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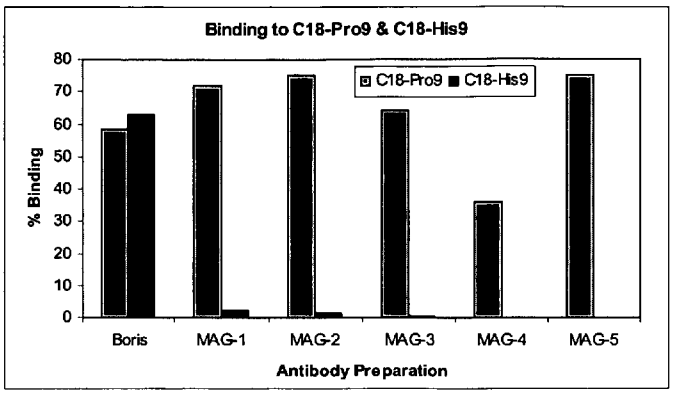
(54) Title: PEPTIDES, ANTIBODIES, AND USES THEREOF FOR IDENTIFYING AND TARGETING PROVASOPRESSIN-EXPRESSING CANCER CELLS

Figure 1



(57) Abstract: This application provides peptides, antibodies, and antigen-binding portions thereof, for identifying and targeting provasopressin-expressing cancer cells. Also provided are methods of using said compositions, for example to image cancer cells *in vivo* and in biological samples. Also provided are methods of using said compositions for treating patients suffering from a provasopressin-associated cancers, including breast cancer and small cell lung cancer.

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PEPTIDES, ANTIBODIES, AND USES THEREOF FOR IDENTIFYING AND TARGETING PROVASOPRESSIN-EXPRESSING CANCER CELLS

RELATED APPLICATIONS

This application claims the benefit of US Provisional Application No. 61/127,089, filed May 9, 2008, the specification of which is hereby incorporated herein by reference in its entirety.

FEDERAL FUNDING

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BACKGROUND

Lung cancer is the leading cause of cancer-related deaths worldwide, and SCLC comprises about 16% of all lung cancer cases in the United States (Travis et al. (1995) *Cancer* 75: 191-202). Currently, SCLC is diagnosed on the basis of gross morphological and histological data, and is too often identified after the disease has reached its advanced stages (Junker et al. (2000) *J. Cancer Res. Clin. Oncol.* 126: 361-368). Although there is a high response rate to present treatments consisting of high-dose chemotherapy with or without radiotherapy, disease recurrence is frequent, and tumors become resistant to these approaches, resulting in 2-year survival rates of only 6-12% (Johnson et al. (1998) *J. Natl. Cancer Inst.* (Bethesda) 90: 1335-1345). Considerable toxicity is also associated with these therapies.

Breast cancer is a leading cause of death among women throughout the world, and accounts for the death of approximately 50,000 women in the United States each year (American Cancer Society. Cancer Facts and Figures, Atlanta, GA: American Cancer Society, 1993). Although there have been many recent advances for effectively treating this disease (Silverstein, M.J. et al., *The Breast Journal* (2002) 8:70-76), successful intervention still heavily relies on early detection through mammography and surgical removal of cancerous tissue. This application provides novel compositions and methods for cancer diagnosis and therapy, including anti-provasopressin antibodies.

The expression of the vasopressin gene is largely restricted to hypothalamic neurons, and it encodes for a protein product of ~17 kDa, to which an N-glycosidic side-chain of ~4 kDa is added, resulting in the ~20 kDa provasopressin (pro-VP) precursor. This protein is normally packaged into secretory vesicles where it undergoes enzymatic cleavage to generate vasopressin (VP), VP-NP, and VAG (North, W.G. *In*: D. Gash and G. Boer (eds.), *Vasopressin: Principles and Properties*, pp. 175-209. New York: Plenum Press, 1987). These components are then secreted into the circulation. SCLC tumors and cultured cells also express the VP gene, however intact provasopressin protein can become localized to the cell surface plasma membrane (Friedmann et al. (1994) *B. J. Cancer* 69: 260-263; North et al. (1993) *Ann. NY Acad. Sci.* 689: 107-121). Polyclonal antibodies raised against VP-NP bind specifically to the surface of cultured SCLC cells, as determined by immunofluorescent analysis (Friedmann et al. (1995) *Neuropeptides* 28: 183-189; North et al. (1983) *Prog. Brain Res.* 60: 217-225; North and Yu (1993) *Regulatory Peptides* 45: 209-216). Thus, the target of these antibodies has been termed neurophysin-related cell surface antigen (NRSA) (North et al. (1993) *Peptides* 14: 303-307). Polyclonal anti-VP-NP antibodies recognize proteins of ~20 kDa and ~40 kDa in total protein extracts from SCLC cultured cells by Western analysis (North et al. (1993) *Peptides* 14: 303-307). The ~20 kDa protein corresponds in size to the provasopressin protein, and the ~40 kDa protein is believed to be a related form (Camier et al. (1979) *FEBS Lett.*, 108: 369-373; Lauber et al. (1979) *FEBS Lett.*, 97: 343-347; Lauber et al. (1981) *Proc. Natl. Acad. Sci. USA*, 78: 6086-6090; Moore and Rosenior. (1983) *Prog. Brain Res.*, 60: 253-256; Nicolas et al. (1980) *Proc. Natl. Acad. Sci.* USA, 77: 2587-2591; Rosenior et al. (1981) *Endocrinology*, 109: 1067-1072). Polyclonal antibodies that have been raised against the vasopressin, VP-NP, or VAG regions of the pro-VP protein display specific staining of SCLC tumor sections, whereas they exhibit a very low incidence of immunoreactivity with the non-neuroendocrine tumors examined (Friedmann et al. (1994) *B. J. Cancer* 69: 260-263; Friedmann et al. (1993) *Cancer Letters* 75: 79-85).

Products of the vasopressin (VP) gene appear to present a universal tumor marker system for breast cancer/ductal carcinoma *in situ* (DCIS), and SCLC that could provide advanced warnings of early post-oncogenic tissue changes, precise methods for identifying and evaluating changes in tumor burden, and new non-surgical methods of treatment that are effective in providing long-term survival for patients (North et al. *Br. Can. Res. Treat.* (1995) 34: 229-235; and North *Exper. Physiol.* (2000) 85S: 27-40). Alternatively, no evidence has been found for expression by normal breast tissues or by various fibrocystic conditions, including atypical hyperdisplasia (North et al., *Endocrin. Pathology*, In Press, June, 2003).

Expression of the VP gene in breast cancer gives rise to surface markers named GRSA (North *Exper. Physiol.* (2000) 85S: 27-40). These markers interact with polyclonal antibodies recognizing provasopressin and seem to have molecular weights of 40 and 20 kilodaltons. Since the antibodies were first found to interact with glycopeptide moiety of provasopressin, the antigen has been called GRSA (i.e., Glycopeptide-Related cell Surface Antigen).

There exists a need in the art for new compositions and techniques for diagnosing and treating cancers such as SCLC and breast cancer.

SUMMARY

This application provides, *inter alia,* peptides, antibodies, and antigen-binding portions thereof, for identifying and targeting provasopressin-expressing cancer cells. This application additionally provide methods of using said compositions, for example to image cancer cells *in vivo* and in biological samples. This application additionally provide methods of using said compositions for treating patients suffering from a provasopressin-associated cancer. Provasopressin-associated cancers include breast cancer, ductal carcinoma *in situ*, and small cell lung cancer.

In some aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody (designated MAG-2) produced by a hybridoma having ATCC Number _____. In some aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody (designated MAG-3) produced by a hybridoma having ATCC Number _____. In some aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody (designated MAG-4) produced by a hybridoma having ATCC Number _____. In some aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody (designated MAG-5) produced by a hybridoma having ATCC Number _____.

In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody (designated MAG-2) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody (designated MAG-3) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody (designated MAG-4) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody (designated MAG-5) produced by a hybridoma having ATCC Number _____.

In certain aspects, this disclosure provides monoclonal antibody or antigen-binding portion thereof that binds to provasopressin with a K_d that is 90% or less the K_d of MAG-1.

In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody (designated MAG-2) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody (designated MAG-3) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody (designated MAG-4) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody (designated MAG-5) produced by a hybridoma having ATCC Number _____.

In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds provasopressin, wherein the antibody or antigen-binding portion thereof is capable of inhibiting growth of a xenograft breast or lung tumor in a mouse. In

certain aspects, the breast or lung tumor is SCLC H345, MCF, 7, or MDA-MB231. In some embodiments, the antibody or antigen-binding portion thereof is capable of inhibiting growth of a xenograft breast or lung tumor in a mouse for 16 days.

In some embodiments, the monoclonal antibody or antigen-binding portion thereof is comprises CDRs that are identical to the CDRs of the antibody (designated MAG-2) produced by the hybridoma having ATCC Number _____. In some embodiments, the monoclonal antibody or antigen-binding portion thereof is comprises CDRs that are identical to the CDRs of the antibody (designated MAG-3) produced by the hybridoma having ATCC Number _____. In some embodiments, the monoclonal antibody or antigen-binding portion thereof is comprises CDRs that are identical to the CDRs of the antibody (designated MAG-4) produced by the hybridoma having ATCC Number _____. In some embodiments, the monoclonal antibody or antigen-binding portion thereof is comprises CDRs that are identical to the CDRs of the antibody (designated MAG-5) produced by the hybridoma having ATCC Number _____. In some such embodiments, the antibody is a humanized antibody. In some such embodiments, the antibody is a chimeric antibody.

In some embodiments, the antibody or antigen-binding portion thereof is a monoclonal antibody (designated MAG-2) produced by the hybridoma having ATCC Number _____. In some embodiments, the antibody or antigen-binding portion thereof is a monoclonal antibody (designated MAG-3) produced by the hybridoma having ATCC Number _____. In some embodiments, the antibody or antigen-binding portion thereof is a monoclonal antibody (designated MAG-4) produced by the hybridoma having ATCC Number _____. In some embodiments, the antibody or antigen-binding portion thereof is a monoclonal antibody (designated MAG-5) produced by the hybridoma having ATCC Number _____.

In certain aspects, the antibody or antigen-binding portion are selected from the group consisting of a single chain variable fragment (scFv), or a F(ab')2 fragment.

In certain aspects, the antibody or antigen-binding portion is a Fab fragment.

In certain aspects, the antibody or antigen-binding portion is an IgG antibody.

In certain aspects, the antibody of antigen-binding portion is an IgG1 or IgG2 antibody.

In certain aspects, the antibody or antigen-binding portion bind with a K_d of less than $3x10^{-8}$ M to a peptide comprising SEQ ID No. 2.

In certain aspects, the antibody or antigen-binding portion binds with a K_d of less than $2x10^{-9}$ M to a peptide comprising SEQ ID No. 2.

In some embodiments, the antibody or antigen-binding portion thereof binds to provasopressin with a K_d of equal to or less than the K_d of one of: MAG-2 (produced by the hybridoma having ATCC Number _____), MAG-3 (produced by the hybridoma having ATCC Number _____), MAG-4 (produced by the hybridoma having ATCC Number _____), and MAG-5 (produced by the hybridoma having ATCC Number _____).

In certain embodiments, this disclosure provides antibody or antigen-binding portion further comprising a label. In certain aspects, the label is selected from the group consisting of a fluorescent label, a radiolabel, a toxin, a metal compound, and biotin. In certain aspects, the fluorescent label is selected from the group consisting of Texas Red, phycoerythrin (PE), cytochrome c, and fluorescent isothiocyanate (FITC). In certain aspects, the radiolabel is selected from the group consisting of ^{32}P, ^{33}P, ^{43}K, ^{47}Sc, ^{52}Fe, ^{57}Co, ^{64}Cu, ^{67}Ga, ^{67}Cu, ^{68}Ga, ^{71}Ge, ^{75}Br, ^{76}Br, ^{77}Br, ^{77}As, ^{77}Br, $^{81}Rb/^{81M}Kr$, ^{87M}Sr, ^{90}Y, ^{97}Ru, ^{99}Tc, ^{100}Pd, ^{101}Rh, ^{103}Pb, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{111}In, ^{113}In, ^{119}Sb ^{121}Sn, ^{123}I, ^{125}I, ^{127}Cs, ^{128}Ba, ^{129}Cs, ^{131}I, ^{131}Cs, ^{143}Pr, ^{153}Sm, ^{161}Tb, ^{166}Ho, ^{169}Eu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{191}Os, ^{193}Pt, ^{194}Ir, ^{197}Hg, ^{199}Au, ^{203}Pb, ^{211}At, ^{212}Pb, ^{212}Bi and ^{213}Bi. In certain aspects, the toxin is selected from the group consisting of ricin, ricin A chain (ricin toxin), Pseudomonas exotoxin (PE), diphtheria toxin (DT), *Clostridium perfringens* phospholipase C (PLC), bovine pancreatic ribonuclease (BPR), pokeweed antiviral protein (PAP), abrin, abrin A chain (abrin toxin), cobra venom factor (CVF), gelonin (GEL), saporin (SAP), modeccin, viscumin and volkensin.

In certain aspects, this disclosure provides a peptide consisting of SEQ ID No. 1.

In certain aspects, this disclosure provides a fusion protein comprising: (1) a peptide consisting of SEQ ID No. 1; and (2) a second peptide, wherein the two peptides are operably linked in a manner that does not produce a wild-type provasopressin amino acid sequence. In certain aspects, the second peptide is a purification tag peptide.

In certain aspects, this disclosure provides a peptide consisting of SEQ ID No. 2.

In certain aspects, this disclosure provides a fusion protein comprising: (1) a peptide consisting of SEQ ID No. 2; and (2) a second peptide, wherein the two peptides are operably linked in a manner that does not produce a wild-type provasopressin amino acid sequence. In certain aspects, the second peptide is a purification tag peptide.

This disclosure also provides a composition comprising the peptide or fusion protein as described herein, bound to a solid substrate.

Furthermore, this disclosure provides a nucleic acid that encodes the peptide or fusion protein described herein. This disclosure also provides a nucleic acid construct comprising the nucleic acid described herein operably linked to a second nucleic acid, wherein the two

nucleic acids are operably linked in a manner that does not produce a wild-type provasopressin nucleic acid sequence. The nucleic acid or nucleic acid construct may be a DNA or an RNA. Also provided is a nucleic acid encoding the antibody or antigen-binding portion disclosed herein. Also provided is a composition comprising the antibody or antigen binding portion as described herein.

Also provided is a pharmaceutical composition comprising the antibody or antigen binding portion thereof as disclosed herein. The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition is formulated for systemic delivery, such as injection; it may also be formulated for local delivery.

In certain aspects, this disclosure provides a kit useful for screening a biological sample for breast cancer, ductal carcinoma in situ, or small cell lung cancer, comprising an antibody or antigen-binding portion disclosed herein.

In certain embodiments, this disclosure provides methods of determining whether a subject has any one of the following cancers: breast cancer, ductal carcinoma *in situ,* or small cell lung cancer, comprising obtaining a biological sample from a patient and contacting the sample with the antibody or antigen-binding portion disclosed herein; wherein binding is indicative that the subject is likely to have breast cancer, ductal carcinoma *in situ,* or small cell lung cancer. In certain aspects, the method of determining whether a subject has any one of the cancers disclosed herein comprises a further comprising a step of contacting the antibody or antigen binding portion to a control tissue, and comparing the binding of the antibody or antigen-binding portion to the sample to the binding of the antibody or antigen-binding portion to a control tissue. In certain aspects, the biological sample is from a patient suspected of having breast cancer. In certain aspects, the biological sample is from patients suspected of having ductal carcinoma *in situ*. In certain aspects, the biological sample is from patients suspected of having small cell lung cancer.

In certain aspects, this disclosure provides a method for distinguishing among fibrocystic lesions, ductal carcinoma *in situ*, and invasive breast cancer in a breast tissue biological samples from a patient suspected of having breast cancer, comprising obtaining a breast tissue biological sample from a patient, contacting the breast tissue biological sample with an antibody or antigen-binding portion disclosed herein, contacting the breast tissue biological sample with an antibody or antigen-binding portion thereof immunoreactive with an angiotensin II type-1 receptor, and determining if the cells of the sample overexpress one or both of provasopressin and angiotensin II type-1 receptor, wherein if the breast tissue

biological sample is positive for provasopressin and negative for the angiotensin II type-1 receptor, that patient is characterized as having invasive breast cancer. In certain aspects, if the breast tissue biological sample is positive for both provasopressin and the angiotensin II type-1 receptor, the patient likely has ductal carcinoma *in situ.* In certain aspects, if the breast tissue biological sample is negative for provasopressin and is positive for the angiotensin II type-1 receptor, the patient is characterized as having a fibrocystic lesion. In certain aspects, the antibody or antigen-binding portion thereof which is immunoreactive with an angiotensin II type-1 receptor is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a humanized antibody, a single chain variable fragment, a Fab fragment, and a $F(ab')_2$ fragment.

In certain aspects, this disclosure provides a kit for distinguishing among fibrocystic lesions, ductal carcinoma *in situ,* and invasive breast cancer in a breast tissue biological samples from a patient suspected of having breast cancer, comprising an antibody or antigen-binding portion thereof of the disclosure and an antibody or antigen-binding portion thereof immunoreactive with an angiotensin II type-1 receptor. In certain aspects, the antibody or antigen-binding portion thereof which is immunoreactive with an angiotensin II type-1 receptor is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a humanized antibody, a single chain variable fragment, a Fab fragment, and a $F(ab')_2$ fragment. In certain aspects, the biological sample is from a patient suspected of having a condition selected from the group consisting of breast cancer, breast ductal carcinoma *in situ,* and atypical ductal hyperplasia.

In certain aspects, this disclosure provides a method of treating a condition selected from the group consisting of breast cancer, breast ductal carcinoma *in situ,* and small cell lung cancer, comprising administering an effective amount of any of pharmaceutical compositions described herein to a subject. In certain aspects, the subject administered an effective amount of any of the pharmaceutical compositions described herein is immunocompromised. In certain embodiments, the immunocompromised paitient is a chemotherapy patient, an elderly patient, a patient having a hereditary immunodeficiency, an HIV positive patient, a patient with leukemia, a patient with lymphoma, or a patient with multiple myeloma. In some embodiments, the method comprises administering an effective amount of a pharmaceutical composition comprising a chemotherapeutic agent. In certain embodiments, the pharmaceutical composition further comprises epinephrine.

In some embodiments, the pharmaceutical compositions are administered concomitantly. They may be administered in a single formulation, for example, or

administered as separate formulations. In some embodiments, the method comprises administering an effective amount of a pharmaceutical composition comprising dexamethasone, IBMX, and 8-bromoadenosine 3',5'-cyclic monophosphate (8br-cAMP). In some embodiments, the method comprises comprising administering an effective amount of a pharmaceutical composition comprising IBMX and forskolin.

This application provides, inter alia, the pharmaceutical compositions herein, for the treatment of a cancer selected from the group consisting of breast cancer, small cell lung cancer, and ductal carcinoma in situ. The pharmaceutical compositions may further comprise IBMX and forskolin. The pharmaceutical compositions may also comprise dexamethasone, IBMX, and 8-bromoadenosine 3',5'-cyclic monophosphate (8br-cAMP).

Furthermore, this application provides a method of detecting a tumor in vivo in a patient suspected of having breast cancer, ductal carcinoma in situ, or small cell lung cancer, comprising: a) administering an antibody or antigen-binding portion as described herein to the patient; and b) detecting the label; wherein detection of the label is indicative that the patient likely has a tumor. The method may further comprise determining the location of the cells overexpressing provasopressin.

Still further, this application provides a method for distinguishing among fibrocystic lesions, ductal carcinoma in situ, and invasive breast cancer in vivo in a patient suspected of having breast cancer, comprising: a) administering a composition comprising an antibody or antigen-binding portion as described herein and a first label to the patient; b) administering a composition comprising a second label and an antibody or antigen-binding portion thereof immunoreactive with an angiotensin II type-1 receptor to the patient; and c) detecting the first label and the second label; wherein fibrocystic lesions are identified as those that overexpress angiotensin II type-1 receptor but not provasopressin, and cancerous lesions are identified as those that overexpress provasopressin. The method may further comprise determining the location of the cells overexpressing provasopressin and/or angiotensin II type-1 receptor.

In addition this disclosure provides the use of the antibody or antigen binding portion thereof as described herein in the manufacture of a medicament for the treatment of a cancer selected from the group consisting of breast cancer, small cell lung cancer, and ductal carcinoma in situ. In addition this disclosure provides the use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for the treatment of a cancer selected from the group consisting of breast cancer, small cell lung cancer, and ductal carcinoma *in situ*.

