the Nhe I and EcoRI sites for mammalian expression. The mouse VL was joined to a human C-kappa segment and an Ig leader sequence. The expression cassette was also cloned into pCI-neo at the Nhe I and EcoR I sites for mammalian expression. The heavy and light corresponding plasmids were co-transfected into 293H cells for antibody expression.

The chimeric 1G9 (ch1G9), chimeric 5H9 (ch5H9), and chimeric 1G9 containing the enhancing mutant (ch1G9-mut) were produced for functional testing. Specifically, the ch1G9 heavy chain (HC) expression plasmid was co-transfected together with ch1G9 light chain (LC) into HEK-293 cells. The same procedure was performed for ch5H9. After three days in culture, the amount of human IgG secreted into the supernatant was quantitated by ELISA. Binding to UPK-Ib was then determined by ELISA. Murine 1G9 and 5H9 were used as control. The results of this experiment, depicted in Figure 3B, indicate that the chimeric antibody bound to the antigen with similar affinity to the mouse antibody.

EXAMPLE 6

Antibody-Dependent Cellular Cytotoxicity (ADCC) Studies

Antibody-Dependent Cellular Cytotoxicity (ADCC) studies were performed using SW780 cells as tumor targets and human peripheral blood mononuclear cells (PBMCs) as effector cells. These assays have been performed several times using several independent sources of human donor cells at an effector:target ratio of about 70:1. Results from three of these studies are shown in Figure 6. The wild type chimeric forms of 1G9 and 5H9 mediated ADCC. The introduction of the enhancing Fc mutation into the human Fc region enhanced killing by 1G9 dramatically.

The ADCC experiments were performed as follows. For anti-UPK-1 antibodies (ch1G9 and ch1G9 containing the Fc enhancing mutant; ch1G9 Fc mut), the bladder transitional cell carcinoma line, SW780, was used as a target. Peripheral blood mononuclear cells (PBMC), purified by Ficoll-Hypaque (Amersham Biosciences) density gradient centrifugation from whole human blood (BRT Laboratories Inc.), were used as effector cells. Target cells ($5x10^6$ in 0.5 mL) were labeled with 100 μCi of Indium (In)-111 oxine (Amersham Health) at room temperature for 30 minutes. Unincorporated In-111 was removed through four sequential washes with assay media (RPMI 1640 media supplemented with 10% Fetal bovine serum, 4 mM glutamine, 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 50 μg/mL penicillin and 100 μg/mL streptomycin). Target cells were opsonized with antibody at various concentrations (30 μg/mL - 0.01 ng/mL) for 30 minutes at 37°C. Labeled target cells (5,000 per well), opsonized with anti-UPK-1 antibody, were combined with PBMC in round-bottom 96 well plates at an E:T ratio of about 75:1. Following an 18 hour incubation at 37°C and 5% CO_2, cell supernatants were harvested using the Skatron supernatant collection system (Molecular Devices), and released radioactivity was quantified using a Wallac 1470 gamma counter (Perkin Elmer). Maximal release and spontaneous release were determined by incubation of target cells with 2% TX-100 and media alone, respectively. Antibody independent cellular cytotoxicity was measured by incubation of target and effector cells in the absence of antibody. Each assay was performed in triplicate. The mean percentage specific lysis was calculated from the formula:

% specific lysis = (experimental CPM - antibody independent CPM)/(maximal release CPM - spontaneous release CPM) x 100.

EXAMPLE 7

Immunohistochemical Analysis of Human Bladder Tumor Samples

In order to determine if UPK-Ib expression in more advanced stages of TCC is common and would serve as a suitable tumor target in such cases, an array of transitional cell carcinoma samples was evaluated using a bladder array containing both normal and tumor sample cores. For these studies, MAb 1A10, one of the MAbs from the original set of 24 UPK-Ib monoclonal antibodies, was used. While this antibody was observed to not recognize UPK-Ib on the surface of live TCC cells, this antibody does detect the molecule in a fixed, denatured form particularly well. Figure 7 shows examples of four independent human tumor tissues representing normal bladder tissue as well as tissue obtained from patients with Grade I-III tumors.

The immunohistochemistry experiments were adapted from standard protocols. For example, frozen blocks of the desired tissue were sliced to a thickness of 9 microns using a cryostat to prepare glass slide non-fixed frozen sections. Each of the frozen sections was subjected to 10 minutes of reaction with 0.5% hydrogen peroxide to effect blocking of endogenous peroxidase. This was washed with 20 mM phosphate buffer containing 0.154 M NaCl (PBS) and then subjected to 10 minutes of reaction with 10% rabbit serum in PBS. After washing again with PBS, a monoclonal antibody was added thereto to carry out 60 minutes of reaction. This was again washed with PBS and allowed to undergo reaction with peroxidase-labeled rabbit anti-mouse immunoglobulin, which has been diluted 200 times with PBS. After 60 minutes of the reaction and subsequent washing with PBS, a color developing kit containing diaminobenzidine and hydrogen peroxide was added as the substrate, and 15 minutes of reaction was carried out. Samples were washed again, subjected to nuclear staining with a hematoxylin solution, dehydrated and then embedded. The treated sections were observed under a microscope at the desired magnification, and a section in which 10% or more of cells were stained was judged positive.

EXAMPLE 8

In vivo efficacy of UPK-Ib antibodies

The next set of experiments are designed to assess *in vivo* efficacy of ch1G9 containing the enhancing mutation in a murine model to determine the ability of the antibody to increase at least ADCC with respect to the target cell in a mammal.

