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the applicant in respect of an application for a patent for an invention entitled Mouse monoclonal antibodies

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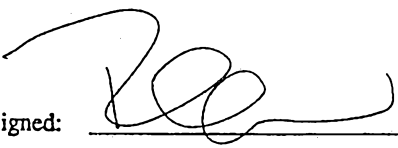
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Signed: 
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Date: 23 April 1993

Status: Patent Attorney

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- (57) Claim

1. A hybridoma having the characteristics of a hybridoma cell lines chosen from among the group having accession numbers ATCC HB 10572, ATCC HB 10673 and/or ATCC 10669 and which produces a monoclonal antibody specific for a cell surface epitope wherein said epitope is characterized by expression on normal primate tissue, malignant human cultured cell lines and human tumors.

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(21) International Application Number: PCT/US91/07226 (22) International Filing Date: 9 October 1991 (09.10.91) (30) Priority data: 596,289 12 October 1990 (12.10.90) US (71) Applicant: THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, U.S. DEPARTMENT OF COMMERCE [US/US]; 5285 Port Royal Road, Springfield, VA 22161 (US). (72) Inventors: PASTAN, Ira, H. ; 11710 Beall Mountain Road, Potomac, MD 20854 (US). WILLINGHAM, Mark, C. ; 4301 Chestnut Street, Bethesda, MD 20814 (US).		(74) Agents: HOLMAN, John, Clarke et al.; Fleit, Jacobson, Cohn, Price, Holman & Stern, The Jenifer Building, 400 Seventh Street, N.W., Washington, DC 20004 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i> 650302
(54) Title: MOUSE MONOCLONAL ANTIBODIES		
(57) Abstract The subject invention relates to monoclonal antibodies and uses thereof. In particular, the invention relates to three monoclonal antibodies, referred to as B1, B3, and B5, which are useful in the treatment and diagnosis of many forms of cancer.		

MOUSE MONOCLONAL ANTIBODIESBACKGROUND OF THE INVENTIONTechnical Field

The subject invention relates to monoclonal
5 antibodies and uses thereof.

In particular, the invention relates to three
monoclonal antibodies, referred to as B1, B3 and B5,
which are useful in the treatment and diagnosis of many
forms of cancer.

10 Background Information

Current therapies for metastatic human cancers,
such as radiation or chemotherapy, center on agents
that selectively kill rapidly growing cancer cells.
Unfortunately, many tumors do not show an unusually
15 fast growth rate compared to important normal tissues,
such as bone marrow or the epithelium of the
gastrointestinal tract. An alternative group of
therapeutic approaches targets unique chemical
structures on the surface of tumor cells for therapy,
20 most often employing antibodies that bind selectively
to these target molecules. One of these therapeutic
approaches employs antibodies that are coupled to cell-
killing agents, such as plant or bacterial toxins.
These antibody-toxin complexes, immunotoxins, have been
25 shown to be capable of selectively killing tumor cells
in model tumor systems in tissue culture and in
laboratory animals (Pastan, et al, Cell, 47:641-48
(1986)) . In spite of many attempts to isolate such
tumor-specific antibodies for human therapy, there are
30 still very few antibodies identified that selectively
bind only to tumor cells and not to other important
normal tissues. Isolation of such tumor-specific
antibodies is, therefore, of importance for the
application of such immuno-directed therapies.

Monoclonal antibody methodology as originally described by Kohler and Milstein (Nature 156:495-97 (1975)) and disclosed in Koprowski, et al. (U.S. Pat. No. 4,172,124) has allowed the isolation of antibodies in pure form for the construction of therapeutic agents. However, two problems have prevented the application of many previously isolated antibodies. First, many monoclonal antibodies reactive with tumor cells also react with important normal human tissues. Secondly, many of the isolated antibodies bind to surface elements that do not efficiently mediate the entry of toxin conjugates into cells by endocytosis. The present invention includes three monoclonal antibodies, B1, B3, and B5, that selectively bind to some human tumors, but not to many important normal tissues. These antibody, when incorporated as the targeting element of an immunotoxin, also has been shown to allow efficient entry of these toxic agents into cells.

Previously, antibodies reactive with the Lewis Y antigen have been isolated and characterized. Recently, two antibodies, BR64 and BR96 have been described (Hellstrom et al., Cancer Res., 50:2183-90 (1990)) that react with Lewis Y antigen, one of which (BR64) is not useful for immunotherapy because of its reactivity to capillaries in human cardiac muscle. BR96, however, shows reactivities that might make an immunotoxin constructed with this antibody potentially useful. The three new monoclonal antibodies, B1, B3, and B5, referred to above, which were isolated using a different cell type for immunizations and using morphologic screening methods, are similar, but not identical, to BR96. These differences in reactivity to tumors, normal tissues, and carbohydrate epitopes make

these three new antibodies potentially useful for the therapy and diagnosis of some forms of human cancer.