The potential uses for anti-provasopressin antibodies and antigen-binding portions thereof are significant, not only as a tumor-targeting agent for the localization and treatment of SCLC, but also for distinguishing SCLC from other forms of lung cancer, and aiding its early diagnosis (Friedmann et al. (1994) *Br. J. Cancer* 69:260-263; Friedmann et al. (1993) *Cancer Lett.* 75: 79-85; North et al. (1989) *Nuc. Med. Commun.* 10: 643-652). Furthermore, said antibodies and antigen binding portion thereof may be used in basic research. For example, they may be used to identify provasopressin in Western blots or immunofluorescence.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a bar graph depicting the percent binding of "Boris" and MAG-1 through -5 to C18-Pro9 and C18-His9. The y-axis represents percent binding to the indicated substrate, and the x-axis represents the six antibodies tested.

Figures 2A and 2B depict the percent binding of Boris and MAG-1 to radiolabeled substrates. Panel A depicts the inhibition of Boris's binding to C18-Pro9 (SEQ ID No. 5) upon addition of either C18-Pro9 or C18-His9 (SEQ ID No. 6). Panel B depicts the inhibition of MAG-1's binding to C18-Pro9 upon the addition of either C18-Pro9 or C18-His9. In both panels, the y axis represents % binding and the x axis represents the concentration of the inhibitor (which is C18-Pro9 or C18-His9). In both panels, C18-Pro9 data points are represented as diamonds and C18-His9 data points are represented as squares.

Figures 3A and 3B depict line graphs where each point represents the percent binding of an antibody to C18-Pro9 in the presence of different concentrations of a competitive binding inhibitor. In panel A, C18-His9 is the inhibitor and in panel B C18-Pro9 is the inhibitor. In both panels, the y axis represents % binding and the x axis represents the concentration of the inhibitor. In panel A, the MAG-1 and MAG-2 curves are essentially identical, and the MAG-1 curve appears hidden behind the MAG-2 curve.

Figures 4A and 4B depict competitive inhibition of binding of Boris (panel A) and MAG-1 (panel B) to Peptide 1 (SEQ ID No. 7), Peptide 2 (SEQ ID No. 8) , and Peptide 3 (SEQ ID No. 1). Each data point represents the percent binding of an antibody to C18-Pro9 in the presence of different concentrations of a competitive binding inhibitor (Peptide 1, Peptide 2, or Peptide 3). In both panels, the y axis represents % binding and the x axis represents the concentration of the inhibitor.

Figure 5 depicts the competitive inhibition of C18 peptide binding with Peptide 4 (SEQ ID No. 2). Each data point represents the percent binding of an antibody to C18-Pro9

in the presence of different concentrations of a competitive binding inhibitor, which is Peptide 4. In both panels, the y axis represents % binding and the x axis represents the concentration of the inhibitor.

Figure 6 depicts a tumor-bearing mouse as well as the images obtained from *in vivo* and *ex vivo* imaging of the tumor. Far left panel, photograph of a tumor-bearing mouse. Second panel from left, *in vivo* image of tumor, detected using a radiolabeled Fab fragment of MAG-1. Third panel from left, *ex vivo* photograph of an excised tumor. Rightmost panel, image of the excised tumor detected using a radiolabeled Fab fragment of MAG-1.

In Figure 7, both T1 images (left two columns) and T2 images (right two columns) of tumors are shown. The top row of images in Figure 7 portrays tumors in the absence of imaging reagents. The center row of images in Figure 7 portrays tumors treated with gadodiamide. The bottom row of images in Figure 7 portrays tumors treated with Gd-DTPA-Fab-MAG-1.

Figure 8 shows ^{99}Tc-DTPA-Fab-MAG-1 image of an MCF-7 tumor xenograft at 24 h post-injection. The small anterior lump on animal is an estrogen pellet used to promote tumor growth.

Figure 9 illustrates the inhibition in SCLC S345 tumor growth in mice caused by administration of ^{90}Y-MAG-1 and MAG-1. The y axis represents % tumor growth, and the x axis represents time.

Figure 10 illustrates that ^{90}Y-MAG-1 inhibits estrogen-dependent and estrogen-independent tumor growth in nude mice. Left panel, MCF-7 tumor. Right panel, MDA-MB231 tumor. In both panels, the y axis represents % tumor growth, and the x axis represents time.

Figure 11 shows that MAG-1 inhibits estrogen-dependent and estrogen-independent tumor growth in nude mice. In both panels, the y axis represents % tumor growth, and the x axis represents time. Left panel, MCF-7 tumor. Right panel, MDA-MB231 tumor.

Figures 12A-12B show that MAG-1 daily treatment (50 μg) for 16 days shrinks and prevents re-growth of MDA-MB-231 breast tumors (12A) and MCF-7 breast tumors (12B): Mean +/- SEM ($p<0.05x10-4$, n=4). Arrow denotes day of last treatment.

Figure 13 show that Fab-MAG-1 daily treatment (20 μg) for 15 days shrinks and prevents re-growth of MDA-MB-231 breast tumors. Arrow denotes day of last treatment.

Figure 14 shows biodistribution of ^{99}Tc-DTPA-Fab-MAG-1 in MCF-7 (■; n=4) and MDA-MB-231 (□; n=4) breast cancer tumors bearing nude mice.

Figures 15A, B, C, and D. Kidney sections (10x) from untreated (A) and long-term MAG-1 treated (B) MDA-MB231 tumor bearing animals and liver sections (10x) from untreated (C) and long-term MAG-1 treated (D) MDA-MB231 tumor bearing animals.

Figures 16A, B, C, and D. (A), (B), and (C) are cross-sections (0.5-1.2 cm) of three tumors from MDA-MB231 cells treated long-term with MAG-1 and stained with hematoxylin and eosin; (D) is a cross-section of saline-treated MDA-MB231 tumor.

DETAILED DESCRIPTION

1. Overview

The present disclosure describes, *inter alia,* the detection of NRSA in cultured SCLC cells and human SCLC tumor tissue using novel antibodies designated MAG-2, MAG-3, MAG-4, and MAG-5. These antibodies bind the C-terminal portion of the VAG region of provasopressin and have similar specificity to MAG-1, which was described in PCT Publication No. WO04/006860. MAG-1 recognizes the ~20 kDa and ~40 kDa NRSA proteins in cultured SCLC cell lysate by Western analysis, while immunofluorescent cytometric and microscopic analyses indicates that it binds to the surface of these cells. Importantly, the ~20 kDa and ~40 kDa NRSA proteins were detected in the lysate of human SCLC tumor biopsy samples by Western analysis using MAG-1, but they were not detected in the lysate of non-tumor human lung tissue. Immunohistochemical analysis revealed that MAG-1 reacts with human SCLC tumor, but not with normal lung tissue. Since NRSA is not typically found on the surface of normal cells, it is an excellent target in an antibody-based approach for tumor localization in the diagnosis and therapy of SCLC.

Breast cancer cells express the vasopressin gene. The vasopressin gene is expressed by DCIS but not by various fibrocystic breast conditions, including ADH. Since metastatic as well as localized breast cancer seems to express provasopressin on the cell surface, suitably labeled or modified forms of monoclonal or polyclonal antibodies against this receptor, through injection and different means of detection, are useful in providing a needed very sensitive and specific means of detecting and localizing metastatic and/or early recurrent disease in patients. These antibodies, and their modified forms, are important new tools for effectively targeting different treatments to tumors in patients. This is potentially especially valuable in treating recurrent and generally estrogen-resistant forms of breast cancer.

2. Definitions

"Administering" is defined herein as a means providing the composition to the patient in a manner that results in the composition coming into contact with the patient's body in a manner that permits a therapeutic effect. Such an administration can be by any route including, without limitation, subcutaneous, intradermal, intravenous, intra-arterial, intraperitoneal, intramuscular, and topical.

The term "amino acid residue" is known in the art. In general the abbreviations used herein for designating the amino acids and the protective groups are based on recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (see Biochemistry (1972) 11:1726-1732). In certain embodiments, the amino acids used are those naturally occurring amino acids found in proteins, or the naturally occurring anabolic or catabolic products of such amino acids which contain amino and carboxyl groups. Particularly suitable amino acid side chains include side chains selected from those of the following amino acids: glycine, alanine, valine, cysteine, leucine, isoleucine, serine, threonine, methionine, glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine, proline, histidine, phenylalanine, tyrosine, and tryptophan.

As used herein, the term "antibody" refers to an immunoglobulin molecule. The term "antibody" encompasses monoclonal and polyclonal antibodies. The antibody may be an IgG, an IgM, an IgE, an IgA or an IgD molecule. In certain embodiments, the antibody is an IgG and is an IgG1, IgG2, IgG3 or IgG4 subtype. The class and subclass of antibodies may be determined by any method known in the art, for example, by using antibodies that are specific for a particular class and subclass of antibody. Such antibodies are available commercially. The class and subclass can be determined by ELISA or Western blot as well as other techniques. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant domains of the heavy and/or light chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various class and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

As used herein, the term "antibodies" encompasses immunoglobulins produced using hybridoma technology or recombinant methods, including immunoglobulins, chimeric immunoglobulins, humanized immunoglobulins such as Composite Human Antibodies™, and single chain antibodies. As used herein, "antibodies" also refers to antigen binding synthetic peptides comprising sequences derived from the sequences of immunoglobulin antigen binding domains. In some embodiments, the anti-vasopressin antibody or antigen-

binding portion thereof contains non-natural amino acid residues and/or is conjugated to additional molecules such as PEG.

As used herein, the "antigen-binding portion thereof" of an anti-provasopressin antibody refers to a portion of an anti-provasopressin antibody that binds to provasopressin with substantial affinity. Substantial affinity includes affinity that is at least 1/100, 1/50, 1/20, 1/10, 1/5, or 1/2 the affinity of the antibody for provasopressin. Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen-binding portions include, *inter alia*, Fab, Fab', F(ab')2, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), single domain antibodies, chimeric antibodies, diabodies and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

As used herein, "biological sample" refers to a sample taken from the body of a patient. Such samples include tissue samples, blood samples, urine samples, and the like.

"Boris", as used herein, refers to the polyclonal antibody preparation reactive with vasopressin-human glycopeptide (VAG), the preparation of which is described in North *et al.* Breast Cancer Res Treat. 1995 Jun;34(3):229-35)

As used herein, the term "cancer" is used to mean a condition in which a cell in a patient's body undergoes abnormal, uncontrolled proliferation. Non-limiting examples of cancers include breast cancer, small cell lung cancer, and ductal carcinoma *in situ*.

A "fusion protein" as used herein refers to a protein (including a peptide or polypeptide) comprising a sequence derived from two or more sources. The two or more sources may be, for example, different organisms, different genes from the same organism, or different portions of the same protein that are not juxtaposed in a wild-type organism, and may comprise one or more non-naturally occurring sequences.

"Homology" is a measure of the identity of nucleotide sequences or amino acid sequences. In order to characterize the homology, subject sequences are aligned so that the highest percentage homology (match) is obtained, after introducing gaps, if necessary, to achieve maximum percent homology. N- or C-terminal extensions shall not be construed as affecting homology. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. Computer program methods to determine identity between two sequences, for example, include DNAStar® software (DNAStar Inc. Madison, WI); the GCG® program package (Devereux, J., *et al. Nucleic Acids Research* (1984) 12(1): 387);

BLASTP, BLASTN, FASTA (Atschul, S.F. *et al., J. Molec Biol* (1990) 215: 403). Homology (identity) as defined herein is determined conventionally using the well-known computer program, BESTFIT® (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI, 53711). When using BESTFIT® or any other sequence alignment program (such as the Clustal algorithm from MegAlign software (DNAStar®) to determine whether a particular sequence is, for example, about 90% homologous to a reference sequence, the parameters are set such that the percentage of identity is calculated over the full length of the reference nucleotide sequence or amino acid sequence and that gaps in homology of up to about 90% of the total number of nucleotides in the reference sequence are allowed.

A "humanized" antibody, as used herein, is an antibody comprising regions, such as CDRs, that were originally produced in a non-human animal and other regions (for example constant regions and/or framework regions) that were originally produced by a human body.. The non-human animal content may be less than 50%, 40%, 30%, 20%, 10%, or 5%. In certain humanized antibodies the six CDRs of the heavy and light chains and a limited number of structural amino acids of the murine monoclonal antibody are grafted by recombinant technology to the CDR-depleted human IgG scaffold. In certain embodiments, chimeric, humanized or primatized (CDR-grafted) antibodies, comprising portions derived from different species or fully human antibodies, are also encompassed by the present disclosure. The various portions of these antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See, e.g., Cabilly et al., U.S. Pat. No. 4,816,567; Cabilly et al., European Patent No. 0,125,023; Boss et al., U.S. Pat. No. 4,816,397; Boss et al., European Patent No. 0,120,694; Neuberger, M. S. et al., WO 86/01533; Neuberger, M. S. et al., European Patent No. 0,194,276 B1; Winter, U.S. Pat. No. 5,225,539; and Winter, European Patent No. 0,239,400 B1. See also, Newman, R. et al., BioTechnology, 10: 1455-1460 (1992), regarding primatized antibody. See, e.g., Ladner et al., U.S. Pat. No. 4,946,778; and Bird, R. E. et al., Science, 242: 423-426 (1988)), regarding single chain antibodies.

As used herein, the term "label" or "labeled" refers to incorporation of another molecule in the antibody, antigen-binding portion thereof, or peptide. In one embodiment, the label is a detectable marker, e.g., incorporation of a radiolabeled amino acid. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used.

Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides, fluorescent labels, enzymatic labels, chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), magnetic agents, and toxins.

"MAG-1" as used herein refers to the monoclonal antibody that is produced by the hybridoma having ATCC Number PTA-5322.

"Nucleic acid construct" refers to a nucleic acid molecule that is not found in nature, although it may comprise one or more naturally occurring portions. In some embodiments, "nucleic acid construct" comprises a sequence derived from two or more sources. The two or more sources may be, for example, different organisms, different genes from the same organism, or different portions of the same gene that are not juxtaposed in a wild-type organism, and may comprise one or more non-naturally occurring sequences.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression. In addition, a polypeptide tag operably linked to a protein may direct the localization of that protein to a specific region of a cell, facilitate affinity purification of that protein, and the like.

A "patient" or "subject" to be treated by the subject methods may mean either a human or non-human animal, such as primates, mammals, and vertebrates.

The phrase "pharmaceutically acceptable carrier" is art-recognized, and includes, for example, pharmaceutically acceptable materials, compositions or vehicles, such as a liquid or solid filler, diluent, solvent or encapsulating material involved in carrying or transporting any subject composition, from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of a subject composition and not injurious to the patient, within a reasonable risk-benefit ratio. In certain embodiments, a pharmaceutically acceptable carrier is non-pyrogenic. Some examples of materials which may serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene

glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The terms "polynucleotide" and "nucleic acid," used interchangeably herein, refer to a polymeric form of nucleotides of any length, including ribonucleotides or deoxynucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

The terms "protein", "polypeptide" and "peptide" are used interchangeably herein when referring to a gene product, e.g., as may be encoded by a coding sequence. A polypeptide is a polymer of amino acids and/or amino acid analogues.

As used herein, "purification tag peptide" refers to a peptide, oligopeptide, or polypeptide having a primary use in affinity purification. "Purification tag peptide" encompasses His, GST, TAP, FLAG, myc, HA, MBP, VSV-G, thioredoxin, V5, avidin, streptavidin, BCCP, Calmodulin, Nus, and S tags.

The term "substantially purified" when referring to a protein or nucleic acid refers to a preparation of a protein or proteins or nucleic acid or nucleic acids which are preferably isolated from, or otherwise substantially free of, other proteins and nucleic acids normally associated with the protein(s) or nucleic acid(s) in a cell or cell lysate. The term "substantially free of other cellular proteins" (also referred to herein as "substantially free of other contaminating proteins") is defined as encompassing individual preparations of each of the component proteins comprising less than 20% (by dry weight) contaminating protein, and preferably comprises less than 5% contaminating protein. Functional forms of each of the component proteins can be prepared as purified preparations by using a cloned gene as described in the attached examples. By "purified", it is meant, when referring to component protein preparations used to generate a reconstituted protein mixture, that the indicated molecule is present in the substantial absence of other biological macromolecules, such as other proteins (particularly other proteins which may substantially mask, diminish, confuse or alter the characteristics of the component proteins either as purified preparations or in their function in the subject reconstituted mixture). The term "purified" as used herein preferably means at least 80% by dry weight, more preferably in the range of 85% by weight, for

example 95-99% by weight, for example at least 99.8% by weight, of biological macromolecules of the same type present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 5000, can be present). The term "pure" as used herein preferably has the same numerical limits as "purified" immediately above.

A "therapeutically effective amount" is defined herein an effective amount of composition for causing the tumor-specific killing of tumor cells in a patient, eliminating one or more tumor cells, preventing said cells from proliferating, or inhibiting the rate of proliferation of said cells, at a reasonable benefit/risk ratio applicable to any medical treatment.

By "treating" a patient suffering from cancer it is meant that at least one of the patient's symptoms is partially or totally alleviated, or remain static following treatment according to the methods herein. A patient that has been treated can exhibit a partial or total alleviation of symptoms and/or tumor load.

3: Peptides

This application provides, for example, a peptide of 8 amino acids consisting of SEQ ID No. 1. The instant disclosure also provides a peptide of 10 amino acids consisting of SEQ ID No. 2. This application also discloses a fusion protein consisting of either of said peptides operably linked to a second peptide, wherein the peptides are operably linked in a manner that does not produce a wild-type provasopressin amino acid sequence. The second peptide may be a purification tag peptide. These peptide or fusion protein may be bound to a solid substrate, for example for use in an ELISA assay.

In certain embodiments, the peptides or fusion proteins are substantially purified peptides or fusion proteins.

In certain embodiments, the fusion proteins disclosed herein comprise a purification tag peptide. Exemplary purification tag peptides include His, GST, TAP, FLAG, myc, HA, MBP, VSV-G, thioredoxin, V5, avidin, streptavidin, BCCP, Calmodulin, Nus, and S tags. In other embodiments, a fusion protein may comprise a fluorescent protein or a protein that binds a fluorescent label. Examples of fluorescent proteins include dsRed, mRFP, YFP, GFP, CFP, BFP, and Venus. An example of a protein that binds a fluorescent label is FlAsH.

The disclosed peptides have a variety of uses. For example, they may be used in an assay (such as an ELISA or Western blot) to test the specificity of putative provasopressin antibodies or antigen-binding portions thereof. In an ELISA, it may be useful to have the

peptide bound to a solid substrate. Such *in vitro* characterization will be useful in predicting the therapeutic utility of different anti-vasopressin antibodies. In addition, these peptides may be used in a quality-control setting to test different batches of therapeutic antibodies (or antigen-binding portions thereof), especially if the therapeutic antibodies or portions thereof are manufactured on a large scale.

4: Antibodies and antigen-binding portions thereof

4.1 Introduction

Native antibodies are multi-subunit animal protein molecules with highly specific antigen-binding properties. Native antibodies are made up of two or more heterodimeric subunits each containing one heavy (H) and one light (L) chain. Every individual native antibody has one type of L chain and one type of H chain, which are held together by disulfide bonds to form a heterodimeric subunit. Typically a native antibody (e.g., an IgG) has two such subunits, which are also held together by disulfide bonds. The disulfide bonds linking together the heterodimeric subunits are located at the hinge regions of the heavy and light chains. The hinge region is particularly sensitive to proteolytic cleavage, such proteolysis yielding two or three fragments (depending on the precise site of cleavage), a non-antigen binding fragment containing only H chain C regions (Fc) and one bivalent (Fab'2) or two monovalent (Fab) antigen binding fragments.

The first domain of each chain is highly variable in amino acid sequence, providing the vast spectrum of antibody binding specificities found in each individual. These are known as variable heavy (VH) and variable light (VL) domains. The second and subsequent (if any) domains of each chain are relatively invariant in amino acid sequence. These are known as constant heavy (CH) and constant light (CL) domains.

Each variable region contains three loops of hypervariable sequence that provide a complementary structure to that of the antigen and are critical in determining the antigen binding specificity of the antibody, as they are the contact sites for binding to the antigen. These loops are known as complementarity determining regions, or CDRs. Each variable domain is made up of three CDRs embedded in four much less variable framework segments (FRs). Together, the sets of collinear CDRs and FRs are in large part responsible for determining the three dimensional conformation of the variable regions of antibody molecules.