The SW780 cell line has been confirmed as capable of forming sub-cutaneous tumors in nude and RAG2 deficient BALB/c animals. Efficacy studies to test for reduction of tumor size or

prevention of tumor formation can be performed as follows. Groups of ten animals are injected subcutaneously with SW780 cells (e.g., about 5 X 10^6 cells per animal). One group will receive 8 doses of 250 μg ch1G9-enhancing Fc mutant administered once per week over the course of the study. Control groups include a PBS control and dosing with an irrelevant antibody (ch4-4-20 with the Fc enhancing mutant, which recognizes fluoresceine and 4D5-Fc enhancing mutant, which recognizes Her2/neu; Her2/neu appears to be expressed at low levels on SW780 cells).

Histology would be performed essentially, as described above. Briefly, bladder tissue is isolated and placed in formalin. After sectioning, the slides are stained with MAb 1A10 to evaluate presence of UPK-Ib as described *supra*.

EXAMPLE 9

Expression UPK-Ib in lung cell lines

The following experiments were designed to assess the expression of UPK-Ib in lung cell lines. By way of example, the human lung tumor cell lines A549 and EKVX were used. The cell lines were cultured on adherent plates under standard culturing conditions. The adherent cells were detached from tissue culture flasks using PBS-EDTA.

In order to assess expression of UPK-Ib on these cell, the cells were spun down and resuspended in FACs buffer (PBS containing 1% BSA) at 4 x 10^6 cells/mL. Antibodies to either UPK1b (chimeric 1G9-enhancing Fc mutant) or CD32B (2B6-enhancing Fc mutant, the negative control) were added to 0.5 mL of cell suspension to a final concentration of 2 μg/mL. Cells were incubated on ice for 30 minutes. Cells were washed three times in ice-cold FACs buffer and resuspended in a secondary antibody solution of goat anti-human IgG conjugated to phycoerythrin. These mixtures were incubated on ice for another 30 minutes, washed three times and resuspended in PBS for analysis on the FACsCalibur. Figure 10 demonstrates that the A549 and EKVX human lung tumor cell lines stained positive for UPK-Ib.

It will be apparent to those skilled in the art that various modifications and variation can be made to the compositions and methods described herein and as provided in the examples above without departing from the spirit or scope of the compositions and methods.

All cited patents and publications referred to in this application are herein incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. An isolated antibody or fragment thereof that specifically binds to a fusion protein comprising an extracellular region of human UPK-Ib and a human IgG Fc region, wherein the antibody or fragment thereof does not recognize the Fc region of the fusion protein.

2. The antibody of claim 1, wherein the extracellular region comprises the amino acid sequence set forth in SEQ ID NO: 13, and wherein the Fc region comprises the amino acid sequence set forth in SEQ ID NO: 14.

3. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises a heavy chain variable region of SEQ ID NO: 1 and a light chain variable region of SEQ ID NO: 3, or a heavy chain variable region of SEQ ID NO: 5 and a light chain variable region of SEQ ID NO: 7.

4. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises a heavy chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO: 2 and a light chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO: 4, or a heavy chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO: 6 and a light chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO: 8.

5. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof specifically binds to UPK-Ib expressed on a cell, wherein the binding occurs *in vitro*, *in vivo, in situ,* unfixed tissue, or any combination thereof.

6. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof is of murine origin.

7. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof is primatized or humanized.

8. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof is selected from the group consisting of Fv, Fab, Fab', $F(ab')_2$, and single chain Fv (scFv).

9. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof further comprises a human IgG wild type Fc.

10. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof further comprises an enhancing Fc mutant.

11. The antibody or fragment thereof of claim 1, further comprising an anti-tumor agent.

12. The chimeric antibody of claim 11, wherein the anti-tumor agent is a chemotherapeutic agent, an anti-cell proliferation agent, or any combination thereof.

13. The chimeric antibody of claim 12, wherein the chemotherapeutic agent is selected from the group consisting of alkylating agents nitrosoureas, antimetabolites, antitumor antibiotics, plant alkyloids, taxanes, hormonal agents, or any combination thereof.

14. The chimeric antibody of claim 12, wherein the anti-proliferation agent is selected from the group consisting of an apoptosis-inducing agent, a cytotoxic agent, or any combinations thereof.

15. A method of diagnosing a disease state in a mammal, the method comprising determining the level of UPK-Ib using an antibody or fragment thereof of claim 1.

16. The method of claim 15, wherein the disease state is bladder cancer, transitional cell carcinoma of the bladder, ovarian cancer, Brenner ovarian cancer, or lung cancer.

17. The method of claim 15, wherein the antibody or fragment thereof comprises a heavy chain variable region of SEQ ID NO:1 and a light chain variable region of SEQ ID NO:3, or a heavy chain variable region of SEQ ID NO:5 and a light chain variable region of SEQ ID NO:7.

18. The method of claim 15, wherein the antibody or fragment thereof comprises a heavy chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:2 and a light chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:4, or a heavy chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:6 and a light chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:8.

19. The method of claim 15, wherein the antibody or fragment thereof is of murine origin.

20. The method of claim 15, wherein the antibody or fragment thereof is primatized or humanized.

21. The method of claim 15, wherein the antibody or fragment thereof is selected from the group consisting of Fv, Fab, Fab', $F(ab')_2$, and single chain Fv (scFv).

22. The method of claim 15, wherein the antibody or fragment thereof further comprises a human IgG wild type Fc.

23. The method of claim 15, wherein the antibody or fragment thereof further comprises an enhancing Fc mutant.

24. A method of inducing antibody-dependent cellular cytotoxicity against a cell expressing UPK-Ib, the method comprising contacting the cell with the antibody of claim 1.