CDRs and FRs are features that have been deduced from structural properties of antibody variable regions. Both amino acid sequence (primary structure) and three dimensional modeling (deduced secondary and tertiary structure) of antibody variable regions have been used by various researchers to define CDRs and, by default, FRs. While the positions of the CDRs are beyond question, not all workers in the art agree upon the precise locations of the boundaries of each CDR in VH or VL regions; there is no clear cut structural marker delineating CDR/FR boundaries. Two definitions of CDR location are currently in general use in the art. These are the "sequence variability" definition of Kabat et al. ("Sequences of Proteins of Immunological Interest," 4th ed. Washington, D.C.: Public Health Service, N.I.H.) and the "structural variability" definition of Chothia and Lesk (J. Mol. Biol. 1987, 196:901).

4.2 Exemplary Provasopressin-binding antibodies

Numerous example of provasopressin-binding antibodies are disclosed herein.

In some aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody (designated MAG-2) produced by a hybridoma having ATCC Number _____. In some aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody (designated MAG-3) produced by a hybridoma having ATCC Number _____. In some aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody (designated MAG-4) produced by a hybridoma having ATCC Number _____. In some aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody (designated MAG-5) produced by a hybridoma having ATCC Number _____.

In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody (designated MAG-2) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or

antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody (designated MAG-3) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody (designated MAG-4) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody (designated MAG-5) produced by a hybridoma having ATCC Number _____.

In certain aspects, this disclosure provides monoclonal antibody or antigen-binding portion thereof that binds to provasopressin with a K_d that is 90% or less the K_d of MAG-1.

In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody (designated MAG-2) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody (designated MAG-3) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody (designated MAG-4) produced by a hybridoma having ATCC Number _____. In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody (designated MAG-5) produced by a hybridoma having ATCC Number _____.

In certain aspects, this disclosure provides a monoclonal antibody or antigen-binding portion thereof that binds provasopressin, wherein the antibody or antigen-binding portion thereof is capable of inhibiting growth of a xenograft breast or lung tumor in a mouse. In certain aspects, the breast or lung tumor is SCLC H345, MCF, 7, or MDA-MB231. In some embodiments, the antibody or antigen-binding portion thereof is capable of inhibiting growth of a xenograft breast or lung tumor in a mouse for 16 days.

In some embodiments, the monoclonal antibody or antigen-binding portion thereof is comprises CDRs that are identical to the CDRs of the antibody (designated MAG-2) produced by the hybridoma having ATCC Number _____. In some embodiments, the monoclonal antibody or antigen-binding portion thereof is comprises CDRs that are identical to the CDRs of the antibody (designated MAG-3) produced by the hybridoma having ATCC Number _____. In some embodiments, the monoclonal antibody or antigen-binding portion thereof is comprises CDRs that are identical to the CDRs of the antibody (designated MAG-4) produced by the hybridoma having ATCC Number _____. In some embodiments, the monoclonal antibody or antigen-binding portion thereof is comprises CDRs that are identical to the CDRs of the antibody (designated MAG-5) produced by the hybridoma having ATCC Number _____. In some such embodiments, the antibody is a humanized antibody. In some such embodiments, the antibody is a chimeric antibody.

In some embodiments, the antibody or antigen-binding portion thereof is a monoclonal antibody (designated MAG-2) produced by the hybridoma having ATCC Number _____. In some embodiments, the antibody or antigen-binding portion thereof is a monoclonal antibody (designated MAG-3) produced by the hybridoma having ATCC Number _____. In some embodiments, the antibody or antigen-binding portion thereof is a monoclonal antibody (designated MAG-4) produced by the hybridoma having ATCC Number _____. In some embodiments, the antibody or antigen-binding portion thereof is a monoclonal antibody (designated MAG-5) produced by the hybridoma having ATCC Number _____.

In certain aspects, the antibody or antigen-binding portion are selected from the group consisting of a single chain variable fragment (scFv), or a F(ab')2 fragment.

In certain aspects, the antibody or antigen-binding portion is a Fab fragment.

In certain aspects, the antibody or antigen-binding portion is an IgG antibody.

In certain aspects, the antibody of antigen-binding portion is an IgG1 or IgG2 antibody.

In certain aspects, the antibody or antigen-binding portion bind with a K_d of less than $3x10^{-8}$ M to a peptide comprising SEQ ID No. 2.

In certain aspects, the antibody or antigen-binding portion binds with a K_d of less than $2x10^{-9}$ M to a peptide comprising SEQ ID No. 2.

In some embodiments, the antibody or antigen-binding portion thereof binds to provasopressin with a K_d of equal to or less than the K_d of one of: MAG-2 (produced by the hybridoma having ATCC Number _____), MAG-3 (produced by the hybridoma having

ATCC Number _____), MAG-4 (produced by the hybridoma having ATCC Number _____), and MAG-5 (produced by the hybridoma having ATCC Number _____).

In certain embodiments, this disclosure provides antibody or antigen-binding portion further comprising a label. In certain aspects, the label is selected from the group consisting of a fluorescent label, a radiolabel, a toxin, a metal compound, and biotin. In certain aspects, the fluorescent label is selected from the group consisting of Texas Red, phycoerythrin (PE), cytochrome c, and fluorescent isothiocyanate (FITC). In certain aspects, the radiolabel is selected from the group consisting of ^{32}P, ^{33}P, ^{43}K, ^{47}Sc, ^{52}Fe, ^{57}Co, ^{64}Cu, ^{67}Ga, ^{67}Cu, ^{68}Ga, ^{71}Ge, ^{75}Br, ^{76}Br, ^{77}Br, ^{77}As, ^{77}Br, $^{81}Rb/^{81M}Kr$, ^{87M}Sr, ^{90}Y, ^{97}Ru, ^{99}Tc, ^{100}Pd, ^{101}Rh, ^{103}Pb, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{111}In, ^{113}In, ^{119}Sb ^{121}Sn, ^{123}I, ^{125}I, ^{127}Cs, ^{128}Ba, ^{129}Cs, ^{131}I, ^{131}Cs, ^{143}Pr, ^{153}Sm, ^{161}Tb, ^{166}Ho, ^{169}Eu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{191}Os, ^{193}Pt, ^{194}Ir, ^{197}Hg, ^{199}Au, ^{203}Pb, ^{211}At, ^{212}Pb, ^{212}Bi and ^{213}Bi. In certain aspects, the toxin is selected from the group consisting of ricin, ricin A chain (ricin toxin), Pseudomonas exotoxin (PE), diphtheria toxin (DT), *Clostridium perfringens* phospholipase C (PLC), bovine pancreatic ribonuclease (BPR), pokeweed antiviral protein (PAP), abrin, abrin A chain (abrin toxin), cobra venom factor (CVF), gelonin (GEL), saporin (SAP), modeccin, viscumin and volkensin.

4.3 Humanized antibodies

One problem that antibody engineering attempts to address is the immune activity of a human patient that occurs in response to a native murine (or other non-human animal) antibody, typically a mAb, that is being administered to the patient for therapeutic purposes. This activity against murine antibodies is characterized by a human anti-mouse antibody (HAMA) response that can have deleterious effects on treatment efficacy and patient health. The HAMA response may be triggered when an antibody has epitopes recognized by human T cells. Thus, the antibodies described herein preferably are not recognized well by human T cells.

It has been found that almost all such human anti-non-human antibody ("HAMA type") activity is directed at the constant domains and at the FR regions of the variable domains of native non-human antibodies. Antibodies from other non-human animals have similar deleterious effects to a patient. The antibodies described herein may be humanized by any means known in the art.

By manipulating the nucleic acid molecules encoding antibody H and L chains it is possible to incorporate non-human variable regions into antibodies otherwise made up of human constant regions. The resulting antibodies are referred to as "chimeric antibodies,"

and are typically less prone to eliciting HAMA type responses than are the non-human antibodies from which the variable regions are derived.

An alternative to eliminating the potential of a non-human antibody to elicit a HAMA type response is to "humanize" it, e.g., to replace its non-human framework regions with human ones. One way of achieving such humanization involves the insertion of polynucleotide fragments encoding the non-human CDRs of the antibody to be humanized into a nucleic acid molecule encoding an otherwise human antibody (with human constant regions if desired) so as to replace the human CDRs and to use the resulting nucleic acid molecule to express the encoded "humanized" antibody. If this process results in a loss of antibody-epitope affinity, selected humanized residues may be mutated back to their identity in the non-human antibody.

Detailed discussions of antibody engineering may be found in numerous recent publications including: Borrebaek, "Antibody Engineering, A Practical Guide," 1992, W.H. Freeman and Co. NY; and Borrebaek, "Antibody Engineering," 2nd ed. 1995, Oxford University Press, NY, Oxford.

A humanized antibody can be an antibody derived from a non-human species, in which certain amino acids in the framework and constant domains of the heavy and light chains have been mutated so as to reduce of abolish an immune response in humans. Alternatively, a humanized antibody may be produced by fusing the constant domains from a human antibody to the variable domains of a non-human species. Examples of how to make humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293. A humanized antibody may comprise portions of immunoglobulins of different origin. For example, at least one portion can be of human origin. For example, the humanized antibody can comprise portions derived from an immunoglobulin of nonhuman origin with the requisite specificity, such as a mouse, and from immunoglobulin sequences of human origin (e.g., a chimeric immunoglobulin), joined together chemically by conventional techniques (e.g., synthetic) or prepared as a contiguous polypeptide using genetic engineering techniques (e.g., DNA encoding the protein portions of the chimeric antibody can be expressed to produce a contiguous polypeptide chain). Alternatively, a humanized antibody may be created in a transgenic or humanized animal expressing the human antibody genes (see Lonberg, N. "Transgenic Approaches to Human Monoclonal Antibodies." Handbook of Experimental Pharmacology 113 (1994): 49-101).

Another example of a humanized immunoglobulin is an immunoglobulin containing one or more immunoglobulin chains comprising a CDR of nonhuman origin (e.g., one or more CDRs derived from an antibody of nonhuman origin) and a framework region derived from a light and/or heavy chain of human origin (e.g., CDR-grafted antibodies with or without framework changes). Chimeric or CDR-grafted single chain antibodies are also encompassed by the term humanized immunoglobulin.

One means of humanization is called Composite Human Antibody™ technology and is described in detail in WO/2006/082406. Briefly, the CDR regions are left intact or essentially intact. The non-human framework is replaced with a composite humanized framework. The composite humanized framework may be a chimera of several fragments from different endogenous human framework alleles. In this manner, a composite framework may be produced that resembles the antibody's non-human framework more closely than any given endogenous human framework.

The humanized antibodies disclosed by this application include those with similar binding affinity to MAG-2, MAG-3, MAG-4, and MAG-5. They also include those with therapeutic effects that are similar to MAG-2, MAG-3, MAG-4, and MAG-5. Furthermore, they include those with CDRs similar to or identical to the CDRs of MAG-2, MAG-3, MAG-4, or MAG-5. Furthermore, they include those with a variable region similar to or identical to the variable region of MAG-2, MAG-3, MAG-4, or MAG-5.

This application also provides human antibodies. Human antibodies do not have any portions derived from non-human animals. In some cases, a human antibody is one that was produced in humanized micem resulting in antibodies that do not contain any mouse sequences. Human antibodies can also be produced *ex vivo*.

4.4 Linkers

It may be necessary in some instances to introduce an unstructured polypeptide linker region between a label and portions of the antibodies, antigen binding portions, or peptides. The linker can facilitate enhanced flexibility, and/or reduce steric hindrance between any two fragments. The linker can also facilitate the appropriate folding of each fragment to occur. The linker can be of natural origin, such as a sequence determined to exist in random coil between two domains of a protein. An exemplary linker sequence is the linker found

between the C-terminal and N-terminal domains of the RNA polymerase a subunit. Other examples of naturally occurring linkers include linkers found in the lcI and LexA proteins.

Within the linker, the amino acid sequence may be varied based on the preferred characteristics of the linker as determined empirically or as revealed by modeling. For instance, in addition to a desired length, modeling studies may show that side groups of certain amino acids may interfere with the biological activity, e.g. DNA binding or transcriptional activation, of the protein. Considerations in choosing a linker include flexibility of the linker, charge of the linker, and presence of some amino acids of the linker in the naturally-occurring subunits. The linker can also be designed such that residues in the linker contact DNA, thereby influencing binding affinity or specificity, or to interact with other proteins. For example, a linker may contain an amino acid sequence which can be recognized by a protease so that the activity of the chimeric protein could be regulated by cleavage. In some cases, particularly when it is necessary to span a longer distance between subunits or when the domains must be held in a particular configuration, the linker may optionally contain an additional folded domain.

In some embodiments it is preferable that the design of a linker involve an arrangement of domains which requires the linker to span a relatively short distance, preferably less than about 10 Angstroms (Å). However, in certain embodiments, depending, e.g., upon the selected domains and the configuration, the linker may span a distance of up to about 50 Angstroms.

Antibodies described herein can be made recombinantly. Linkers may be added to the nucleic acid sequences of the heavy and light chains to increase flexibility of the antibody. In the case of a scFv, the linkers are added to connect the Vh and Vl chains and the varying composition can effect solubility, proteolytic stability, flexibility, and folding. In one embodiment, a linker of has the amino sequence GSTSG (SEQ ID NO: 3). In another embodiment, a linker has the amino sequence GGSSRSS (SEQ ID NO: 4). Linkers are well-known in the art and can comprise varied amino acid residues depending on the flexibility needed in the resulting recombinant protein to allow for biological activity.

5: Pharmaceutical compositions

The disclosed antibodies and antigen-binding portions thereof can be used, for example, for immuno-based targeting of tumors and delivery of chemotoxic/radiologic agents. SCLC tumors can be localized and imaged using an antibody to the provasopressin protein. Thus, antibodies, antigen-binding portions thereof, and their derivatives could be

radiolabeled, conjugated to or used in conjunction with chemotoxic agents, or serve as an attractor for endogenous immune system cells to kill NRSA/GRSA-expressing tumors. Since all SCLC, breast cancer, and DCIS cells appear to express NRSA/GRSA, treatments that target this antigen would provide for significantly more potent therapy than currently available strategies for these diseases.

Cancer vaccines are based on tumor antigens, such as NRSA and GRSA. Because of its unique expression in certain cancers, vaccine strategies based on NRSA/GRSA, such as anti antibodies or utilizing antigenic motifs on the NRSA/GRSA structure, could be developed that would enable the initial prevention and/or recurrence of these diseases.

5.1 Labels

The antibodies, antigen-binding portions thereof, and peptides disclosed herein may be labeled. As used herein, "label" is used to mean a detectable label which is used to visualize the binding of an antibody to its target protein or receptor. Alternatively, antibodies, antigen-binding portions thereof, and peptides may be labeled with, for example, a radiolabel, an iron-related compound, a fluorescent label, or a toxin which would kill or inhibit proliferation of the cell to which it binds. Radiolabels and toxins are well known in the art.

Non-limiting examples of radiolabels include, for example, ^{32}P, ^{33}P, ^{43}K, ^{47}Sc, ^{52}Fe, ^{57}Co, ^{64}Cu, ^{67}Ga, ^{67}Cu, ^{68}Ga, ^{71}Ge, ^{75}Br, ^{76}Br, ^{77}Br, ^{77}As, ^{77}Br, $^{81}Rb/^{81M}Kr$, ^{87M}Sr, ^{90}Y, ^{97}Ru, ^{99}Tc, ^{100}Pd, ^{101}Rh, ^{103}Pb, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{111}In, ^{113}In, ^{119}Sb ^{121}Sn, ^{123}I, ^{125}I, ^{127}Cs, ^{128}Ba, ^{129}Cs, ^{131}I, ^{131}Cs, ^{143}Pr, ^{153}Sm, ^{161}Tb, ^{166}Ho, ^{169}Eu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{191}Os, ^{193}Pt, ^{194}Ir, ^{197}Hg, ^{199}Au, ^{203}Pb, ^{211}At, ^{212}Pb, ^{212}Bi and ^{213}Bi.

Non-limiting examples of toxins include, for example, ricin A chain (ricin toxin), Pseudomonas exotoxin (PE), diphtheria toxin (DT), Clostridium perfringens phospholipase C (PLC), bovine pancreatic ribonuclease (BPR), pokeweed antiviral protein (PAP), abrin, abrin A chain (abrin toxin), cobra venom factor (CVF), gelonin (GEL), saporin (SAP), modeccin, viscumin and volkensin.

Non-limiting examples of fluorescent labels include, for example, FITC, Texas Red, phycoerythrin (PE), cytochrome c, Cy3, and Cy5.

Non-limiting examples of iron-related compounds include, for example, magnetic iron-oxide particles, ferric or ferrous particles, Fe_2O_3, and Fe_3O_4. Iron-related compounds and methods of labeling antibodies and polypeptides can be found, for example, in U.S.

Patents 4,101,435 and 4,452,773, and U.S. published applications 20020064502 and 20020136693, all of which are hereby incorporated by reference in their entirety.

Additionally, other labels, such as biotin followed by streptavidin-alkaline phosphatase (AP), horseradish peroxidase (HRP) are contemplated.

Methodology for labeling proteins, such as antibodies, antigen binding portions thereof, and peptides are well known in the art. When the antibodies, antigen binding portions thereof, and peptides are labeled with a radiolabel or toxin, the antibodies, antigen binding portions thereof, and peptides can be prepared as pharmaceutical compositions which are useful for therapeutic treatment of patients exhibiting increased levels of provasopressin wherein the pharmaceutical compositions are administered to the patient in an effective amount.

In some embodiments, the antibodies, antigen binding portions, or peptides are coupled to a polymer or a functionalized polymer (e.g., a polymer conjugated to another molecule). Examples include water soluble polymers, such as polyglutamic acid or polyaspartic acid, conjugated to a drug such as a chemotherapeutic or antiangiogenic agent, including, for example, paclitaxel or docetaxel.

In certain embodiments, particularly where the cytotoxic moiety is chemically cross-linked to the antibody, antigen binding portion, or peptide moieties, the linkage is hydrolysable, e.g., such as may be provided by use of an amide or ester group in the linking moiety.

In certain embodiments, the subject antibodies, antigen binding portions thereof, or peptides can be coupled with an agent useful in imaging tumors. Such agents include: metals; metal chelators; lanthanides; lanthanide chelators; radiometals; radiometal chelators; positron-emitting nuclei; microbubbles (for ultrasound); liposomes; molecules microencapsulated in liposomes or nanosphere; monocrystalline iron oxide nanocompounds; magnetic resonance imaging contrast agents; light absorbing, reflecting and/or scattering agents; colloidal particles; fluorophores, such as near-infrared fluorophores. In many embodiments, such secondary functionality will be relatively large, e.g., at least 25 amu in size, and in many instances can be at least 50, 100 or 250 amu in size.

In certain preferred embodiments, the secondary functionality is a chelate moiety for chelating a metal, e.g., a chelator for a radiometal or paramagnetic ion. In preferred embodiments, it is a chelator for a radionuclide useful for radiotherapy or imaging procedures.

Radionuclides useful within the compositions and methods herein include gamma-emitters, positron-emitters, Auger electron-emitters, X-ray emitters and fluorescence-emitters, with beta- or alpha-emitters preferred for therapeutic use. Examples of radionuclides useful as toxins in radiation therapy include: ^{32}P, ^{33}P, ^{43}K, ^{47}Sc, ^{52}Fe, ^{57}Co, ^{64}Cu, ^{67}Ga, ^{67}Cu, ^{68}Ga, ^{71}Ge, ^{75}Br, ^{76}Br, ^{77}Br, ^{77}As, ^{77}Br, $^{81}Rb/^{81M}Kr$, ^{87M}Sr, ^{90}Y, ^{97}Ru, ^{99}Tc, ^{100}Pd, ^{101}Rh, ^{103}Pb, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{111}In, ^{113}In, ^{119}Sb ^{121}Sn, ^{123}I, ^{125}I, ^{127}Cs, ^{128}Ba, ^{129}Cs, ^{131}I, ^{131}Cs, ^{143}Pr, ^{153}Sm, ^{161}Tb, ^{166}Ho, ^{169}Eu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{191}Os, ^{193}Pt, ^{194}Ir, ^{197}Hg, ^{199}Au, ^{203}Pb, ^{211}At, ^{212}Pb, ^{212}Bi and ^{213}Bi. Appropriate therapeutic radionuclides include ^{188}Re, ^{186}Re, ^{203}Pb, ^{212}Pb, ^{212}Bi, ^{109}Pd, ^{64}Cu, ^{67}Cu, ^{90}Y, ^{125}I, ^{131}I, ^{77}Br, ^{211}At, ^{97}Ru, ^{105}Rh, ^{198}Au and ^{199}Ag, ^{166}Ho or ^{177}Lu. Conditions under which a chelator will coordinate a metal are described, for example, by Gansow et al., U.S. Pat. Nos. 4,831,175, 4,454,106 and 4,472,509. As used herein, "radionuclide" and "radiolabel" are interchangeable.

^{99m}Tc is one appropriate radioisotope for diagnostic applications, as it is readily available to all nuclear medicine departments, is inexpensive, gives minimal patient radiation doses, and has good nuclear imaging properties. It has a half-life of six hours which means that rapid targeting of a technetium-labeled antibody is desirable. Accordingly, in certain preferred embodiments, the modified antibodies, antigen binding portions, and peptides include a chelating agent for technium.

In still other embodiments, the secondary functionality can be a radiosensitizing agent, e.g., a moiety that increases the sensitivity of cells to radiation. Examples of radiosensitizing agents include nitroimidazoles, metronidazole and misonidazole (see: DeVita, V. T. Jr. in Harrison's Principles of Internal Medicine, p.68, McGraw-Hill Book Co., N.Y. 1983, which is incorporated herein by reference). The modified antibodies, antigen binding portions, and peptides that comprise a radiosensitizing agent as the active moiety are administered and localize at the target cell. Upon exposure of the individual to radiation, the radiosensitizing agent is "excited" and causes the death of the cell.

There are a wide range of moieties which can serve as chelators and which can be derivatized to the antibodies, antigen binding portions, and peptides described herein. For instance, the chelator can be a derivative of 1,4,7,10-tetraazacyclododecanetetraacetic acid (DOTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) and 1-p-Isothiocyanato-benzyl-methyl-diethylenetriaminepentaacetic acid (ITC-MX). These chelators typically have groups on the side chain by which the chelator can be used for attachment to subject antibodies, antigen binding portions, and peptides. Such

groups include, e.g., benzylisothiocyanate, by which the DOTA, DTPA or EDTA can be coupled to, e.g., an amine group.

In one embodiment, the chelate moiety is an "N_xS_y" chelate moiety. As defined herein, the term "N_xS_y chelates" includes bifunctional chelators that are capable of coordinately binding a metal or radiometal and, preferably, have N_2S_2 or N_3S cores. Exemplary N_xS_y chelates are described, e.g., in Fritzberg et al. (1988) PNAS 85:4024-29; and Weber et al. (1990) Bioconjugate Chem. 1:431-37; and in the references cited therein.

The Jacobsen *et al.* PCT application WO 98/12156 provides methods and compositions, i.e. synthetic libraries of binding moieties, for identifying compounds which bind to a metal atom. The approach described in that publication can be used to identify binding moieties which can subsequently be added to antibodies, antigen binding portions, and peptides to derive the modified antibodies, antigen binding portions, and peptides described herein.

A problem frequently encountered with the use of conjugated proteins in radiotherapeutic and radiodiagnostic applications is a potentially dangerous accumulation of the radiolabeled moiety fragments in the kidney. When the conjugate is formed using a acid- or base-labile linker, cleavage of the radioactive chelate from the protein can advantageously occur. If the chelate is of relatively low molecular weight, as most of the subject modified antibodies, antigen binding portions, and peptides are expected to be, it is not retained in the kidney and is excreted in the urine, thereby reducing the exposure of the kidney to radioactivity. However, in certain instances, it may be advantageous to utilize acid- or base-labile linkers in the subject ligands for the same reasons they have been used in labeled proteins.

Accordingly, certain of the subject labeled/modified antibodies, antigen binding portions thereof, and peptides can be synthesized, by standard methods known in the art, to provide reactive functional groups which can form acid-labile linkages with, e.g., a carbonyl group of the ligand. Examples of suitable acid-labile linkages include hydrazone and thiosemicarbazone functions. These are formed by reacting the oxidized carbohydrate with chelates bearing hydrazide, thiosemicarbazide, and thiocarbazide functions, respectively.

Alternatively, base-cleavable linkers, which have been used for the enhanced clearance of the radiolabel from the kidneys, can be used. See, for example, Weber et al. 1990 Bioconjug. Chem. 1:431. The coupling of a bifunctional chelate to antibodies, antigen binding portions, and peptides via a hydrazide linkage can incorporate base-sensitive ester moieties in a linker spacer arm. Such an ester-containing linker unit is exemplified by

ethylene glycolbis(succinimidyl succinate), (EGS, available from Pierce Chemical Co., Rockford, Ill.), which has two terminal N-hydroxysuccinimide (NHS) ester derivatives of two 1,4-dibutyric acid units, each of which are linked to a single ethylene glycol moiety by two alkyl esters. One NHS ester may be replaced with a suitable amine-containing BFC (for example 2-aminobenzyl DTPA), while the other NHS ester is reacted with a limiting amount of hydrazine. The resulting hyrazide is used for coupling to the antibodies, antigen binding portions, and peptides, forming an ligand-BFC linkage containing two alkyl ester functions. Such a conjugate is stable at physiological pH, but readily cleaved at basic pH.

Antibodies, antigen binding portions thereof, and peptides labeled by chelation can be subject to radiation-induced scission of the chelator and to loss of radioisotope by dissociation of the coordination complex. In some instances, metal dissociated from the complex can be re-complexed, providing more rapid clearance of non-specifically localized isotope and therefore less toxicity to non-target tissues. For example, chelator compounds such as EDTA or DTPA can be infused into patients to provide a pool of chelator to bind released radiometal and facilitate excretion of free radioisotope in the urine.

In still other embodiments, the antibodies, antigen binding portions, and peptides are coupled to a Boron addend, such as a carborane. For example, carboranes can be prepared with carboxyl functions on pendant side chains, as is well known in the art. Attachment of such carboranes to an amine functionality, e.g., as may be provided on the antibodies, antigen binding portions, and peptides, can be achieved by activation of the carboxyl groups of the carboranes and condensation with the amine group to produce the conjugate. Such modified antibodies, antigen binding portions, and peptides can be used for neutron capture therapy.

The subject antibodies, antigen-binding portions thereof, and peptides may also be modified with dyes, for example, useful in photodynamic therapy, and used in conjunction with appropriate non-ionizing radiation. The use of light and porphyrins is also contemplated and their use in cancer therapy has been reviewed by van den Bergh, Chemistry in Britain, 22: 430-437 (1986).

One embodiment includes antibodies, antigen binding portions thereof, and peptides labeled with a fluorescent label. Common fluorescent labels include, for example, FITC, PE, Texas Red, fluorescent nanodots, rhodamine, and the like. Techniques for labeling polypeptides and proteins are well-known in the art.

One embodiment includes antibodies, antigen binding portions thereof, and peptides labeled with a metal compound, such as iron, which can be used in MRI imaging and/or for treatment. Iron-containing compounds include both ferrous and ferric-containing

compounds, such as ferric-oxides. Specific examples include Fe_2O_3 and Fe_3O_4. Iron-containing compounds and methods of making iron-coupled antibodies and fragments thereof are described in U.S. Patents 4,101,435 and 4,452,773 and published U.S. patent applications 20020064502 and 20020136693, all of which are hereby incorporated by reference in their entireties.

5.2 Chemotherapeutic compounds

In certain embodiments, the subject antibodies, antigen binding portions thereof, and peptides can be covalently or non-covalently coupled to a cytotoxin, chemotherapeutic agent, or other cell proliferation inhibiting compound, in order to localize delivery of that agent to a tumor cell. For instance, the agent can be selected from the group consisting of alkylating agents, enzyme inhibitors, proliferation inhibitors, lytic agents, DNA or RNA synthesis inhibitors, membrane permeability modifiers, DNA intercalators, metabolites, dichloroethylsulfide derivatives, protein production inhibitors, ribosome inhibitors, inducers of apoptosis, and neurotoxins.

Chemotherapeutics useful as active moieties which when conjugated to antibodies, antigen binding portions, and peptides are specifically delivered to tumorigenic cells are typically, small chemical entities produced by chemical synthesis. Chemotherapeutics include cytotoxic and cytostatic drugs. Chemotherapeutics may include those which have other effects on cells such as reversal of the transformed state to a differentiated state or those which inhibit cell replication. Examples of known, useful cytotoxic agents are listed, for example, in Goodman et al., "The Pharmacological Basis of Therapeutics," Sixth Edition, A. G. Gilman et al, eds./Macmillan Publishing Co. New York, 1980. These include taxanes, such as paclitaxel (Taxol®) and docetaxel (Taxotere®); nitrogen mustards, such as mechlorethamine, cyclophosphamide, melphalan, uracil mustard and chlorambucil; ethylenimine derivatives, such as thiotepa; alkyl sulfonates, such as busulfan; nitrosoureas, such as carmustine, lomustine, semustine and streptozocin; triazenes, such as dacarbazine; folic acid analogs, such as methotrexate; pyrimidine analogs, such as fluorouracil, cytarabine and azaribine; purine analogs, such as mercaptopurine and thioguanine; vinca alkaloids, such as vinblastine and vincristine; antibiotics, such as dactinomycin, daunorubicin, doxorubicin, bleomycin, mithramycin and mitomycin; enzymes, such as L-asparaginase; platinum coordination complexes, such as cisplatin; substituted urea, such as hydroxyurea; methyl hydrazine derivatives, such as procarbazine; adrenocortical suppressants, such as mitotane; hormones and antagonists, such as adrenocortisteroids (prednisone), progestins

(hydroxyprogesterone caproate, medroprogesterone acetate and megestrol acetate), estrogens (diethylstilbestrol and ethinyl estradiol), antiestrogens (tamoxifen), and androgens (testosterone propionate and fluoxymesterone).

Drugs that interfere with intracellular protein synthesis can also be used; such drugs are known to those skilled in the art and include puromycin, cycloheximide, and ribonuclease.

Most of the chemotherapeutic agents currently in use in treating cancer possess functional groups that are amenable to chemical cross-linking directly with an amine or carboxyl group of an agent described herein. For example, free amino groups are available on methotrexate, doxorubicin, daunorubicin, cytosinarabinoside, bleomycin, gemcitabine, fludarabine, and cladribine while free carboxylic acid groups are available on methotrexate, melphalan, and chlorambucil. These functional groups, that is free amino and carboxylic acids, are targets for a variety of homobifunctional and heterobifunctional chemical cross-linking agents which can crosslink these drugs directly to a free amino group of an antibody, antigen binding portion thereof, or peptide.

Peptide and polypeptide toxins are also useful as active moieties, and the present disclosure specifically contemplates embodiments wherein the antibodies, antigen binding portions, and peptides are coupled to a toxin. In certain preferred embodiments, the antibodies, antigen binding portions, or peptides and the toxin are both polypeptides and are provided in the form of a fusion protein. Peptide and polypeptide toxins are generally complex toxic products of various organisms including bacteria, plants, etc. Examples of toxins include but are not limited to: ricin, ricin A chain (ricin toxin), Pseudomonas exotoxin (PE), diphtheria toxin (DT), Clostridium perfringens phospholipase C (PLC), bovine pancreatic ribonuclease (BPR), pokeweed antiviral protein (PAP), abrin, abrin A chain (abrin toxin), cobra venom factor (CVF), gelonin (GEL), saporin (SAP), modeccin, viscumin and volkensin.

Chemotherapeutic agents include chemotherapeutic drugs that are commercially available.

Merely to illustrate, the chemotherapeutic can be an inhibitor of chromatin function, a topoisomerase inhibitor, a microtubule inhibiting drug, a DNA damaging agent, an antimetabolite (such as folate antagonists, pyrimidine analogs, purine analogs, and sugar-modified analogs), a DNA synthesis inhibitor, a DNA interactive agent (such as an intercalating agent), and/or a DNA repair inhibitor.

Chemotherapeutic agents may be categorized by their mechanism of action into, for example, the following groups: anti-metabolites/anti-cancer agents, such as pyrimidine analogs (5-fluorouracil, floxuridine, capecitabine, gemcitabine and cytarabine) and purine analogs, folate antagonists and related inhibitors (mercaptopurine, thioguanine, pentostatin and 2-chlorodeoxyadenosine (cladribine)); antiproliferative/antimitotic agents including natural products such as vinca alkaloids (vinblastine, vincristine, and vinorelbine), microtubule disruptors such as taxane (paclitaxel, docetaxel), vincristin, vinblastin, nocodazole, epothilones and navelbine, epidipodophyllotoxins (etoposide, teniposide), DNA damaging agents (actinomycin, amsacrine, anthracyclines, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin, cyclophosphamide, cytoxan, dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethylmelamineoxaliplatin, iphosphamide, melphalan, merchlorehtamine, mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, teniposide, triethylenethiophosphoramide and etoposide (VP16)); antibiotics such as dactinomycin (actinomycin D), daunorubicin, doxorubicin (adriamycin), idarubicin, anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin) and mitomycin; enzymes (L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine); antiplatelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa), alkyl sulfonates-busulfan, nitrosoureas (carmustine (BCNU) and analogs, streptozocin), trazenes - dacarbazinine (DTIC); antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate); platinum coordination complexes (cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide; hormones, hormone analogs (estrogen, tamoxifen, goserelin, bicalutamide, nilutamide) and aromatase inhibitors (letrozole, anastrozole); anticoagulants (heparin, synthetic heparin salts and other inhibitors of thrombin); fibrinolytic agents (such as tissue plasminogen activator, streptokinase and urokinase), aspirin, dipyridamole, ticlopidine, clopidogrel, abciximab; antimigratory agents; antisecretory agents (breveldin); immunosuppressives (cyclosporine, tacrolimus (FK-506), sirolimus (rapamycin), azathioprine, mycophenolate mofetil); anti-angiogenic compounds (TNP-470, genistein) and growth factor inhibitors (vascular endothelial growth factor (VEGF) inhibitors, fibroblast growth factor (FGF) inhibitors); angiotensin receptor blocker; nitric oxide donors; anti-sense oligonucleotides; antibodies (trastuzumab, rituximab); cell cycle inhibitors and differentiation inducers (tretinoin); mTOR inhibitors, topoisomerase

inhibitors (doxorubicin (adriamycin), amsacrine, camptothecin, daunorubicin, dactinomycin, eniposide, epirubicin, etoposide, idarubicin, irinotecan (CPT-11) and mitoxantrone, topotecan, irinotecan), corticosteroids (cortisone, dexamethasone, hydrocortisone, methylpednisolone, prednisone, and prenisolone); growth factor signal transduction kinase inhibitors; mitochondrial dysfunction inducers, toxins such as Cholera toxin, ricin, Pseudomonas exotoxin, Bordetella pertussis adenylate cyclase toxin, or diphtheria toxin, and caspase activators; and chromatin disruptors. Preferred dosages of the chemotherapeutic agents are consistent with currently prescribed dosages.

5.3 Amino acid analogs

In certain embodiments, an antibody, antigen-binding portion thereof, or peptide as described herein may comprise one or more amino acid analogs, derivatives and congeners of any specific amino acid referred to herein, as well as C-terminal or N-terminal protected amino acid derivatives (e.g. modified with an N-terminal or C-terminal protecting group). For example, one may use an amino acid analog wherein a side chain is lengthened or shortened while still providing a carboxyl, amino or other reactive precursor functional group for cyclization, as well as amino acid analogs having variant side chains with appropriate functional groups). For instance, the subject compound can include an amino acid analog such as, for example, cyanoalanine, canavanine, djenkolic acid, norleucine, 3-phosphoserine, homoserine, dihydroxy-phenylalanine, 5-hydroxytryptophan, 1-methylhistidine, 3-methylhistidine, diaminopimelic acid, ornithine, or diaminobutyric acid. Other naturally occurring amino acid metabolites or precursors having side chains which are suitable herein will be recognized by those skilled in the art and may be used according to the disclosures herein.

5.4 Combinations of provasopressin antibodies and angiotensin antibodies

In some embodiments, the anti-provasopressin antibodies and antigen-binding portions thereof described herein may be co-administered with antibodies (or antigen-binding portions thereof) immunoreactive with the angiotensin II type-1 receptor. For example, polyclonal antibodies against the angiotensin II type-1 receptors are commercially available from Santa Cruz Biotechnology (Polyclonal AT_2, Santa Cruz, CA). Monoclonal antibodies, humanized antibodies, scFv antibodies, and antigen binding portions immunoreactive with the angiotensin II type-1 receptor are also contemplated.

5.5 Combinations of provasopressin antibodies and vasopressin V2 receptor antibodies

In some embodiments, the antibodies and antigen-binding portions thereof may be co-administered with antibodies immunoreactive with the vasopressin V2 receptor. Due to a splicing error, cancer cells often produce an abnormal form of the V2 receptor, which is a good tumor cell marker for targeted therapy.

5.6 Pharmaceutical additives

In certain embodiments, the subject antibodies, antigen-binding portions thereof, or peptides are formulated with a pharmaceutically acceptable carrier. The antibodies or antibody variants can be administered alone or as a component of a pharmaceutical formulation (composition). They may be formulated for administration in any convenient way for use in human or veterinary medicine. Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

The subject formulations include those suitable for oral, dietary, topical, parenteral (e.g., intravenous, intraarterial, intramuscular, subcutaneous injection), inhalation (e.g., intrabronchial, intranasal or oral inhalation, intranasal drops), rectal, and/or intravaginal administration. Other suitable methods of administration can also include rechargeable or biodegradable devices and slow release devices such as slow release polymeric devices. The pharmaceutical compositions can also be administered as part of a combinatorial therapy with other agents (either in the same formulation or in a separate formulation).

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect.

In certain embodiments, methods of preparing these formulations or compositions include combining another type of anti-cancer therapeutic agent and a carrier and, optionally, one or more accessory ingredients. In general, the formulations can be prepared with a liquid carrier, or a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Pharmaceutical compositions suitable for parenteral administration may comprise one or more antibodies, antigen-binding portions thereof, or peptides in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants, such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

Injectable depot forms are made by forming microencapsule matrices of one or more antibodies in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes. polymeric nanoparticles or microemulsions which are compatible with body tissue.

In certain embodiments, the pharmaceutical composition is administered by subcutaneous, intravenous, intranasal, parenteral, transdermal, intracheal, intravenous, intramuscular, intracranial, intrathecal or intravitreal injection; by oral administration, eye drops, pessary, or inhalation.

To achieve the desired effects, the antibodies, antigen-binding portions thereof, or peptides can be administered in a variety of unit dosage forms. The dose will vary according to the particular antibody or antigen-binding portion thereof. For example, different antibodies may have different masses and/or affinities, and thus require different dosage levels. Antibodies prepared as Fab' fragments will also require differing dosages than the equivalent intact immunoglobulins, as they are of considerably smaller mass than intact immunoglobulins, and thus require lower dosages to reach the same molar levels in the patient's blood.

The dose will also vary depending on the manner of administration, the particular symptoms of the patient being treated, the overall health, condition, size, and age of the patient, and the judgment of the prescribing physician. Dosage levels of the antibodies for human subjects are generally between about 1 mg per kg and about 100 mg per kg per patient per treatment, and preferably between about 5 mg per kg and about 50 mg per kg per patient per treatment. In terms of plasma concentrations, the antibody concentrations are preferably in the range from about 25 μg/ml to about 500 μg/ml.

A human dose may be determined readily if an effective dose in a non-human animal is known. For instance, Table 2 (below) may be used to convert non-human animal drug dosages to human dosages.

Table 2: Conversion of Animal Doses to Human Equivalent Doses (HED) Based on Body Surface Area (see e.g., Guidance for Industry Reviewers: Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, on the world wide web at fda.gov/ohrms/dockets/98fr/02d-0492-gdl0001-vol1.pdf).

		To convert animal dose in mg/kg to HED[a] in mg/kg, either:	
Species	To convert animal dose in mg/kg to dose in mg/m^2, multiple by km below:	Divide animal dose by:	Multiply animal dose by:
Human	37	--	--
Human Child (20 kg)	25	--	--
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16

Ferret	7	5.3	0.19
Guinea Pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Monkeys[b]	12	3.1	0.32
Marmoset	6	6.2	0.16
Squirrel Monkey	7	5.3	0.19
Baboon	20	1.8	0.54
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95

[a]Assumes 60 kg human. For species not listed or for weights outside the standard ranges, human equivalent dose can be calculated from the formula: HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{0.33}$.

[b]For example, cynomolgus, rhesus, stumptail.

Subject to the judgment of the physician, a typical therapeutic treatment includes a series of doses, which will usually be administered concurrently with the monitoring of clinical endpoints (such as tumor load or presence of provasopressin fragments in the bloodstream) with the dosage levels adjusted as needed to achieve the desired clinical outcome. Other protocols can, of course, be used if desired as determined by the physician.

Administration of the compositions described herein may be performed by an intravascular route, e.g., via intravenous infusion by injection. Other routes of administration may be used if desired. Formulations suitable for injection are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, Pa., 17th ed. (1985). Such formulations must be sterile and non-pyrogenic, and generally will include a pharmaceutically effective carrier, such as saline, buffered (e.g., phosphate buffered) saline, Hank's solution, Ringer's solution, dextrose/saline, glucose solutions, and the like. The formulations may contain pharmaceutically acceptable auxiliary substances as required, such as, tonicity adjusting agents, wetting agents, bactericidal agents, preservatives, stabilizers, and the like.