25. The method of claim 24, wherein the cell is a cancer cell.

26. The method of claim 25, wherein the cancer cell is selected from the group consisting of a bladder cell, an ovarian cell, a lung cell, or any combination thereof.

27. The method of claim 24, wherein the cell is a human cell.

28. The method of claim 24, wherein the antibody or fragment thereof comprises a heavy chain variable region of SEQ ID NO:1 and a light chain variable region of SEQ ID NO:3,

or a heavy chain variable region of SEQ ID NO:5 and a light chain variable region of SEQ ID NO:7.

29. The method of claim 24, wherein the antibody or fragment thereof comprises a heavy chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:2 and a light chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:4, or a heavy chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:6 and a light chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:8.

30. The method of claim 24, wherein the antibody or fragment thereof is of murine origin.

31. The method of claim 24, wherein the antibody or fragment thereof is primatized or humanized.

32. The method of claim 24, wherein the antibody or fragment thereof is selected from the group consisting of Fv, Fab, Fab', $F(ab')_2$, and single chain Fv (scFv).

33. The method of claim 24, wherein the antibody or fragment thereof further comprises a human IgG wild type Fc.

34. The method of claim 24, wherein the antibody or fragment thereof further comprises an enhancing Fc mutant.

35. A method of treating a cancer associated with abnormal expression level of UPK-Ib, the method comprising contacting a cell expressing UPK-Ib with the antibody of claim 1.

36. The method of claim 35, wherein the cancer is select from the group consisting of bladder cancer, transitional cell carcinoma of the bladder, ovarian cancer, Brenner ovarian cancer, lung cancer, or any combination thereof.

37. The method of claim 35, wherein the antibody or fragment thereof comprises a heavy chain variable region of SEQ ID NO:1 and a light chain variable region of SEQ ID NO:3,

or a heavy chain variable region of SEQ ID NO:5 and a light chain variable region of SEQ ID NO:7.

38. The method of claim 35, wherein the antibody or fragment thereof comprises a heavy chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:2 and a light chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:4, or a heavy chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:6 and a light chain variable region encoded by the nucleic acid sequence set forth in SEQ ID NO:8.

39. The method of claim 35, wherein the antibody or fragment thereof is of murine origin.

40. The method of claim 35, wherein the antibody or fragment thereof is primatized or humanized.

41. The method of claim 35, wherein the antibody or fragment thereof is selected from the group consisting of Fv, Fab, Fab', $F(ab')_2$, and single chain Fv (scFv).

42. The method of claim 35, wherein the antibody or fragment thereof further comprises a human IgG wild type Fc.

43. The method of claim 35, wherein the antibody or fragment thereof further comprises an enhancing Fc mutant.

44. The method of claim 35, further comprising an additional cancer therapy, wherein the therapy is surgery, radiation, gene therapy, hormone therapy, immunotherapy, or any combination thereof.

45. The method of claim 44, wherein the therapy and the antibody or fragment thereof is administered concomitantly.

46. The method of claim 44, wherein the therapy and the antibody or fragment thereof is administered in succession.

47. The method of claim 44, wherein the antibody or fragment thereof is administered prior to the therapy.

48. The method of claim 44, wherein the antibody or fragment thereof is administered subsequent to the therapy.

49. An isolated or purified humanized UPK-Ib specific antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 1 or a glycosylation variant, fusion molecule or a chemical derivative thereof.

50. An isolated or purified humanized UPK-Ib specific antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 3 or a glycosylation variant, fusion molecule or a chemical derivative thereof.

51. An isolated or purified humanized UPK-Ib specific antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5 or a glycosylation variant, fusion molecule or a chemical derivative thereof.

52. An isolated or purified humanized UPK-Ib specific antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 7 or a glycosylation variant, fusion molecule or a chemical derivative thereof.

QRDFFTPNLFLKQMLERYQNNSPPNNDDQWKNNGVTKTWDRLMLQDNCCGVNGP SDWQKYTSAFRTENNDADYPWPRQCCVMNNLKEPLNLEACKLGVPGFYHNQGCYE LISGPMNRHAWGVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK

FIG. 1

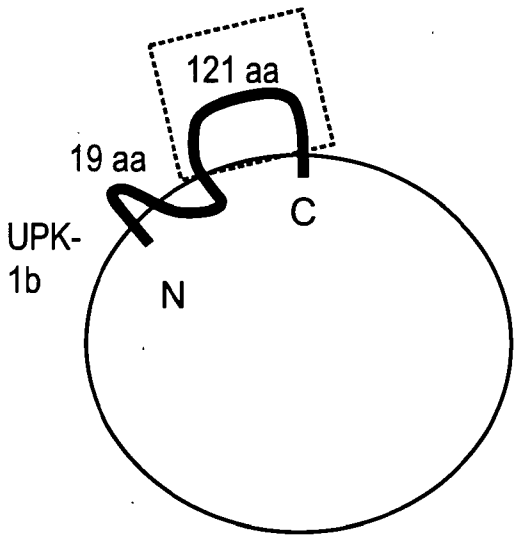
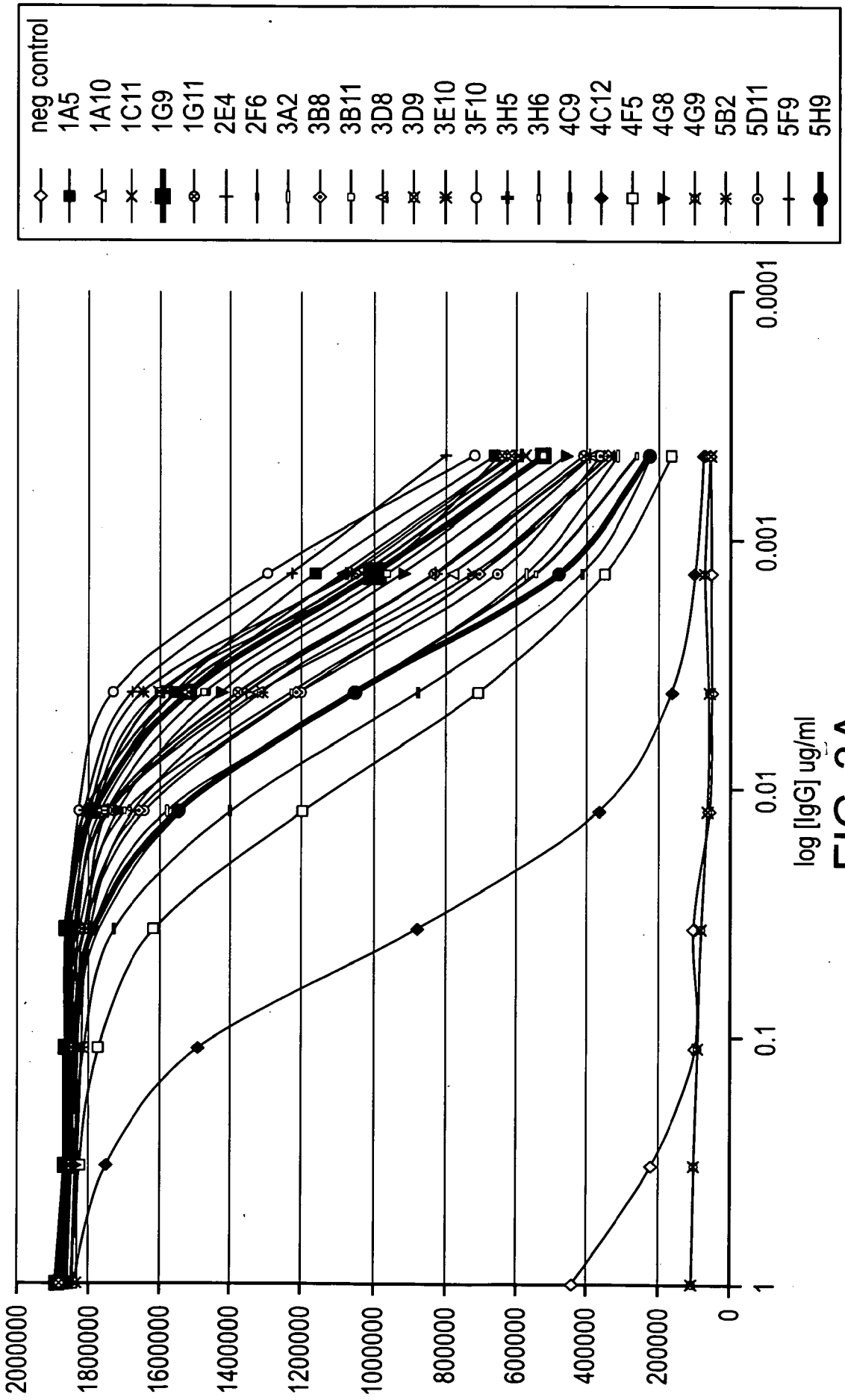


FIG. 2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1A10
1C11
1G9
1G11
2E4
2F6
3A2
3B8
3B11
3D8
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3E10
3F10
3H5
3H6
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4C12
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4G9
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5D11
5F9
5H9
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
1
0.1
0.01
0.001
0.0001
log [IgG] ug/ml