6: Diagnostic Methods

This application provides, *inter alia,* a rapid, inexpensive, sensitive, and specific method for: 1) early detection of cancer; and 2) identifying and localizing cancer, including

metastatic and/or recurrent disease, in patients. Cancers that may be imaged using the methods herein include breast cancer, ductal carcinoma *in situ*, and small cell lung cancer. The present disclosure also provides valuable tools for developing new immuno-targeted treatments, applicable to patients with cancer, that are effective with both primary disease, and with recurrent drug-resistant disease. In this respect it should be useful to all hospitals and physicians examining and treating patients with breast cancer, ductal carcinoma *in situ*, and small cell lung cancer. Detection kits are simple enough to be set up in any local hospital laboratory, and anti-provasopressin antibodies and antigen-binding portions thereof can readily be made available to all hospitals treating patients with breast cancer.

Also taught herein are methods for distinguishing DCIS from ADH using antibodies against VP or against VP-associated glycopeptide (VAG) or Copeptin; and by performing RT-PCR for VP mRNA on RNA from biopsied material and fixed material from stored tissue blocks.

A further discovery is the ability to distinguish between fibrocystic lesions and cancerous lesions. If a test sample is positive for provasopressin (as detected by, for example MAG-2, MAG-3, MAG-4, or MAG-5) and negative for the angiotensin II type-1 receptor, that patient likely has invasive breast cancer. If the test sample is positive for both provasopressin and the angiotensin II type-1 receptor, the patient likely has ductal carcinoma *in situ*. If a test sample is negative for provasopressin and is positive for the angiotensin II type-1 receptor, the patient likely has a fibrocystic lesion, such as atypical ductal hyperplasia. This method can form a powerful tool whereby health care providers can conclusively distinguish non-invasive fibrocystic tissue from cancerous lesions in test samples from patients suspected of having cancer. The relationship between angiotensin and cancer progression is described in De Paepe *et al.* (Histochem Cell Biol. 2001 Sep;116(3):247-54.), entitled "Growth stimulatory angiotensin II type-1 receptor is upregulated in breast hyperplasia and in situ carcinoma but not in invasive carcinoma."

6.1. *Methods of phenotyping breast and lung samples.*

Provasopressin is a marker of breast cancer cells and may be used to distinguish between cancerous cells and non-cancerous cells. In addition, angiotensin II type-1 is a known marker that may be used to distinguish between cells in various stages of breast cancer. Angiotensin tends to be overexpressed in early phases of breast cancer (e.g. fibrocystic lesions and DCIS) while provasopressin is overexpressed in later phases (e.g.

DCIS and invasive cancer). Thus, combinations of provasopressin staining and angiotensin staining may be used to "stage" a cancer.

This application discloses, *inter alia,* a method for distinguishing among fibrocystic lesions, ductal carcinoma *in situ*, and invasive breast cancer in a breast tissue biological samples from a patient suspected of having breast cancer, comprising: a) obtaining a breast tissue biological sample from a patient; b) contacting the breast tissue biological sample with an antibody or antigen-binding portion as described herein; d) contacting the breast tissue biological sample with an antibody or antigen-binding portion thereof immunoreactive with an angiotensin II type-1 receptor; e) determining if the cells of the rendered sample overexpress one or both of provasopressin and angiotensin II type-1 receptor compared to a non-cancerous control breast tissue; wherein if the breast tissue biological sample is positive for provasopressin and negative for the angiotensin II type-1 receptor, that patient is characterized as having invasive breast cancer.

One embodiment comprises a method of phenotyping tissue samples from patients suspected of having breast cancer, DCIS, or SCLC comprising the steps of (i) obtaining a biological sample from a patient, (ii) rendering the biological sample amenable to immunoassay, (iii) contacting the rendered sample with an anti-provasopressin antibody or antigen-binding portion thereof under conditions that allow for binding of the antibody or antigen-binding portion to provasopressin, and (iv) determining if the cells of the rendered sample overexpress provasopressin compared to a control tissue. In one embodiment, if provasopressin is over-expressed in the biological sample, the patient is likely to have invasive breast cancer, DCIS, or small cell lung cancer.

6.2. *Methods of phenotyping breast tissue samples from patients to distinguish fibrocystic and cancerous lesions.*

One embodiment comprises a diagnostic assay wherein fibrocystic tissue can be distinguished from cancerous lesions in breast biological samples. The method may comprise the steps of obtaining one or more biological sample(s) from a patient, rendering the biological sample amenable to immunoassay, contacting a rendered sample with an antibody, antigen-binding portion thereof, or peptide immunoreactive with provasopressin under conditions that allow for binding to provasopressin, contacting a rendered sample with an antibody immunoreactive with an angiotensin II type-1 receptor, and determining if the cells of the rendered samples express one or both of provasopressin and angiotensin II type-1 receptor.

Methods are known in the art for rendering a sample amenable to immunoassay. For example, a sample may be fixed, permeabolized, or blocked.

In one embodiment, determining if the cells of the rendered samples express one or both of provasopressin and angiotensin II type-1 receptor is accomplished wherein the antibodies and/or peptide are labeled with a detectable label. If the antibodies or antigen-binding portions thereof are unlabeled, a secondary antibody can be added to the rendered samples wherein the secondary antibody is labeled with a detectable label. Visualization of the detectable labels can be accomplished using immunohistochemistry methodology as described in the Examples of the instant specification.

In certain embodiments, the antibody or antigen-binding portion thereof which is immunoreactive with an angiotensin II type-1 receptor is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a humanized antibody, a single chain variable fragment, a Fab fragment, and a $F(ab')_2$ fragment.

6.3 *Methods of phenotyping blood samples for non-invasive or less-invasive cancer detection.*

Certain tumors may be detected by measuring blood levels of provasopressin components. This may be done with anti-provasopressin antibodies, antigen-binding portions thereof. Such antibodies include, for example, MAG-2, MAG-3, MAG-4, and MAG-5. These antibodies and antigen-binding portions thereof would be useful in the clinical screening assay to measure provasopressin (or fragments of provasopressin) levels in the blood of patients suspected of having certain tumors, or who have had those tumors in the past. This would be a useful, non-invasive or less invasive test to possibly justify further, more invasive tests/biopsies, and aid in monitoring recurrence of disease.

6.4 In vivo diagnostic techniques

This application additionally discloses a method of detecting a tumor in a patient suspected of having breast cancer, ductal carcinoma *in situ*, or small cell lung cancer comprising: a) administering a pharmaceutical composition comprising an antibody or antigen-binding portion described herein to the patient, b) detecting the label, and c) determining if the patient has cells that overexpress provasopressin compared to a control; wherein if the patient has cells that overexpress provasopressin, the patient is identified as likely having a tumor. Optionally, the method further comprises determining the location of the cells overexpressing provasopressin.

Furthermore, this application discloses a method for phenotyping breast tissue samples from patients to distinguish fibrocystic and cancerous lesions comprising: a) administering a pharmaceutical composition comprising an antibody or antigen-binding portion described herein and a first label to the patient (optionally, said antibody or antigen-binding portion are covalently or noncovalently bound to the first label), b) administering a pharmaceutical composition comprising a second label and an antibody or antigen-binding portion thereof immunoreactive with an angiotensin II type-1 receptor to the patient (optionally, said antibody or antigen-binding portion are covalently or noncovalently bound to the second label), c) detecting the first label and the second label, and d) determining if the patient has cells that overexpress one or both of provasopressin and angiotensin II type-1 receptor compared to a control, wherein fibrocystic lesions are identified as those that overexpress angiotensin II type-1 receptor but not provasopressin, and cancerous lesions are identified as those that overexpress provasopressin. The method may further comprise determining the location of the cells overexpressing provasopressin and/or angiotensin II type-1 receptor.

With the use of antibodies directed against various portions NRSA/GRSA, current imaging techniques, such as mammography, could be greatly enhanced, and new imaging protocols for diseases such as SCLC/breast cancer could be developed and effectively implemented for clinical use. These types of techniques would be especially useful for the detection of metastatic disease. These techniques could also assist a surgeon preparing to surgically remove a tumor or tumors, by identifying the location of the tumor or tumors.

One of skill in the art will appreciate that there are certain cells (some neurons, for example) that normally express provasopressin in a healthy patient. Clearly, binding of an anti-provasopressin antibody to cells that are expressing normal, non-cancerous amounts of provasopressin is not indicative of cancer. Rather, overexpression of provasopressin is indicative of cancer. One of skill in the art will understand that overexpressing refers to expression at higher levels than that seen in a non-cancerous cell of the same tissue type as the cancerous cell.

7. *Therapeutic Methods*

7.1 *Methods of therapy with anti-provasopressin antibodies*

Herein is disclosed, *inter alia*, a method of treating a condition selected from the group consisting of breast cancer, breast ductal carcinoma *in situ*, and small cell lung cancer, comprising administering an effective amount of the pharmaceutical compositions described

herein to a subject. These pharmaceutical compositions include anti-provasopressin antibodies, and antigen-binding portions thereof. Such anti-provasopressin antibodies and portions thereof include those that bind to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody produced by the hybridomas described herein. Also included are those that that bind to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody produced by one of the hybridomas described herein. Also included are those that bind provasopressin, wherein the antibody or antigen-binding portion thereof is capable of inhibiting growth of a xenograft breast or lung tumor in a mouse. Specific examples include MAG-2, MAG-3, MAG-4, and MAG-5.

In certain embodiments, an antibody (or antigen-binding portion thereof) suitable for therapeutic use is a humanized antibody or antigen-binding portion thereof. The antibodies and antigen-binding portions may be humanized by any means known in the art, such as CDR grafting or generation of a chimeric antibody. Specific point mutations may also be made during the humanization process.

Antibodies can be used for targeting provasopressin (NRSA/GRSA) on tumors. Previous work indicates that SCLC tumors can be localized and imaged in humans using radiolabeled antibody directed against the neurophysin portion of provasopressin. Subsequent studies show that polyclonal antibodies, monoclonal antibodies, and antibody Fab fragments directed against different regions of the provasopressin protein bind specifically to cultured SCLC and breast cancer cells, as well as to human tumor sections, but not to tissue that is devoid of tumor. We describe polyclonal and monoclonal antibodies, and their Fab fragment derivatives, to NRSA/GRSA. They can bind to cultured human cancer cells and human cancer tissue. Since the NRSA/GRSA is not typically found in normal cells, it is anticipated that it can serve as an excellent target for tumor localization in the early detection, diagnosis, and treatment of cancers that express the vasopressin gene. NRSA/GRSA also provides for a attractive candidate for use in vaccine development strategies for the prevention of those cancers that express the vasopressin gene.

Single-chain antibodies fragments and small binding peptides can be used for targeting provasopressin (NRSA/GRSA) on tumors. We also disclose single-chain variable region fragments (scFv) antibodies that bind to NRSA/GRSA. The use of such smaller molecules will provide added benefits (tumor penetration, ease of manufacturing) for *in vivo* tumor targeting. Although the expression of vasopressin by various tumors has been known

for some time, targeting the precursor protein with antibodies and antibody fragments is a novel concept.

An effective therapeutic response is achieved when the patient experiences partial or total alleviation or reduction of signs or symptoms of illness, and specifically includes, without limitation, prolongation of survival. In certain embodiments, a therapeutic response is achieved when the patient's symptoms remain static, and the tumor burden does not increase.

7.2 Methods of combination therapy

Herein is disclosed, *inter alia*, a method of treating a condition selected from the group consisting of breast cancer, breast ductal carcinoma *in situ*, and small cell lung cancer, comprising administering an effective amount of a pharmaceutical composition comprising an anti-provasopressin antibody (such as MAG-2, MAG-3, MAG-4, or MAG-5), and antigen-binding portions thereof to a subject, and further comprising administration of an effective amount of a second pharmaceutical composition. The second pharmaceutical compositions may comprise a chemotherapeutic agent, and optionally comprising epinephrine. The pharmaceutical compositions may be administered concomitantly, in a single formulation, or in separate formulations. Alternatively, the second pharmaceutical composition may comprise one or more of dexamethasone, IBMX, and 8-bromoadenosine 3',5'-cyclic monophosphate (8br-cAMP). In one embodiment, the second pharmaceutical composition comprises each of dexamethasone, IBMX, and 8-bromoadenosine 3',5'-cyclic monophosphate (8br-cAMP). Alternatively, the second pharmaceutical composition may comprise one or more of IBMX and forskolin. In one embodiment, the second pharmaceutical composition comprises both of IBMX and forskolin.

Subject antibodies, antigen binding portions, and peptides can be used in combination therapy with chemotherapeutic agents. This application also discloses that the subject antibodies and antigen-binding portions thereof in combination with a cocktail of chemotherapeutic agents is effective at inhibiting proliferation of cancerous cells when administered in an effective amount.

One of ordinary skill in the art could prepare a formulation of any of the chemotherapeutic agents as described above to be administered with a preparation of one of the disclosed antibodies or antigen-binding portions thereof to treat a breast cancer, DCIS, or SCLC.

8. Kits

One embodiment includes for a kit useful for screening a biological sample for invasive breast cancer or small cell lung cancer comprising a preparation of an antibody, antigen binding portion, or peptide immunoreactive with provasopressin or fragment of provasopressin (such as SEQ ID No. 1 or 2), wherein the antibody immunoreactive with provasopressin indicates the presence of carcinogenic, invasive breast cancer or small cell lung cancer tissue. If the biological sample is positive for provasopressin, an invasive form of cancer, such as breast cancer or small cell lung cancer, as been identified

The kit can further comprise a preparation of an antibody, or an antigen binding portion thereof, immunoreactive with an angiotensin II type-1 receptor. If the biological sample is negative for the angiotensin II type-1 receptor, a sample has been confirmed as invasive breast cancer.

One embodiment includes for a kit useful for screening a biological sample for breast ductal carcinoma *in situ* comprising a preparation of an antibody, antigen binding portion, or peptide immunoreactive with provasopressin or fragment of provasopressin (such as SEQ ID No. 1 or 2), and a preparation of an antibody immunoreactive with an angiotensin II type-1 receptor.

In some aspects, if the biological sample is positive for both provasopressin and the angiotensin II type-1 receptor, the biological sample contains carcinogenic breast ductal carcinoma *in situ* cells.

One embodiment includes for a kit useful for screening a biological sample for atypical ductal hyperplasia comprising a preparation of an antibody, antigen binding portion thereof, or peptide, immunoreactive with provasopressin or fragment of provasopressin (such as SEQ ID No. 1 or 2), and a preparation of an antibody immunoreactive with an angiotensin II type-1 receptor.

In some embodiments, if the biological sample is negative for provasopressin and positive for the angiotensin II type-1 receptor, the biological sample contains hyperplastic cells.

One embodiment of the kits include preparations of antibodies or antigen binding portions immunoreactive with provasopressin or fragment of provasopressin (such as SEQ ID No. 1 or 2). Antibodies and antigen binding portions can be lyophilized or in solution. Additionally, the preparations can contain stabilizers to increase the shelf-life of the kits, e.g., bovine serum albumin (BSA). Wherein the antibodies and antigen binding portions are

lyophilized, the kit can contain further preparations of solutions to reconstitute the preparations. Acceptable solutions are well known in the art, e.g., PBS.

Kits can further include the components for an ELISA assay for measuring provasopressin and fragments thereof as tumor markers in body fluids. Samples to be tested in this application include, for example, plasma, urine, lymph, breast ductal secretions and products thereof. Alternatively, preparations of the kits may be used in immunoassays, such as immunohistochemistry to test patient tissue biopsy sections.

The compositions of the kit can be formulated in single or multiple units for either a single test or multiple tests. In certain embodiments, the preparations of the kit are free of pyrogens. The kits can include instructions for the use of the compositions in an immunoassay.

The practice of the present invention will employ, where appropriate and unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, virology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are described in the literature. See, for example, Molecular Cloning: A Laboratory Manual, 3rd Ed., ed. by Sambrook and Russell (Cold Spring Harbor Laboratory Press: 2001); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Using Antibodies, Second Edition by Harlow and Lane, Cold Spring Harbor Press, New York, 1999; Current Protocols in Cell Biology, ed. by Bonifacino, Dasso, Lippincott-Schwartz, Harford, and Yamada, John Wiley and Sons, Inc., New York, 1999.

EXAMPLES

Example 1: Production and characterization of anti-provasopressin antibodies

Antibodies were raised against an 18 amino acid fragment (named C18-Pro9) found in the C-terminal end of provasopressin (SEQ ID No. 5: Val-Gln-Leu-Ala-Gly-Ala-Pro-Glu-Pro-Phe-Glu-Pro-Ala-Gln-Pro-Asp-Ala-Tyr). Five monoclonal antibodies were obtained. These antibodies were named MAG-1, MAG-2, MAG-3, MAG-4, and MAG-5. The production of MAG-1 was described in WO04/006860. MAG-2 through -5 were produced using similar methods to MAG-1.

To study the conformation requirement for C18 (VAG) peptide binding, the C18 peptide was modified with a substitution of a proline with a histidine residue at position 9. It is well established that a proline residue will provide a more rigid structure than a histidine

residue in the same position of a peptide. The amino acid sequences of both the native C18-Pro9 and the modified C18-His9 are listed in Figure 1. Boris, a polyclonal antibody preparation reactive with vasopressin-human glycopeptide (VAG) (described in North *et al.* Breast Cancer Res Treat. 1995 Jun;34(3):229-35) and MAG-1 and MAG-1 related monoclonal antibodies (Table 1) are then used to test their abilities to bind *directly* to C18-Pro9 and C18-his9.

SEQ ID No. 5:

C18-Pro9: Val-Gln-Leu-Ala-Gly-Ala-Pro-Glu-***Pro***-Phe-Glu-Pro-Ala-Gln-Pro-Asp-Ala-Tyr

SEQ ID No. 6:

C18-His9: Val-Gln-Leu-Ala-Gly-Ala-Pro-Glu-***His***-Phe-Glu-Pro-Ala-Gln-Pro-Asp-Ala-Tyr

Table 1
List of MAG Monoclonal Antibodies

Hybridoma	***Monoclonal Antibody***
8D12A4	MAG-1
8D12A12	MAG-2
4E5A5	MAG-3
4E5A11	MAG-4
8C10A11	MAG-5

As indicated in Figure 1, Boris binds equally well to both the native and modified C18 peptides. In contrast, all MAGs bind only to the native C18-Pro9, but not the modified C18-His9. The histidine substitution completely abolishes the ability of MAG-1 and the MAG-1 related monoclonal antibodies to bind directly to the modified peptide. This suggests that there is a conformation requirement for the binding of MAG-1 to the native C18 peptide since MAG-1 does not bind to the modified ^{125}I-C18-His9 directly. More importantly, these results indicate that there may be 2 or more epitopes on the native C18-Pro9. This is important because the binding to the MAG-1 epitope is associated with the MAG-1 in vivo activities.

Example 2: Competitive Binding Inhibition

To further analyze the binding characteristics of Boris, MAG-1 and the MAG-1 related monoclonal antibodies to C18-Pro9, competitive inhibition studies were conducted with the unlabelled C18-Pro9 and C18-His9. The results of Boris and MAG-1 binding to the C18-Pro9 are summarized in Figure 2A and 2B. As indicated in Figure 2A, there is a 7.1 fold decrease in the concentration of C18-Pro9 to inhibit Boris' binding to ^{125}I-C18-Pro9 when compared to that of C18-His9. In contrast, there is almost a 50-fold difference in concentration with MAG-1 binding to C18-Pro9. These results emphasize the epitope difference between Boris and the MAG-1 related antibodies.

Similarly, all the other MAG-1 related monoclonal antibodies behave like MAG-1 in the competitive inhibition of binding studies. As indicated in Figures 3A and 3B, MAG-1 and MAG-2 have different binding characteristics to the native C18-Pro9 from the rest of MAG monoclonal antibodies tested. A much higher concentration of C18-His9 is required to inhibit MAG-1 and MAG-2 from binding to the radioactive C18-Pro9.

The dissociation constants of Boris and the monoclonal antibodies binding to C18-Pro9 and C18-His9 are summarized in Table 3.

Table 3

Dissociation Constants of Binding to C18-Pro9 and C18-His9

Antibody Preparation	***Kd (M)***		***Ratio (Kd1/Kd2)***
	C18-Pro9 (Kd1)	***C18-His9(Kd2)***	
Boris	3.86×10^{-10}	2.73×10^{-9}	7.1
MAG-1	1.50×10^{-8}	6.38×10^{-7}	51.5
MAG-2	1.50×10^{-8}	6.38×10^{-7}	51.5
MAG-3	2.70×10^{-8}	1.85×10^{-7}	6.7
MAG-4	3.25×10^{-9}	1.85×10^{-7}	56.9
MAG-5	3.41×10^{-9}	1.25×10^{-7}	36.7

Based on these studies, it is apparent that MAG-1 and MAG-2 are the most highly related, while MAG-3, MAG-4 and MAG-5 are separate and distinct from MAG-1 and MAG-2. In addition the binding data shows that, MAG-1, MAG-2, MAG-4, and MAG-5 are highly related to each other. However, all of MAG-1, MAG-2, MAG-3, MAG-4, and MAG-5 have very similar binding specificities overall.

Methods: Direct Binding Assay

About 6 - 8,000 cpm of an $^{I-125}$peptide (pg) in 50 µl of 0.05 M phosphate buffer containing 1 mM EDTA, 0.2M cysteine and 1.25 mg/ml BSA were added to each of 1.5 ml of eppendorf tubes. Each antibody preparation, in a volume of 50 µl, tested for binding to the $^{I-125}$peptide was added to each tube. 0.05 M phosphate buffer was added to each tube to a final volume of 300 µl. The solution in each tube was then vortexed for about 20 sec and the tubes were incubated at room temperature overnight. After incubation, 50 µl of Y-globins (16 mg/ml) and 500 µl of 25% polyethylene glycol (PEG) were added to each tube, which was vortexed for 20 sec. The tubes were then centrifuged at 12,000 rpm for 4 min in a cold room. The supernatant in each tube was removed from each tube and the radioactivity in each pellet was then determined in a gamma counter.

Methods: Competitive Inhibition of Binding Assay

To determine the ability of each tested peptide to inhibit the binding of an antibody preparation to bind to the $^{I-125}$peptide, various concentrations of the tested inhibiting peptide, ranging from 0.2 ng to 10 µg, were added to the tubes, each of which contained the $^{I-125}$peptide and the antibody preparation as described in the Direct Binding Assay. Additional 0.05 M phosphate buffer was then added to a final volume of 300 µl. The solution in each tube was vortexed for about 20 sec and the tubes were incubated at room temperature overnight. After incubation, 50 µl of Y-globins (16 mg/ml) and 500 µl of 25% polyethylene glycol (PEG) were added to each tube, which was vortexed for 20 sec. The tubes were then centrifuged at 12,000 rpm for 4 min in a cold room. The supernatant was removed from each tube and the radioactivity in each pellet was then determined in a gamma counter.

Example 3. Epitope Mapping of C18-Pro9

To map the epitopes on the native C18-Pro9, four peptides are prepared for the competitive inhibition of binding studies to ^{125}I-C18-Pro9. The amino acid sequences of these four (4) peptides are listed below.

Amino Acid Sequence of Peptides

SEQ ID No. 5:

C18-Pro9: Val-Gln-Leu-Ala-Gly-Ala-Pro-Glu-***Pro***-Phe-Glu-Pro-Ala-Gln-Pro-Asp-Ala-Tyr

SEQ ID No. 7:

Peptide 1: Ala-Pro-Glu-***Pro***-Phe-Glu-Pro-Ala-Gln-Pro-Asp-Ala-Tyr

SEQ ID No. 8:

Peptide 2: Ala-Pro-Glu-***Pro***-Phe-Glu-Pro-Ala-Gln-Pro-Asp

SEQ ID No. 1:

Peptide 3: Ala-Pro-Glu-***Pro***-Phe-Glu-Pro-Ala

SEQ ID No. 2:

Peptide 4: Val-Gln-Leu-Ala-Gly-Ala-Pro-Glu-***Pro***-Phe

Peptides 1, 2 and 3 inhibit the binding of Boris, a polyclonal antibody preparation, to the native C18-Pro9 (Figure 4A and 4B). The inhibition curves of the three peptides are similar, but the results suggest the additional amino acid residues at the carboxyl end of Peptide 3 have little effect, if any, on the conformation of the peptide in reducing its ability to inhibit the binding of Boris to the C18-Pro9. On the other hand, these three peptides do not inhibit the binding of MAG-1 to the C18-Pro9. These results suggest that Boris binds an on Peptide 3. More importantly, MAG-1 does not bind to this epitope. Similarly, MAG-2, MAG-3, MAG-4, and MAG-5 exhibit the same characteristics in binding to the three peptides as MAG-1 (Data not shown).

On the other hand, Peptide 4 inhibits the binding of MAG-1 to the C18-Pro9 peptide, indicating that the MAG-1 binding epitope is conferred by the Peptide 4 (Figure 5). Similarly, the binding of MAG-1 related monoclonal antibodies to the C18-Pro9 is inhibited by the Peptide 4. Unexpectedly, Boris does not show significant binding to Peptide 4.

The dissociation and association constants of the binding of Boris and MAG monoclonal antibodies to C18-Pro9, C18-His9, Peptide 1, Peptide 2, Peptide 3 and Peptide 4 are summarized in Tables 4 and 5. It should be noted that the sensitivity of the competitive inhibition of binding assay is less than 1.61×10^{-6} M and 6.21×10^{5} M^{-1} for the dissociation constant and association constant respectively.

Table 4

Dissociation Constants (Kd)

Peptide	*Kd (M)*					
	Boris	***MAG-1***	***MAG-2***	***MAG-3***	***MAG-4***	***MAG-5***
C18-Pro9	3.86×10^{-10}	1.50×10^{-8}	1.17×10^{-8}	2.7×10^{-8}	3.25×10^{-9}	3.41×10^{-9}
C18-His9	2.73×10^{-9}	6.38×10^{-7}	6.03×10^{-7}	1.85×10^{-7}	1.85×10^{-7}	1.25×10^{-7}
Peptide 1	7.53×10^{-8}	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$
Peptide 2	1.74×10^{-7}	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$
Peptide 3	2.30×10^{-7}	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$	$> 1.61 \times 10^{-6}$
Peptide 4	1.76×10^{-6}	3.77×10^{-8}	6.81×10^{-8}	4.76×10^{-8}	1.87×10^{-8}	1.54×10^{-8}

Table 5
Association Constants (Ka)

Peptide	*Ka (M^{-1})*					
	Boris	***MAG-1***	***MAG-2***	***MAG-3***	***MAG-4***	***MAG-5***
C18-Pro9	2.59×10^{9}	7.02×10^{7}	8.55×10^{7}	3.70×10^{7}	3.08×10^{8}	2.93×10^{8}
C18-His9	3.66×10^{8}	1.58×10^{6}	1.66×10^{6}	5.41×10^{6}	5.41×10^{6}	8.00×10^{6}
Peptide 1 (13)	1.32×10^{7}	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$
Peptide 2 (11)	5.74×10^{6}	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$
Peptide 3 (8)	4.34×10^{6}	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$	$< 6.21 \times 10^{5}$
Peptide 4 (10)	5.68×10^{5}	2.65×10^{7}	1.4×10^{7}	2.10×10^{7}	5.34×10^{7}	6.49×10^{7}

Based on the competitive inhibition studies, we have detected two separate and distinct epitopes on the C18-Pro9 peptide (Table 5). The conformation of epitope 1 is conferred by Peptide 3, while that of the epitope 2 is conferred by Peptide 4. Interestingly, Boris binds only to the epitope 1 and does not bind to the epitope 2 and vice versa for MAG-1. Similarly, all the other MAG-1 related monoclonal antibodies (that is, MAG-2, MAG-3, MAG-4, and MAG-5) bind to the epitope 2 but not to the epitope 1. This is unexpected because one might have predicted that Boris, a polyclonal antibody preparation, would bind to all epitopes on the C18 peptide.

MAG-1 does not bind to the modified C18-His9 directly. Therefore, the substitution of the proline residue with a histidine residue at position 9 of the C18 peptide abolishes the ability of MAG-1 to directly bind to epitope 2. It is known that a proline residue provides a rigid structure in a peptide. The substitution of Pro9 with His9 has changed the conformation required for direct binding of MAG monoclonal antibodies to bind to the epitope 2. It is also important to note that the last 5 amino acid residues at the carboxyl terminal of the C18 peptide has no effect on the conformation of epitope 1, required for binding.

Table 5

Epitope of C18-Pro9

Epitope	*Epitope Amino Acid Sequence*	*Binding*	
		Boris	*MAG-1*
Epitope 1	Ala-Pro-Glu-***Pro***-Phe-Glu-Pro-Ala	++	-
Epitope 2	Val-Gln-Leu-Ala-Gly-Ala-Pro-Glu-***Pro***-Phe	-	+++

Example 4: In vivo imaging

A xenograft nude mouse system was used to assay the ability of MAG-1 Fab fragments to image SCLC tumors *in vivo*. MAG-1 Fab fragments conjugated to ^{99}Tc were administered to mice bearing SCLC H345 tumors. Figure 6 (left panels) depicts the tumor bearing mouse as well as the images obtained from *in vivo* imaging of the tumor. Next, the tumor was excised from the mouse and imaging was repeated (right panels). An essentially identical image was obtained *in vivo* and *ex vivo*, demonstrating the efficacy of anti-provasopressin antibodies and antigen-binding portions thereof in imaging tumors. ^{99}Tc signal was detected with an eZ-SCOPE gamma-camera.

Tumor xenografts in nude mice were imaged by MRI (Figure 7). Both T1 images (left two columns) and T2 images (right two columns) were collected. The top row of images in Figure 7 portrays tumors in the absence of imaging reagents. The center row of images in Figure 7 portrays tumors treated with gadodiamide, a contrast agent. The bottom row of images in Figure 7 portray tumors treated with Gd-DTPA-Fab-MAG-1 as a contrast agent. Contrast is markedly improved in the tumors treated with Fab MAG-1, indicating the efficacy of anti-provasopressin antibodies and antigen-binding portions thereof in imaging tumors.

Breast tumors were also imaged *in vivo* using MAG-1 Fab fragments. Mice bearing MDA-MB231 tumors were injected IP with 300 μCi / 2 μg protein of ^{99}Tc-DTPA-Fab-MAG-1. After the last imaging, the animals were sacrificed and the tumors and various organs were harvested after perfusion of the animals with PBS. The radioactivity in each tissue was then determined using a gamma counter. Distinct images of breast cancer tumors were obtained at 24 h after injection of ^{99}Tc-DTPA-Fab-MAG-1 as shown in Figure 8. It should be noted that there are two adjacent MCF-7 tumors growing in this animal and both concentrated radiolabel. In general, for both MCF-7 and MDA-MB 231 tumors, the signal to noise ratio is $\geq$7.5, peaking at 13 for new growth.

Methods: Production of Modified forms of Antibody

The Fab fragments of MAG-1 (from 1.0 gm Ab Stock) were prepared by the action of solid-phase ficin (Pierce Chemical Co.) on a reduced form of the antibody, modification by reaction with CHX-A"-DTPA (Ma, D., McDevitt, M.R., Barendswaard, E., Lai, L., Curcio, M.J., Pellegrini, V., Brechbiel, M.W., Scheinberg, D.A. *Radioimmunotherapy for model B cell malignancies using ^{90} Y-labeled anti-CD19 and anti-CD20 monoclonal antibodies.* Leukemia 16:60-66, 2002), and isolation by VAG18-Agarose chromatography. Typically, for DTPA-CHX-A"-Fab fragment preparation, MAG-1 (or MOPC21, an irrelevant monoclonal antibody of the same subclass that does not bind to VAG) is digested at 37°C for 4 hours in "digestion buffer" that contains reducing agent (20 mM cysteine). The digestion mixture is eluted with "binding buffer", and the intact IgG and Fc fragments removed by Protein A chromatography. In these studies The recommendations of the manufacturer [Macrocyclics] were followed, essentially. DTPA-CHX-A''-Fab fragment is then prepared by overnight complexing in metal-free HEPES/150 mM NaCl buffer at ambient temperature using a 50-fold excess of commercially available CHX-A''-DPTA The product is then bound to Antigen-Agarose, eluted with 0.2 M acetic acid/0.5 M NaCl into five volumes of 0.2 M phosphate buffer, pH 7.5, dialyzed against PBS, and tested for purity by SDS-electrophoresis. The binding of DTPA- CHX-A"-Fab form, and an assessment of its affinity for antigen is determined by a previously published method using ^{125}I-antigen (North, W.G., F.T.J. LaRochelle, J. Melton, and R.C. Mills. *Isolation and partial characterization of two neurophysins: Their use in the development of specific radioimmunoassays.* J. Clin. Endocrinol. 51:884-891, 1980; North, W.G., F.T.J. LaRochelle, and G.R. Hardy *Radioimmunoassays for individual rat neurophysins.* J. Endocrinol. 96:373-386, 1983.) Similarly, chelated derivatives of intact antibodies, DTPA-CHX-A"-MAG-1 and DPTA-CHX-A"-MOPC21 (irrelevant antibody used as control) are prepared using the same protocol. An antibody can be modified using either DPTA-anhydride or CHX-A as a chelating agent.

Methods: Labelling of DTPA- CHX-A''-Fab and DPTA- CHX-A''-MAG-1 with ^{99}Technetium and ^{90}Yttrium

Pertechnate ($^{99}TcO_4$) is obtained in PBS from a generator and reduced to a form that will bind to chelation group with stannous chloride under alkaline conditions (pH 8.0) for 30 minutes. This is then mixed with DPTA- CHX-A''-Fab (of MAG-1 or MOPC21) in phosphate buffer at 37C for 30 minutes. This procedure has been found to bind >20 mCi/mg

DPTA- CHX-A''-MAG-1 and >20 mCi/0.5 mg of DPTA- CHX-A''-Fab as measured by thin-layer chromatography and molecular sieve chromatography. ^{90}Yttrium chloride (10 mCi/50 μl) from NEN is mixed with DPTA- CHX-A''-MAG-1 (or MOPC21) in phosphate buffer for one hour at 37C to generate a labeled product with >95% of the isotope bound to antibody with a specific activity of approximately 12 mCi/mg DTPA-MAG-1 (or MOPC21) as measured by TLC, second antibody precipitation, and MS chromatography.

Methods: Radioimaging of Tumors in Mice with ^{99}Technetium-DPTA-CHX-A''-Fab MAG-1

Cultured breast cancer cells of MCF-7 and MDA-Mb231 ($2x10^7$-10^8) are injected subcutaneously into athymic nude mice, that in the case of estrogen-responsive cells (e.g. MCF-7) have been primed several days beforehand by *s.c.* implantation of an estrogen pellet. When the cells have produced palpable masses of 0.5 to 1.5 cm (2-4 weeks), animals receive an intravenous injection of ^{99}Tc- DTPA- CHX-A''-Fab MAG-1. Gamma-camera scintigraphy of mice is performed at 10 minutes, 1 hour, 4 hours, and 24 hours post-injection. Images is obtained at a distance of 4 cm from the animals with a portable gamma camera (Anzai Medical Co., Ltd, Japan). For some animals a comparison of the amounts of radiolabel in normal tissues (skin, brain, liver, kidneys, gut, adrenals, spleen, ovaries/testes, uterus/vas deferens, skeletal muscle, spine, sub-arachnoid glands) with those in identified tumor is made at different times post-injection. ^{99m}Tc- labeling of tissues and tumor is expressed as percentage of the injected dose per gram (net weight) of tissue normalized to 25 grams body weight as a function of time. For these studies up to 200 μCi/25 g body weight is typically used (but for many studies, 50 μCi/25 g body weight is used).

Example 5: In vivo therapeutic use of MAG-1

Figure 8 shows the effect of ^{90}Y-MAG-1 (left panel) or MAG-1 on the growth rate of mice bearing SCLC H345 tumors. On the graphs of Figure 9, the arrow marks the point at which treatment was stopped. As is shown in Figure 9, tumor growth is markedly slowed upon administration of unlabeled MAG-1 (right panel) or ^{90}Y-MAG-1 (left panel) compared to control mice.

Next, the effect of non-radiolabeled MAG-1 on breast tumor growth was determined *in vivo*. Intact MAG-1 and intact ^{90}Y-MAG-1 were administered to test animals. ^{90}Y-labelling of the antibody was performed using known methods as described above. Percent

tumor growth was monitored over the course of treatment by recording the volume of the tumor, measured with a caliper.

Tumor-bearing mice were produced as follows. One estrogen-dependent (MCF-7) and one estrogen-independent (MDA-MB231) cell line ($2x10^7$cells per mouse) were grown as subcutaneous tumor xenografts for 14 days (tumor diameter of 0.5 to 0.75 cm) in nu/nu mice. On Day 14, animals receiving these cancer cells were divided into five groups (N=8 per group) according to the Table 6 provided below. The control group 1 received no additional treatment (4x saline vehicle only). Groups 2 received an intraperitoneal dose comprising 4 x 50 μCi of ^{90}Y-DTPA-MAG-1 in a total of 50 μg of MAG-1 per 25 gm body weight every other day while Group 3 receives 4 x 50 μg per animal of unlabeled MAG-1 intraperitoneally every other day. Group 4 and 5 receives labeled or unlabeled MOPC-21 IgG_1 (Bioexpress, Inc.) with the same treatment schedule as Group 2 and 3 respectively. MOPC21 is an irrelevant monoclonal antibody that does not bind to the C18 (VAG) peptide. Thereafter, tumor size was measured in blinded fashion daily. Body weight was measured concurrently on a daily basis to evaluate toxicity. At the end of the study, liver and kidney and remaining tumor were fixed, blocked, sectioned, and stained, for a review of organ and tumor toxicity. A total of 80 animals were used for these studies: 40 mice in five groups (Table 6) with tumors derived from MCF-7 and 40 mice with tumors derived from MDA-MB231.

Table 6: Animal studies

Group	# of Animals	Treatment			
		^{90}Y-MAG-1	Unlabeled MAG-1	^{90}Y-MOPC21 (non-specific)	Unlabeled MOPC21
1	8	-	-	-	-
2	8	+	-	-	-
3	8	-	+	-	-
4	8	-	-	+	-
5	8	-	-	-	+

As shown in Figure 10, treatment with ^{90}Y-MAG-1 essentially halts growth of estrogen dependent and estrogen-independent tumors, even at time points after the cessation of treatment (marked with an arrow in Figures 10 and 11). ^{90}Y labeled MOPC21 does not produce the same effect, indicating that the therapeutic benefit is not simply a result of administering radioactivity to the animal. Furthermore, as illustrated in Figure 11, even MAG-1 that is not radiolabeled causes a striking reduction in tumor growth, and in fact

causes the tumor to shrink. Again, a MOPC21 control antibody shows that these effects are specific to a provasopressin-binding therapeutic.

Next, the percent MCF-7 tumor growth was monitored under different dosage regimens. MCF-7 is an estrogen-dependent tumor. In one group of mice, MAG-1 was administered 4 times (once every other day for 8 days). In the second group, MAG-1 was administered 16 times (once per day for 16 days). As shown in Figure 12B, while the control (untreated) group showed significant tumor growth, tumor growth was essentially halted in mice that received MAG-1 4 times. Administration of MAG-1 16 times yielded even more drastic results, with the tumor shrinking to less than half its original size over the course of three days and remaining small for the duration of treatment. Figure 12A shows that MAG-1 had a similar effect on MDA-MB-231 tumors (which are estrogen-independent). In Figures 12A and 12B, the arrow indicates the end of treatment with the 4 times dosing regimen. Thus, provasopressin-binding antibodies are effective therapy for estrogen dependent and estrogen-independent tumors.

Figures 12A and 12B also illustrate the size of tumors after cessation of treatment. Tumor size was measured beginning on the day of treatment (timepoint zero on the x axis of Figures 12A and 12B). As illustrated by Figures 12A and 12B, after approximately 5 days tumor growth resumed in the mice treated 4 times with MAG-1. However, in mice treated 16 times, the tumor did not grow over the course of the experiment. Thus, MAG-1 need not be administered indefinitely in order to achieve a sustained decrease in tumor size.

Example 6: In vivo therapeutic use of a Fab fragment of MAG-1

The ability of Fab-MAG-1 to treat breast cancer MDA-MB231 xenografts was determined in nude mice. The tumor bearing mice were treated i.p. with 20 ug of Fab fragment of MAG-1 daily per animal for 15 days. Tumor growth was determined daily during treatment. In addition, the tumor volumes were also monitored for another 20 days after the last treatment. Similar to the treatment with intact MAG-1, Fab-MAG-1 inhibited tumor growth. In addition, the treatment shrank the tumors substantially and there was not tumor growth 20 days after treatment (Figure 13).