FIG. 3A

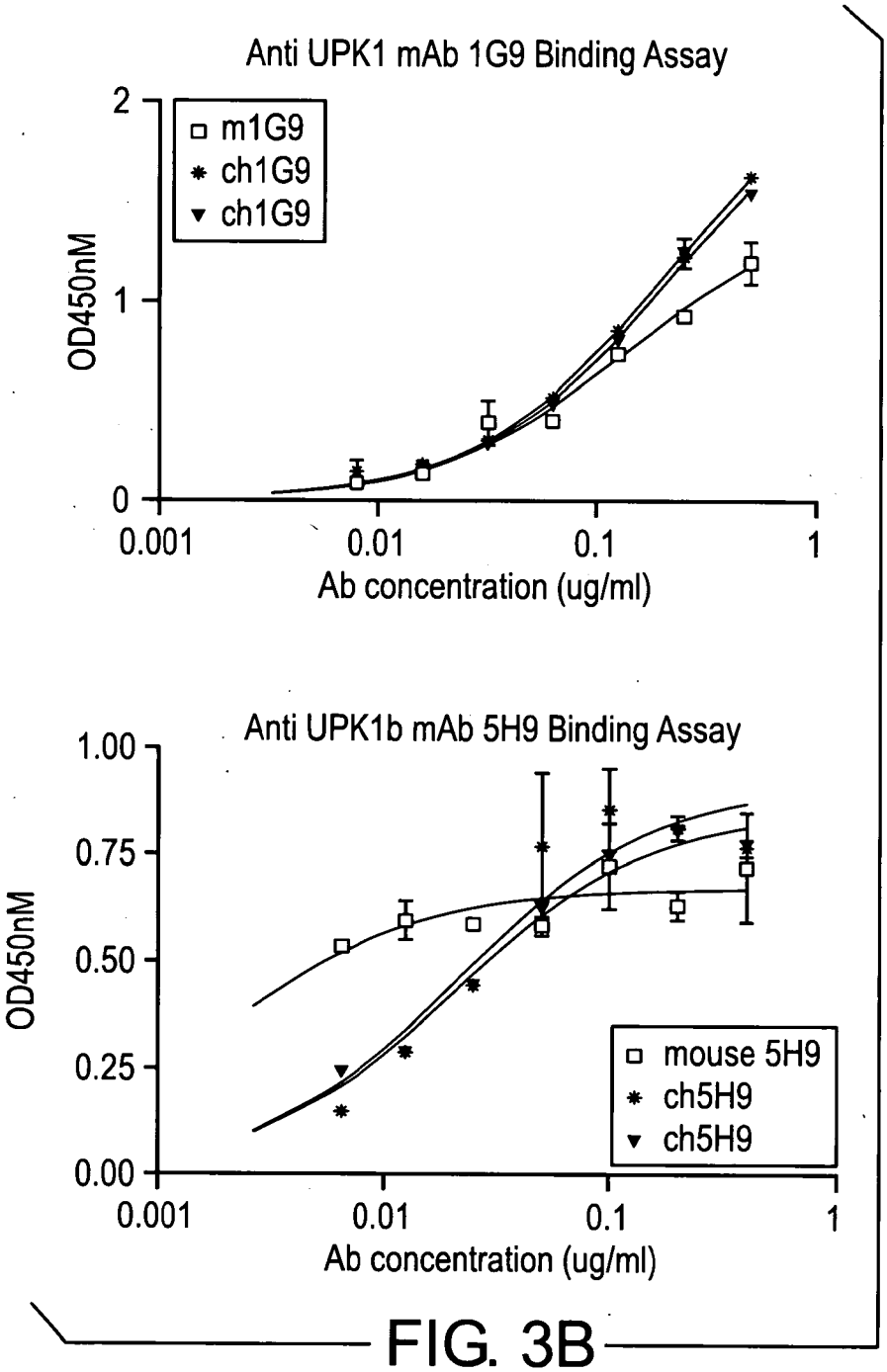
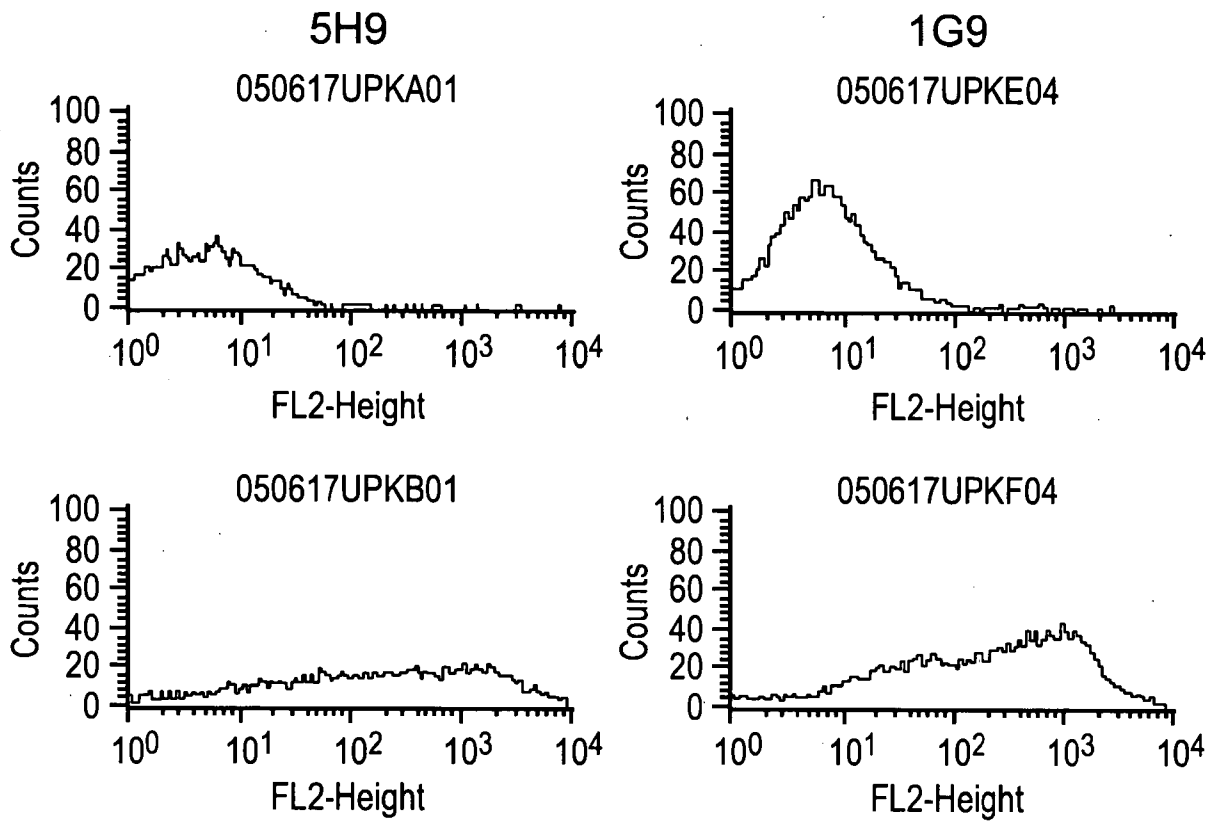

Anti UPK1 mAb 1G9 Binding Assay
m1G9
ch1G9
ch1G9
OD450nM
2
1
0
0.001
0.01
0.1
1
Ab concentration (ug/ml)
Anti UPK1b mAb 5H9 Binding Assay
1.00
0.75
0.50
0.25
0.00
OD450nM
mouse 5H9
ch5H9
ch5H9
0.001
0.01
0.1
1
Ab concentration (ug/ml)

FIG. 3B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050617UPKA01
1G9
050617UPKE04
050617UPKB01
050617UPKF04
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FL2-Height
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80
60
40
20
0
10^0 10^1 10^2 10^3 10^4

FIG. 4A

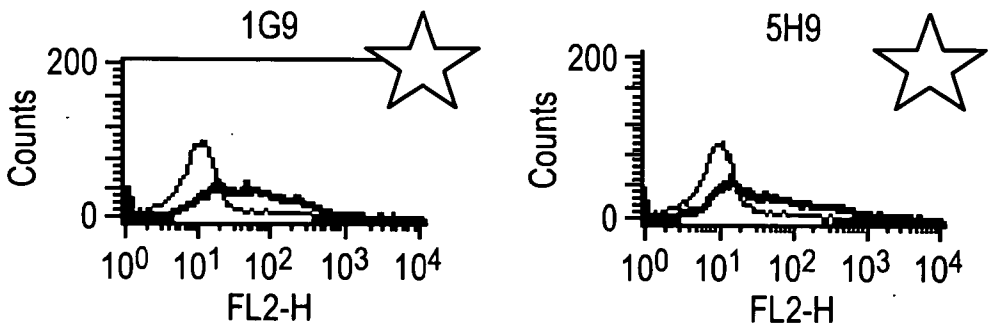
1G9
5H9
Counts
FL2-H
200
0
10^0 10^1 10^2 10^3 10^4

FIG. 4B

1G9 VH
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DSETHYNQKFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARSGAGTGPWL
AYWGQGTLVTVSA

1G9 VL
DIVMTQSPSSLTVTAGEKVTMTCKSSQSLLNSGNQKNYLTWYQQKPGQPPNPLI
YWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPFTFGSGTK
LEIK

5H9 VH
EVQLVESGGGLVQPKGSLKLSCAASGFSFNTYAMNWVRQAPGKGLEWVARIRS
KSNNYATYYADSVKDRFTISRDDSESMLYLQMNNLKTEDTAMYYCVRQGDGY
YVGWFAYWGQGTLVTVSA

5H9 VL
DIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSRLH
SGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPFTFGSGTKLEIK

FIG. 5A

1G9 VH

CAGGTCCAACTGCAGCAGCCTGGGGCTGAGCTGGTGAGGCCTGGGTCTTCAGTGAA
GGTGTCCTGCAAGGCCTCTGGCTACACCTTCAGCAGCTATTGGATGCATTGGGTGAA
GCAAAGGCCTATACAAGGCCTTGAATGGATTGGTAACATTGACCCTTCTGATAGTGA
AACTCACTACAATCAAAAGTTCAAGGACAAGGCCACATTGACCGTAGACAAATCCT
CCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATT
ACTGTGCAAGATCGGGGGCTGGGACGGGCCCCTGGCTTGCTTACTGGGGCCAAGGG
ACTCTGGTCACTGTCTCTGCA

1G9 VL

GACATTGTGATGACACAGTCTCCATCCTCCCTGACTGTGACAGCAGGAGAGAAGGTC
ACTATGACCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAAATCAAAAGAACTA
CTTGACCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAATCCGTTGATCTACTGGGC
ATCCACTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGGAACAG
ATTTCACTCTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTC
AGAATGATTATAGTTATCCATTCACGTTCGGCTCGGGGACAAAGTTGGAAATAAAA

5H9 VH

GAGGTGCAGCTTGTTGAGTCTGGTGGAGGATTGGTGCAGCCTAAAGGGTCATTGAA
ACTCTCATGTGCAGCCTCTGGATTCAGCTTCAATACCTACGCCATGAACTGGGTCCG
CCAGGCTCCAGGAAAGGGTTTGGAATGGGTTGCTCGCATAAGAAGTAAAAGTAATA
ATTATGCAACATATTATGCCGATTCAGTGAAAGACAGATTCACCATCTCCAGAGATG
ATTCAGAAAGCATGCTCTATCTGCAAATGAACAACTTGAAAACTGAGGACACAGCC
ATGTATTACTGTGTGAGACAGGGGGATGGTTACTACGTGGGCTGGTTTGCTTACTGG
GGCCAAGGGACTCTGGTCACTGTCTCTGCA

5H9 VL

GATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTC
ACCATCAGTTGCAGGGCAAGTCAGGACATTAGCAATTATTTAAACTGGTATCAGCAG
AAACCAGATGGAACTGTTAAACTCCTGATCTACTACACATCAAGATTACACTCAGGA
GTCCCATCAAGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGC
AACCTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCCA
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FIG. 5B