The remarkable efficacy of the Fab fragment alone in reducing tumor load shows that the constant region of the antibody is not essential for therapeutic activity. This observation suggests that the mere binding of the Fab fragment to the cancer cells is sufficient for therapeutic activity, without involvement of the subject's immune system. Thus, a

provasopressin antibody may be an effective therapy even in immunocompromised patients, such as patients receiving chemotherapy and elderly patients. A provasopressin antibody may be an effective therapy against tumors which form an immunosuppressive microenvironment, for example by inducing local hypoxia. In contrast to provasopressin antibody therapeutics, many previously proposed forms of cancer immunotherapy rely on the host's immune system to clear cancer cells. For example, alemtuzumab (which binds to CD52) promotes complement fixation and antibody-dependent cell-mediated cytotoxicity.

Example 7: Biodistribution of ^{99}Tc-DTPA-Fab-MAG-1

Radiolabeled Fab fragment of MAG-1 was injected into tumor-bearing mice, and its biodistribution was analyzed. Both MCF-7 and MDA-MB231 tumors accumulated approximately 1% of the injected ^{99}Tc-DTPA-Fab-MAG-1 24 hr following injection of the imaging agent (Figure 14). After this time period most of the radiolabel was cleared so that <10% remained in the animals, with the highest activities, as would be expected, remaining in the kidneys (~4.5% and ~3.0% initial dose per gram of tissue for mice bearing MCF-7 and MDA-MB-231 tumors respectively). It is important to note that < 0.1% of activity was found in the brain of animals despite neurons in the hypothalamus highly expressing the vasopressin gene.

Histological evaluation of normal tissues such as the liver and kidneys revealed there was no damage to these tissues by extensive treatment with MAG-1 (Figure 15A-D), while for tumors there was massive necrosis such that <75% of the tumor cells were destroyed (Figure 16A-D).

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

POLYPEPTIDE SEQUENCES

SEQ ID No. 1 (Peptide 3): Ala-Pro-Glu-Pro-Phe-Glu-Pro-Ala

SEQ ID No. 2 (Peptide 4): Val-Gln-Leu-Ala-Gly-Ala-Pro-Glu-Pro-Phe

SEQ ID No. 3: GSTSG

SEQ ID No. 4: GGSSRSS

SEQ ID No. 5 (C18-Pro9): Val-Gln-Leu-Ala-Gly-Ala-Pro-Glu-***Pro***-Phe-Glu-Pro-Ala-Gln-Pro- Asp-Ala-Tyr

SEQ ID No. 6 (C18-His9): Val-Gln-Leu-Ala-Gly-Ala-Pro-Glu-***His***-Phe-Glu-Pro-Ala-Gln-Pro- Asp-Ala-Tyr

SEQ ID No. 7: (Peptide 1) Ala-Pro-Glu-***Pro***-Phe-Glu-Pro-Ala-Gln-Pro-Asp-Ala-Tyr

SEQ ID No. 8: (Peptide 2) Ala-Pro-Glu-***Pro***-Phe-Glu-Pro-Ala-Gln-Pro-Asp

We claim:

1. A monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody produced by a hybridoma having ATCC Number _____.

2. A monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody produced by a hybridoma having ATCC Number _____.

3. A monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody produced by a hybridoma having ATCC Number _____.

4. A monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein the antibody or antigen-binding portion thereof has a binding affinity corresponding to the binding affinity of a monoclonal antibody produced by a hybridoma having ATCC Number _____.

5. A monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody produced by a hybridoma having ATCC Number _____.

6. A monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody produced by a hybridoma having ATCC Number _____.

7. A monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody produced by a hybridoma having ATCC Number _____.

8. A monoclonal antibody or antigen-binding portion thereof that binds to provasopressin, wherein binding to the provasopressin is inhibited by a monoclonal antibody produced by a hybridoma having ATCC Number _____.

9. The monoclonal antibody or antigen-binding portion thereof of any of claims 5-8, wherein the monoclonal antibody or antigen-binding portion thereof binds to provasopressin with K_d that is 90% or less the K_d of MAG-1.

10. A monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody produced by a hybridoma having ATCC Number _____.

11. A monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody produced by a hybridoma having ATCC Number _____.

12. A monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody produced by a hybridoma having ATCC Number _____.

13. A monoclonal antibody or antigen-binding portion thereof, which is not MAG-1, that binds to provasopressin, wherein the antibody or antigen-binding portion thereof binds to an epitope that is bound by a monoclonal antibody produced by a hybridoma having ATCC Number _____.

14. A monoclonal antibody or antigen-binding portion thereof that binds provasopressin, wherein the antibody or antigen-binding portion thereof is capable of inhibiting growth of a xenograft breast or lung tumor in a mouse.

15. The monoclonal antibody or antigen-binding portion thereof of claim 14, wherein the breast or lung tumor is SCLC H345, MCF, 7, or MDA-MB231.

16. The monoclonal antibody or antigen-binding portion thereof of claim 14, wherein the antibody or antigen-binding portion thereof is capable of inhibiting growth of a xenograft breast or lung tumor in a mouse for 16 days.

17. The monoclonal antibody or antigen-binding portion thereof of claim 1, 5, 10, or 14, wherein the antibody or antigen-binding portion thereof is a humanized antibody and comprises CDRs that are identical to the CDRs of the antibody produced by the hybridoma having ATCC Number _____.

18. The monoclonal antibody or antigen-binding portion thereof of claim 2, 6, 11, or 14 wherein the antibody or antigen-binding portion thereof is a humanized antibody and comprises CDRs that are identical to the CDRs of the antibody produced by the hybridoma having ATCC Number _____.

19. The monoclonal antibody or antigen-binding portion thereof of claim 3, 7, 12, or 14 wherein the antibody or antigen-binding portion thereof is a humanized antibody and

comprises CDRs that are identical to the CDRs of the antibody produced by the hybridoma having ATCC Number _____.

20. The monoclonal antibody or antigen-binding portion thereof of claim 4, 8, 13, or 14 wherein the antibody or antigen-binding portion thereof is a humanized antibody and comprises CDRs that are identical to the CDRs of the antibody produced by the hybridoma having ATCC Number _____.

21. The monoclonal antibody or antigen-binding portion thereof of any of claims 1, 5, 10, or 14, which is a monoclonal antibody produced by the hybridoma having ATCC Number _____.

22. The monoclonal antibody or antigen-binding portion thereof of claim 2, 6, 11, or 14, which is a monoclonal antibody produced by the hybridoma having ATCC Number _____.

23. The monoclonal antibody or antigen-binding portion thereof of claim 3, 7, 12, or 14, which is a monoclonal antibody produced by the hybridoma having ATCC Number _____.

24. The monoclonal antibody or antigen-binding portion thereof of claim 4, 8, 13, or 14, which is a monoclonal antibody produced by the hybridoma having ATCC Number _____.

25. The monoclonal antibody or antigen-binding portion of any of claims 1-24, which is selected from the group consisting of a single chain variable fragment (scFv), or a F(ab')2 fragment.

26. The monoclonal antibody or antigen-binding portion of any of claims 1-24, which is a Fab fragment.

27. The monoclonal antibody or antigen-binding portion of any of claims 1-24, wherein the antibody is an IgG antibody.

28. The monoclonal antibody of antigen-binding portion of claim 27, wherein the antibody is an IgG1 or IgG2 antibody.

29. The monoclonal antibody or antigen-binding portion of any of claims 1-24, which binds, with a K_d of less than $3x10^{-8}$ M, to a peptide comprising SEQ ID No. 2.

30. The monoclonal antibody or antigen-binding portion of any of claims 1-24, which binds, with a K_d of less than $2x10^{-9}$ M, to a peptide comprising SEQ ID No. 2.

31. The monoclonal antibody or antigen-binding portion of any of claims 1-16, which is a humanized antibody, a human antibody, or a chimeric antibody.

32. The monoclonal antibody or antigen-binding portion of any of claims 1-24, which binds to provasopressin with a K_d of equal to or less than the K_d of one of: MAG-2 (produced by the hybridoma having ATCC Number _____), MAG-3 (produced by the hybridoma having ATCC Number _____), MAG-4 (produced by the hybridoma having ATCC Number _____), and MAG-5 (produced by the hybridoma having ATCC Number _____).

33. The monoclonal antibody or antigen-binding portion of any of claims 1-24, further comprising a label.

34. The monoclonal antibody or antigen-binding portion of claim 33, wherein the label is selected from the group consisting of a fluorescent label, a radiolabel, a toxin, a metal compound, and biotin.

35. The monoclonal antibody or antigen-binding portion of claim 34, wherein the fluorescent label is selected from the group consisting of Texas Red, phycoerythrin (PE), cytochrome c, and fluorescent isothiocyanate (FITC).

36. The monoclonal antibody or antigen-binding portion of claim 34, wherein the radiolabel is selected from the group consisting of ^{32}P, ^{33}P, ^{43}K, ^{47}Sc, ^{52}Fe, ^{57}Co, ^{64}Cu, ^{67}Ga, ^{67}Cu, ^{68}Ga, ^{71}Ge, ^{75}Br, ^{76}Br, ^{77}Br, ^{77}As, ^{77}Br, $^{81}Rb/^{81M}Kr$, ^{87M}Sr, ^{90}Y, ^{97}Ru, ^{99}Tc, ^{100}Pd, ^{101}Rh, ^{103}Pb, ^{105}Rh, ^{109}Pd, ^{111}Ag, ^{111}In, ^{113}In, ^{119}Sb ^{121}Sn, ^{123}I, ^{125}I, ^{127}Cs, ^{128}Ba, ^{129}Cs, ^{131}I, ^{131}Cs, ^{143}Pr, ^{153}Sm, ^{161}Tb, ^{166}Ho, ^{169}Eu, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189}Re, ^{191}Os, ^{193}Pt, ^{194}Ir, ^{197}Hg, ^{199}Au, ^{203}Pb, ^{211}At, ^{212}Pb, ^{212}Bi and ^{213}Bi.

37. The monoclonal antibody or antigen-binding portion of claim 34, wherein the toxin is selected from the group consisting of ricin, ricin A chain (ricin toxin), Pseudomonas exotoxin (PE), diphtheria toxin (DT), *Clostridium perfringens* phospholipase C (PLC), bovine pancreatic ribonuclease (BPR), pokeweed antiviral protein (PAP), abrin, abrin A chain (abrin toxin), cobra venom factor (CVF), gelonin (GEL), saporin (SAP), modeccin, viscumin and volkensin.

38. A peptide consisting of SEQ ID No. 1.

39. A fusion protein comprising: (1) the peptide of claim 38; and (2) a second peptide, wherein the two peptides are operably linked in a manner that does not produce a wild-type provasopressin amino acid sequence.

40. The fusion protein of claim 39, wherein the second peptide is a purification tag peptide.

41. A peptide consisting of SEQ ID No. 2.

42. A fusion protein comprising: (1) the peptide of claim 41; and (2) a second peptide, wherein the two peptides are operably linked in a manner that does not produce a wild-type provasopressin amino acid sequence.

43. The fusion protein of claim 42, wherein the second peptide is a purification tag peptide.

44. A composition comprising the peptide or fusion protein of any of claims 38-43, bound to a solid substrate.

45. A nucleic acid that encodes the peptide or fusion protein of any of claims 38-43.

46. A nucleic acid construct comprising the nucleic acid of claim 45 operably linked to a second nucleic acid, wherein the two nucleic acids are operably linked in a manner that does not produce a wild-type provasopressin nucleic acid sequence.

47 The nucleic acid or nucleic acid construct of claim 45 or 46, which is a DNA or an RNA.

48. A nucleic acid encoding the antibody or antigen-binding portion of any of claims 1-37.

49. A composition comprising the antibody or antigen binding portion of any one of claims 1-37.

50. A pharmaceutical composition comprising the antibody or antigen binding portion thereof of any one of claims 1-37.

51. The pharmaceutical composition of claim 50, further comprising a pharmaceutically acceptable carrier.

52. The pharmaceutical composition of claim 50, which is formulated for systemic delivery.

53. The pharmaceutical composition of claim 52, which is formulated for injection.

54. The pharmaceutical composition of claim 50, which is formulated for local delivery.

55. A kit useful for screening a biological sample for breast cancer, ductal carcinoma in situ, or small cell lung cancer, comprising an antibody or antigen-binding portion of any of claims 1-37.

56. A method of determining whether a subject has any one of the following cancers: breast cancer, ductal carcinoma *in situ,* or small cell lung cancer, comprising:

a) obtaining a biological sample from a patient; and

b) contacting the sample with the antibody or antigen-binding portion of any of claims 1-37;

wherein binding is indicative that the subject is likely to have breast cancer, ductal carcinoma *in situ,* or small cell lung cancer.

57. The method of claim 56, further comprising a step of contacting the antibody or antigen binding portion to a control tissue, and comparing the binding of the antibody or antigen-binding portion to the sample to the binding of the antibody or antigen-binding portion to a control tissue.

58. The method of claim 56, wherein the biological sample is from a patient suspected of having breast cancer.

59. The method of claim 56, wherein the biological sample is from patients suspected of having ductal carcinoma *in situ.*

60. The method of claim 56, wherein the biological sample is from patients suspected of having small cell lung cancer.

61. A method for distinguishing among fibrocystic lesions, ductal carcinoma *in situ*, and invasive breast cancer in a breast tissue biological samples from a patient suspected of having breast cancer, comprising:

a) obtaining a breast tissue biological sample from a patient;

b) contacting the breast tissue biological sample with an antibody or antigen-binding portion of any of claims 1-37;

d) contacting the breast tissue biological sample with an antibody or antigen-binding portion thereof immunoreactive with an angiotensin II type-1 receptor;

e) determining if the cells of the sample overexpress one or both of provasopressin and angiotensin II type-1 receptor;

wherein if the breast tissue biological sample is positive for provasopressin and negative for the angiotensin II type-1 receptor, that patient is characterized as having invasive breast cancer.

62. The method of claim 61, wherein if the breast tissue biological sample is positive for both provasopressin and the angiotensin II type-1 receptor, the patient likely has ductal carcinoma *in situ.*

63. The method of claim 61, wherein if the breast tissue biological sample is negative for provasopressin and is positive for the angiotensin II type-1 receptor, the patient is characterized as having a fibrocystic lesion.

64. The method of claim 61, wherein the antibody or antigen-binding portion thereof which is immunoreactive with an angiotensin II type-1 receptor is selected from the

group consisting of a polyclonal antibody, a monoclonal antibody, a humanized antibody, a single chain variable fragment, a Fab fragment, and a $F(ab')_2$ fragment.

65. A kit for distinguishing among fibrocystic lesions, ductal carcinoma *in situ*, and invasive breast cancer in a breast tissue biological samples from a patient suspected of having breast cancer, comprising:

a) the antibody or antigen-binding portion thereof of any of claims 1-37; and

b) an antibody or antigen-binding portion thereof immunoreactive with an angiotensin II type-1 receptor.

66. The kit of claim 65, wherein the antibody or antigen-binding portion thereof which is immunoreactive with an angiotensin II type-1 receptor is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a humanized antibody, a single chain variable fragment, a Fab fragment, and a $F(ab')_2$ fragment.

67. The kit of claim 65, wherein the biological sample is from a patient suspected of having a condition selected from the group consisting of breast cancer, breast ductal carcinoma *in situ*, and atypical ductal hyperplasia.

68. A method of treating a condition selected from the group consisting of breast cancer, breast ductal carcinoma *in situ*, and small cell lung cancer, comprising administering an effective amount of the pharmaceutical composition of any of claims 50-54 to a subject.

69. The method of claim 68, wherein the subject is immunocompromised.

70. The method of claim 69, wherein the subject is a chemotherapy patient, an elderly patient, a patient having a hereditary immunodeficiency, an HIV positive patient, a patient with leukemia, a patient with lymphoma, or a patient with multiple myeloma.

71. The method of claim 68, further comprising administering an effective amount of a pharmaceutical composition comprising a chemotherapeutic agent.

72. The method of claim 71, wherein the pharmaceutical composition further comprises epinephrine.

73. The method of claim 71, wherein the pharmaceutical compositions are administered concomitantly.

74. The method of claim 71, wherein the pharmaceutical compositions are administered in a single formulation.

75. The method of claim 71, wherein the pharmaceutical compositions are administered as separate formulations.

76. The method of claim 68, further comprising administering an effective amount of a pharmaceutical composition comprising dexamethasone, IBMX, and 8-bromoadenosine 3',5'-cyclic monophosphate (8br-cAMP).

77. The method of claim 68, further comprising administering an effective amount of a pharmaceutical composition comprising IBMX and forskolin.

78. A pharmaceutical composition of any of claims 50-54, for the treatment of a cancer selected from the group consisting of breast cancer, small cell lung cancer, and ductal carcinoma *in situ*.

79. The pharmaceutical composition of claim 78, further comprising IBMX and forskolin.

80. The pharmaceutical composition of claim 78, further comprising dexamethasone, IBMX, and 8-bromoadenosine 3',5'-cyclic monophosphate (8br-cAMP).

81. A method of detecting a tumor *in vivo* in a patient suspected of having breast cancer, ductal carcinoma *in situ*, or small cell lung cancer, comprising:

a) administering the antibody or antigen-binding portion of any of claims 1-37 to the patient; and

b) detecting the label;

wherein detection of the label is indicative that the patient has a tumor.

82. The method of claim 81, further comprising determining the location of the cells overexpressing provasopressin.

83. A method for distinguishing among fibrocystic lesions, ductal carcinoma *in situ*, and invasive breast cancer *in vivo* in a patient suspected of having breast cancer, comprising:

a) administering a composition comprising the antibody or antigen-binding portion of any of claims 1-37 and a first label to the patient;

b) administering a composition comprising a second label and an antibody or antigen-binding portion thereof immunoreactive with an angiotensin II type-1 receptor to the patient; and

c) detecting the first label and the second label;

wherein fibrocystic lesions are identified as those that overexpress angiotensin II type-1 receptor but not provasopressin, and cancerous lesions are identified as those that overexpress provasopressin.

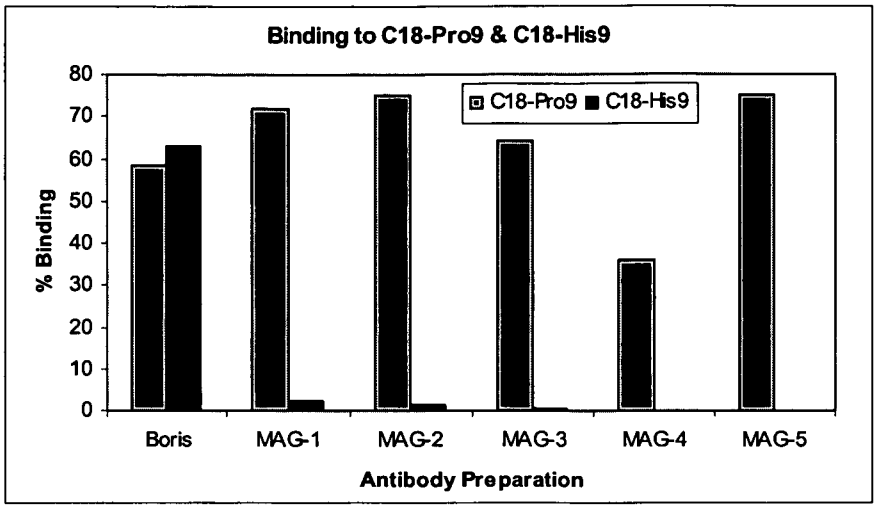
84. The method of claim 83, further comprising determining the location of the cells overexpressing provasopressin and/or angiotensin II type-1 receptor.

85 Use of the antibody or antigen binding portion thereof of any one of claims 1-37 in

the manufacture of a medicament for the treatment of a cancer selected from the group consisting of breast cancer, small cell lung cancer, and ductal carcinoma *in situ*.

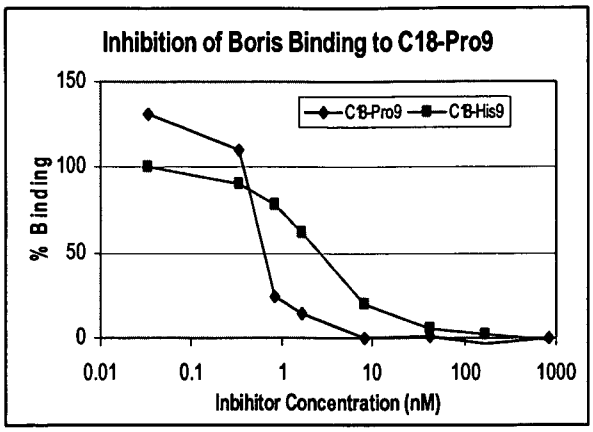
86. Use of the pharmaceutical composition of any of claims 50-54 in the manufacture of a medicament for the treatment of a cancer selected from the group consisting of breast cancer, small cell lung cancer, and ductal carcinoma *in situ*.

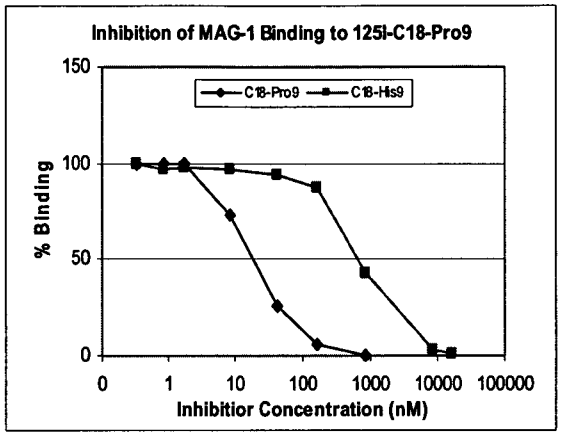
Figure 1


Binding to C18-Pro9 & C18-His9
C18-Pro9
C18-His9
% Binding
80
70
60
50
40
30
20
10
0
Boris
MAG-1
MAG-2
MAG-3
MAG-4
MAG-5
Antibody Preparation

5

Figure 2

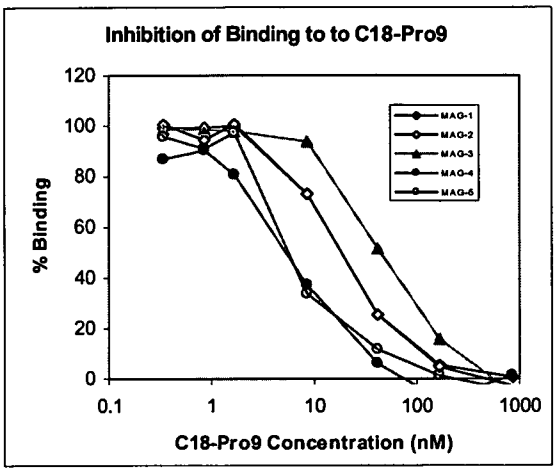




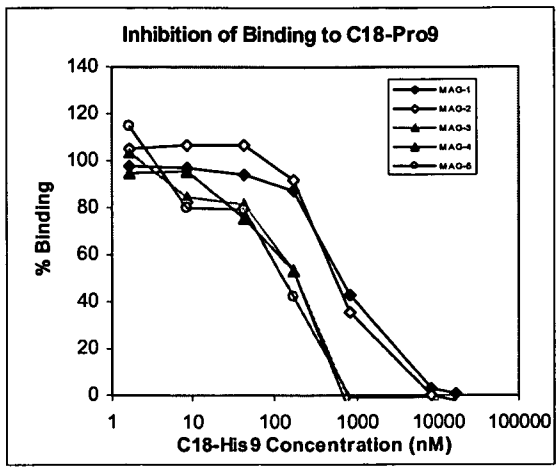
2A 2B

5

Figure 3



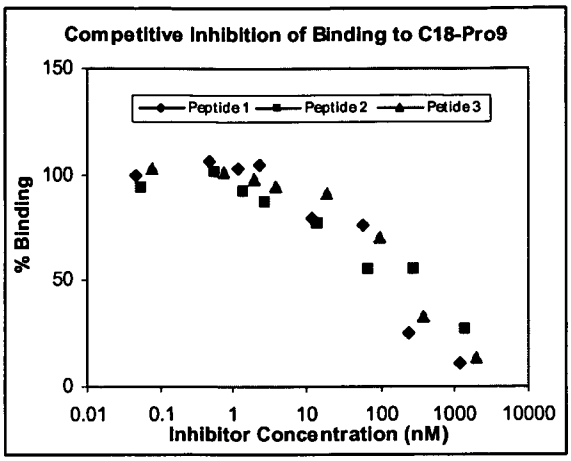
3A

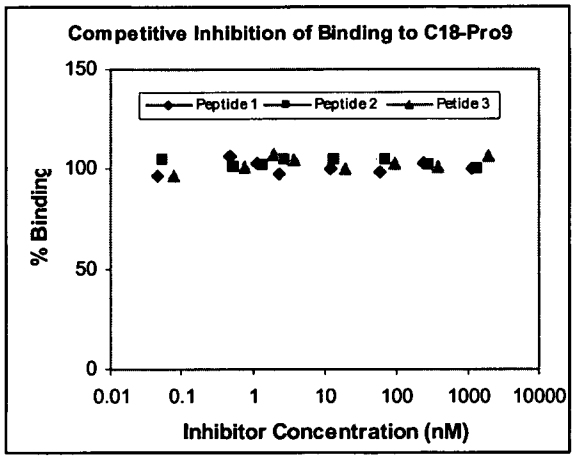


3B

5

Figure 4





4A 4B

5

Figure 5

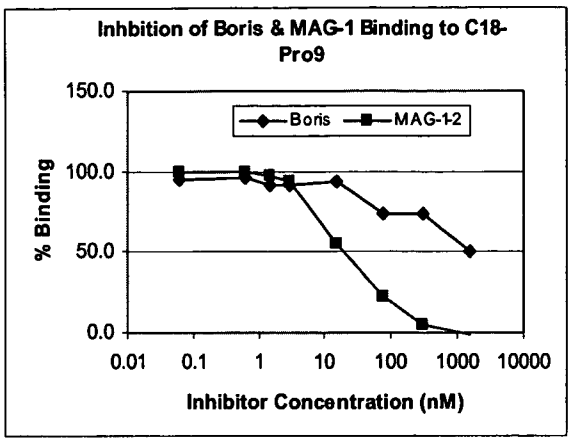
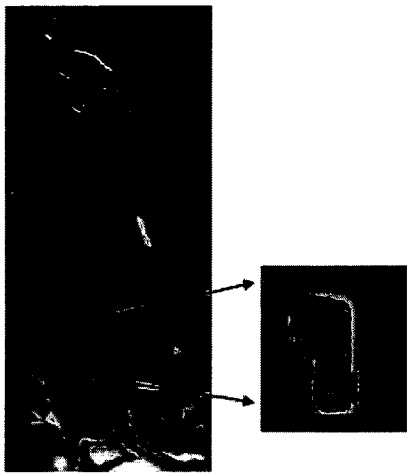
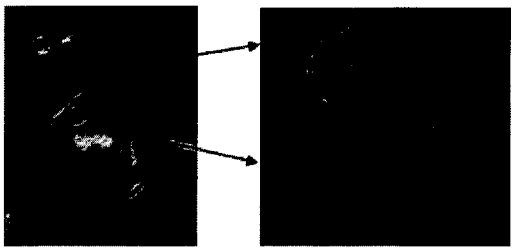

Inhbition of Boris & MAG-1 Binding to C18-Pro9
150.0
100.0
50.0
0.0
% Binding
Boris
MAG-1-2
0.01
0.1
1
10
100
1000
10000
Inhibitor Concentration (nM)

Figure 6



Tumor bearing Nude Mouse



Excised Tumor

Figure 7

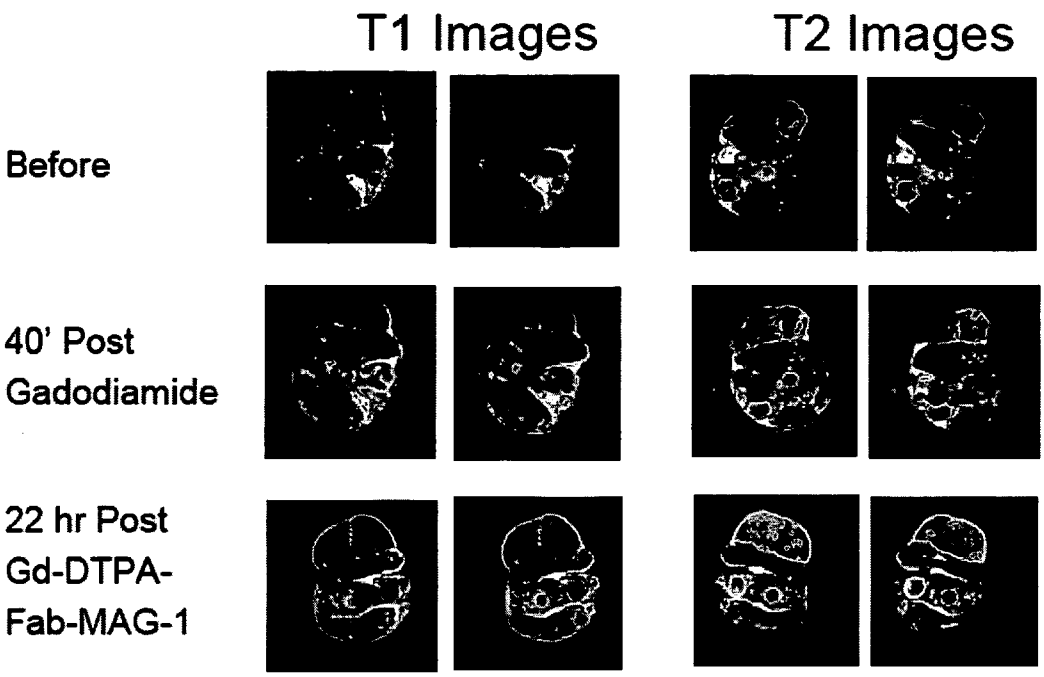

T1 Images
T2 Images
Before
40' Post Gadodiamide
22 hr Post Gd-DTPA-Fab-MAG-1

Figure 8

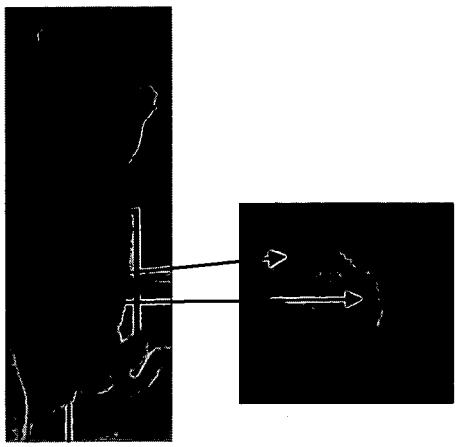
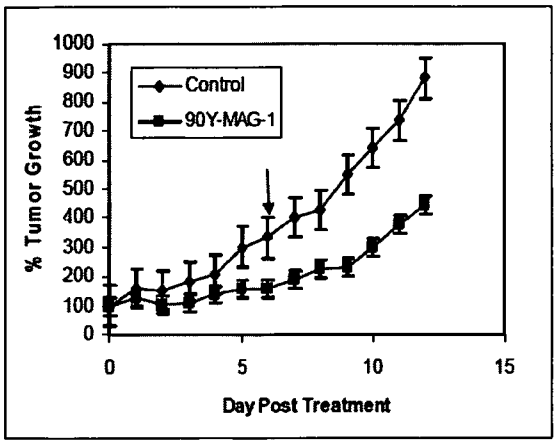
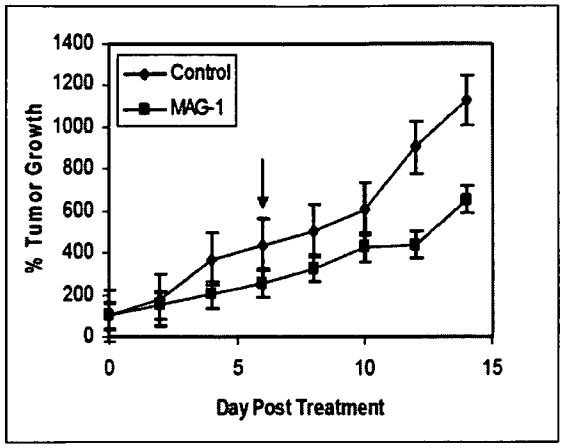


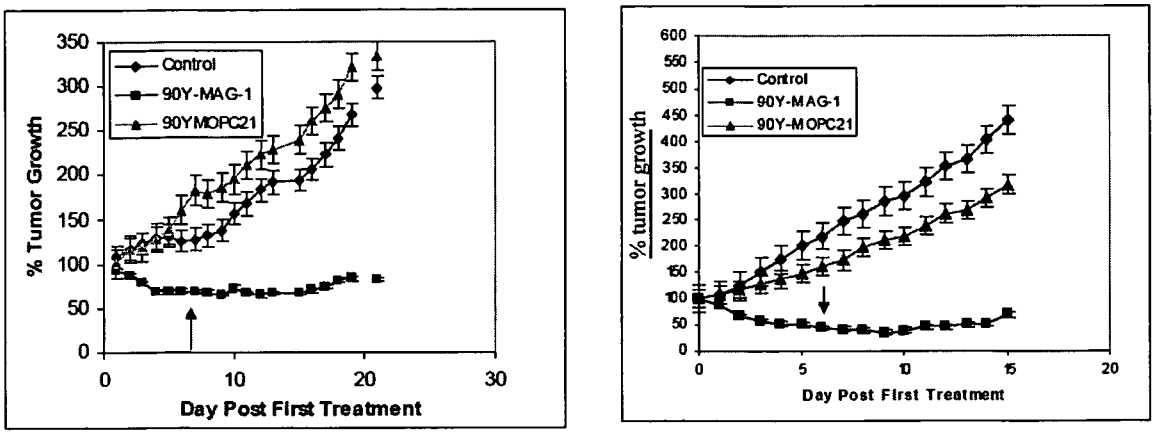
Figure 9


% Tumor Growth
1000
900
800
700
600
500
400
300
200
100
0
Control
90Y-MAG-1
0
5
10
15
Day Post Treatment


% Tumor Growth
1400
1200
1000
800
600
400
200
0
Control
MAG-1
0
5
10
15
Day Post Treatment

5

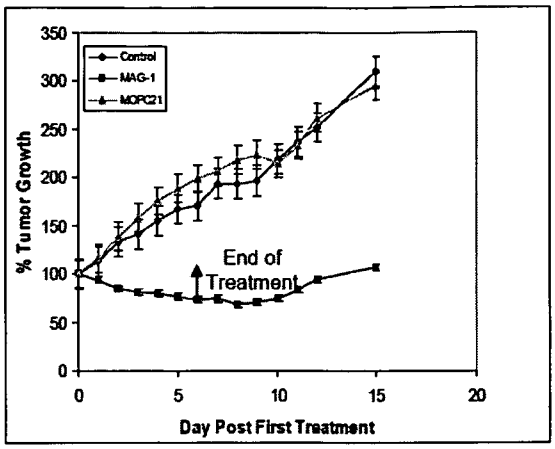
Figure 10



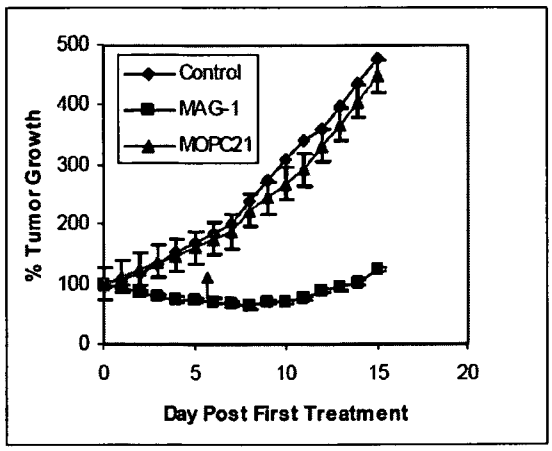
MCF-7 Tumor *MDA-MB231 Tumor*

Left panel: MAG-1 Inhibits MCF-7 tumor growth: MAG-1 (■) ($p<0.0005$, both controls, n=8), Control (●) and MOPC-21 (▲). Right panel: ^{90}Y-MAG-1 Inhibits MCF-7 tumor growth: ^{90}Y-MAG-1(■)) ($p<0001$, both controls), Control (●) and 90Y-MOPC-21 (▲). Arrow denotes last treatment. MOPC-21 is an irrelevant (control) monoclonal antibody that does not bind to the C18 peptide.

Figure 11

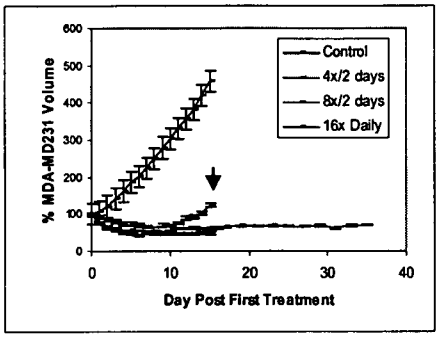


MCF-7 Tumor

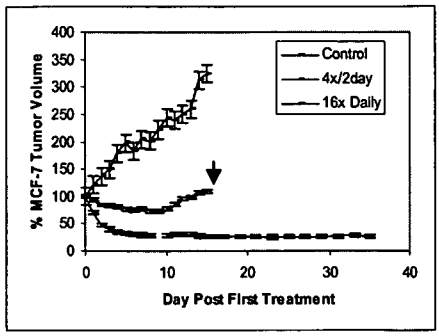


MDA-MB231 Tumor

Figure 12



12A



12B

Figure 13

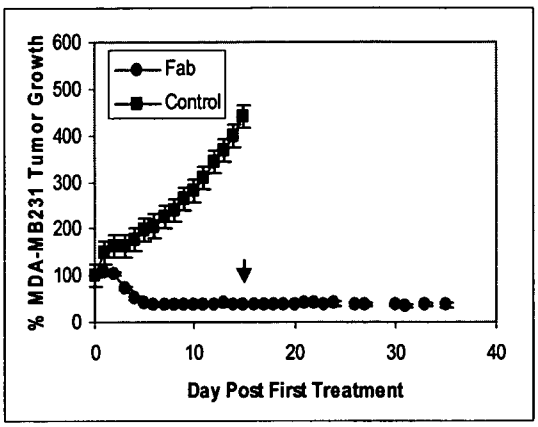
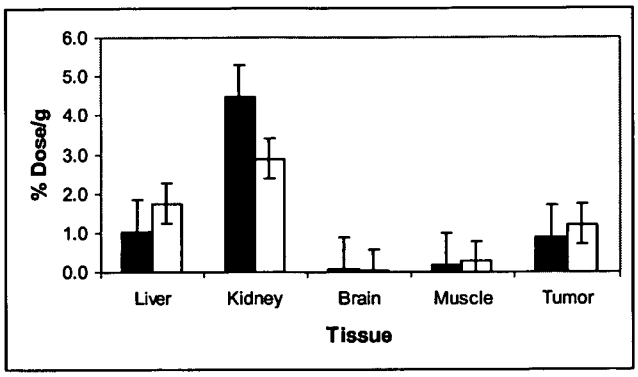

% MDA-MB231 Tumor Growth
600
500
400
300
200
100
0
Fab
Control
0
10
20
30
40
Day Post First Treatment

Figure 14


6.0
5.0
4.0
3.0
2.0
1.0
0.0
% Dose/g
Liver
Kidney
Brain
Muscle
Tumor
Tissue

15

Figure 15

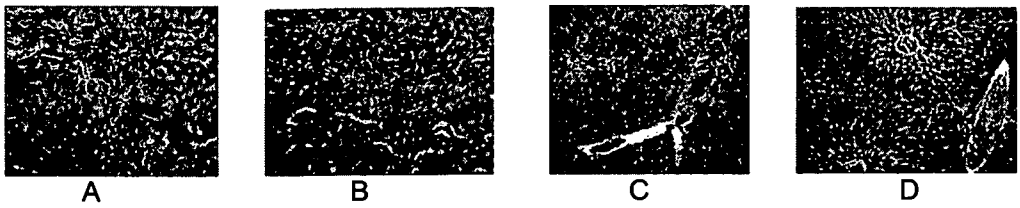
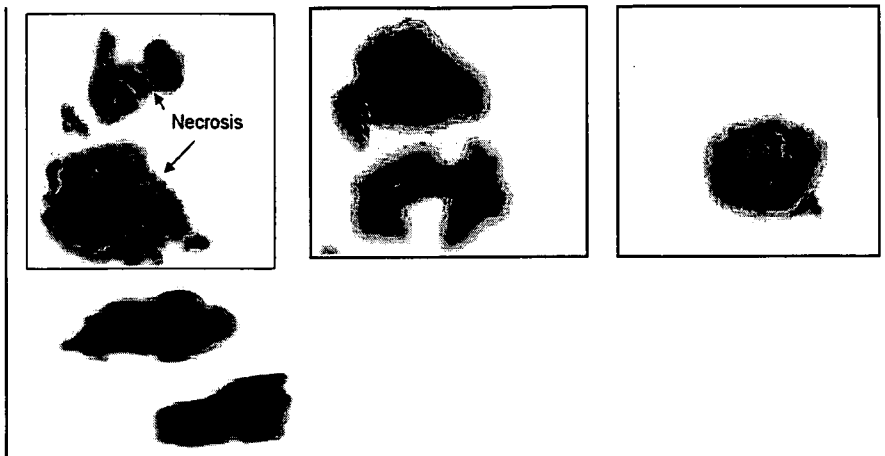


Figure 16



A B C D