ADCC data for UPK Antibodies

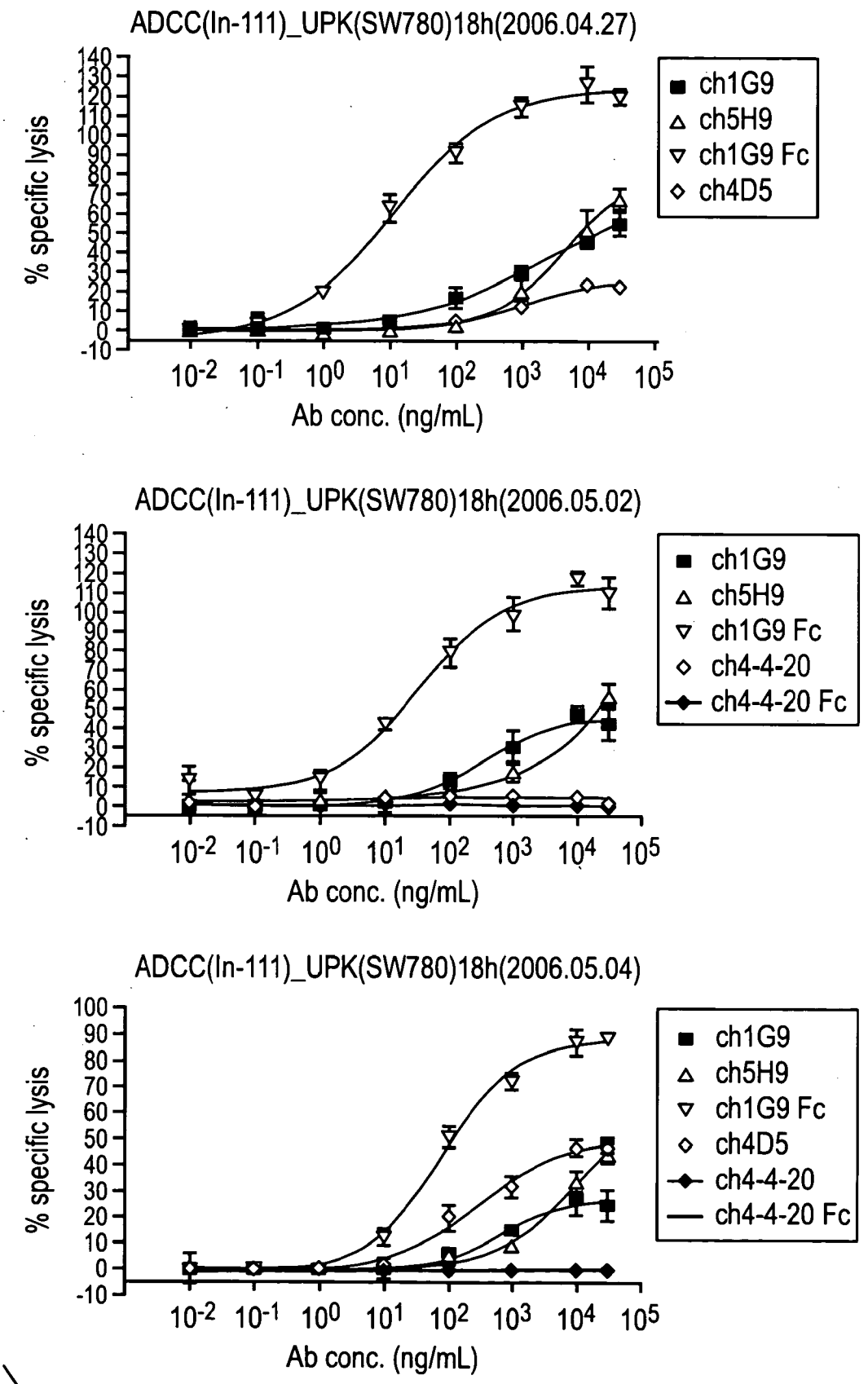

FIG. 6

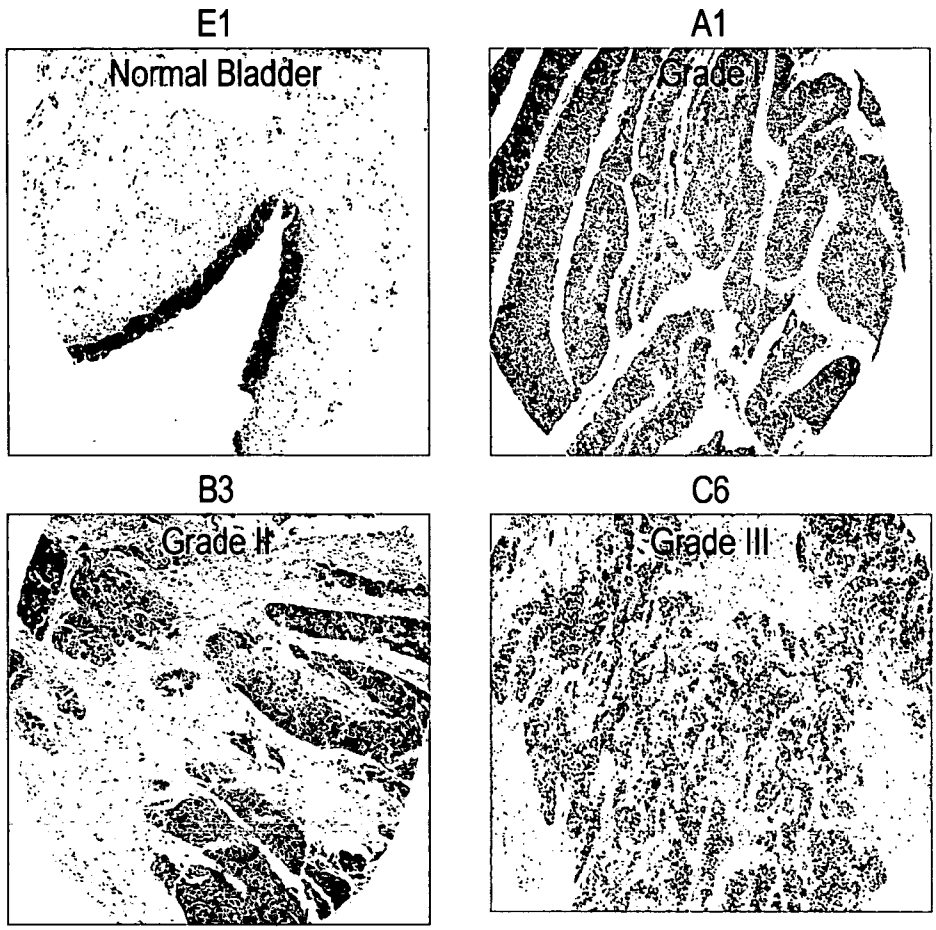

FIG. 7

FIG. 8

Positive cases/ degree of expression						
Bladder	(+) Diffuse	(++) Diffuse	(+++) Diffuse	(+) Focal	(++) Focal	(+++) Focal
Normal	4	17	9	2	--	--
Atypical hyperplasia	--	1	1	1	--	--
TCC Grade I	--	8	--	--	--	--
TCC Grade II	3	9	1	1	--	1
TCC Grade III	1	6	--	2	1	1

Focal: One or a limited group of cells.
Diffuse: Staining of all epithelial cells, normal or malignant
(+): mild staining
(++): moderate staining
(+++): strong staining

TQRDFFTPNLFLKQMLERYQNNSPPNNDDQWKNNGVTKTWD
RLMLQDNCCGVNGPSDWQKYTSAFRTENNDADYPWPRQCCV
MNNLKEPLNLEACKLGVPGFYHNQGCYELISGPMNRHAWGdi
ddddklevepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshed
pevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpap
iektiskakgqprepqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennykttpp
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FIG. 9

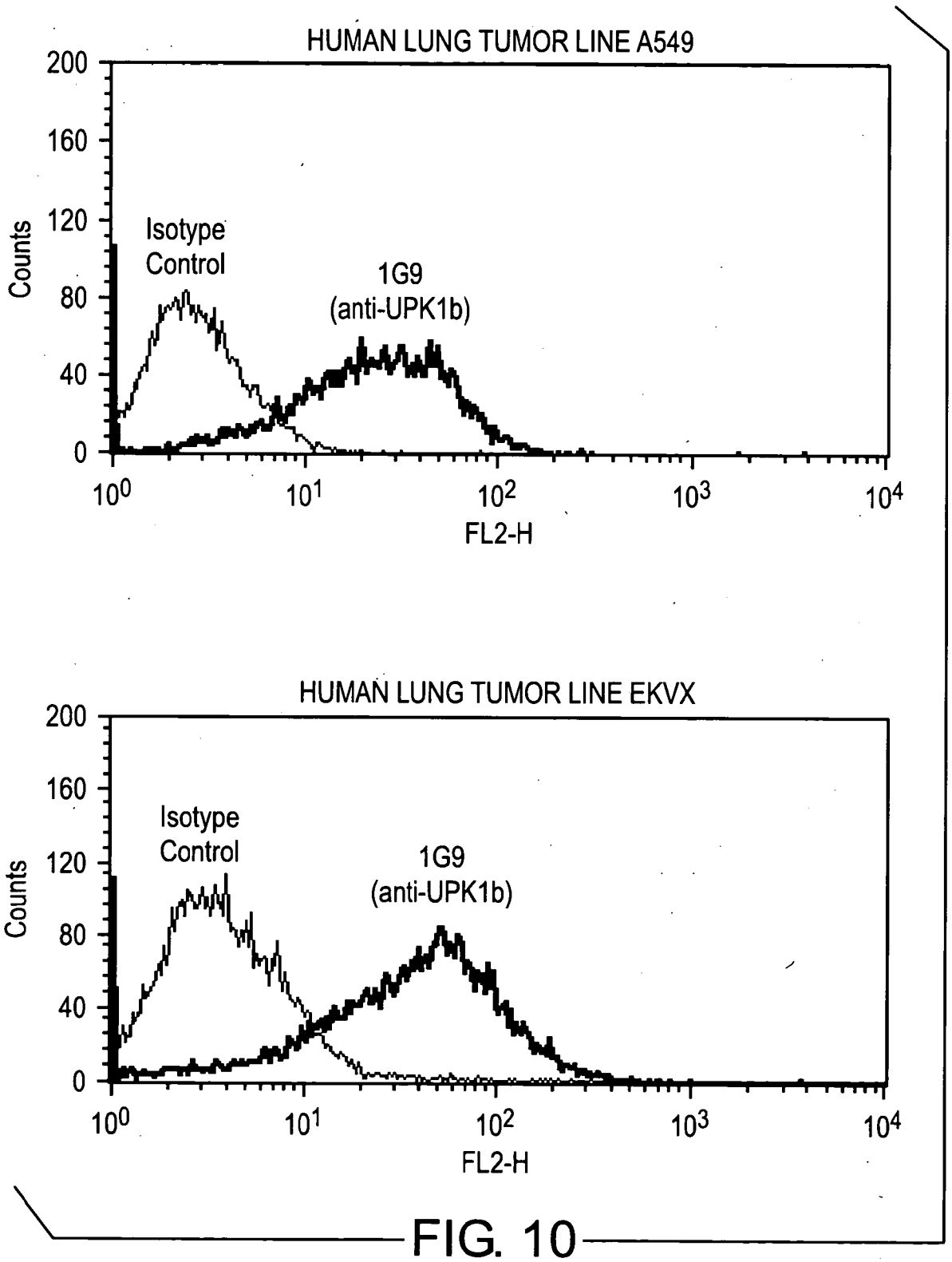
HUMAN LUNG TUMOR LINE A549
200
160
120
80
40
0
Counts
Isotype Control
1G9 (anti-UPK1b)
10^0
10^1
10^2
10^3
10^4
FL2-H
HUMAN LUNG TUMOR LINE EKVX
200
160
120
80
40
0
Counts
Isotype Control
1G9 (anti-UPK1b)
10^0
10^1
10^2
10^3
10^4
FL2-H

FIG. 10