SUMMARY OF THE INVENTION

The subject invention relates to three monoclonal
5 antibodies, referred to as B1, B3 and B5, and to uses thereof.

B1, B3 and B5 exhibit a strong reactivity toward various mucin-producing, as well as non-mucin-producing primate carcinomas. Thus, these antibodies will be
10 useful in the design of targeted therapeutic agents utilized in the diagnosis and treatment of human cancers.

In particular, the present invention relates to a hybridoma which produces a monoclonal antibody specific
15 for a cell surface epitope wherein said epitope is characterized by expression on normal primate tissue, malignant human cultured cell lines and human tumors.

The present also includes a monoclonal antibody specific the cell surface epitope having the above
20 properties. The class of said monoclonal antibody is IgG or IgM.

The malignant human cultured cell lines, referred to above, are selected from the group consisting of A431, MCF-7, HTB 20, and HTB 33. The normal primate
25 tissue is derived from, for example, the esophagus, bladder or stomach. The human tumor noted above is derived from colon, gastric or ovarian carcinomas.

The present invention also relates to three separate hybridomas having the accession numbers ATCC
30 HB 10572 deposited October 12, 1990, HB 10673 deposited October 12, 1990, and HB 10669 deposited October 10, 1990, respectively.

The monoclonal antibody produced by the hybridoma of accession number ATCC HB 10572 is B1.

The monoclonal antibody produced by the hybridoma of accession number ATCC HB 10673 is B3, and the monoclonal antibody produced by the hybridoma of accession number ATCC HB 10669 is B5.

5 Furthermore, the present invention also includes a method of treating cancer comprising administering to a patient, in need of said treatment, an amount of a conjugate of the monoclonal antibody sufficient to effect said treatment. The monoclonal antibody may be
10 conjugated with, for example, a toxin, radionuclide or chemotherapeutic drug. The toxin may be, for instance, Pseudomonas exotoxin. The chemotherapeutic drug may be, for example, vinblastin or daunomycin.

 The present invention also includes a method of
15 diagnosing cancer in a patient comprising the steps of:
 drawing a blood sample from said patient;
 adding a monoclonal antibody to said sample in an amount sufficient to react with cancer shed antigen to form an antigen-antibody complex; and
20 detecting whether cancer is present in said patient by measuring the presence or absence of said complex.

 Furthermore, the present invention also includes a method of diagnosing cancer in a patient comprising the steps of
25 removing a tissue or fluid sample from said patient;
 adding the monoclonal antibody to the sample; and
 visualizing the presence of the antibody in the sample.

 The present invention also includes a
30 pharmaceutical composition comprising the monoclonal antibody in a concentration sufficient to inhibit tumor growth, together with a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents the antitumor activity of BE-PE^{428 57} in mice. Nude mice (20g) were injected with 3×10^6 cells subcutaneously on day 0. Treatment with 0.75 ug per dose was given I.P. on days 4, 6 and 8.

DETAILED DESCRIPTION OF THE INVENTION

In order to produce the B1 and B3 monoclonal antibodies of the present invention, mice can be tolerized to normal human kidney membranes and immunized with MCF-7 cells (May et al., American Type Culture Collection Catalog of Cell Lines and Hybridomas, (May et al., ATCC 1988) 6th Ed. (1989), Matthew et al., J. Immunol Methods 100:73-82 (1987) and Willingham et al., Proc. Natl Acad. Sci. USA 84:2474-78 (1987)). In contrast, in order to produce the B5 monoclonal antibodies, mice are not tolerized and can be immunized with A431 cells (May et al., supra). Spleens from the immunized mice are then removed, and the suspended cells can be fused, for example, with AG8 mouse myeloma cells, using polyethylene glycol. Appropriate clones can be selected after screening procedures have been carried out. One screening procedure may involve selecting clones which react with human colon and gastric cancers and not with normal human liver, kidney or colon tissues. This selection process is important for isolating clones that react with tumors, rather than normal tissue, for the use of such antibodies in selective human immunotherapy of cancer.

After subcloning of such antibodies, the isotype of the clones can be determined. The present inventors have established that the isotype for the B1 and B3 clones is IgG_{1k}, whereas the isotype for the B5 clone is



IgM. Antibody can be purified from the supernatant of the clones.

Once the antibodies are produced, their properties may then be characterized. For example, one
5 may characterize precisely which primate tissue epitopes are reactive with the B1, B3 and B5 antibodies.

Reactivity is defined as detectable binding to the surface of living cells using immunohistochemical
10 methods. Such a determination is necessary so that target agents may be designed which are toxic to tumors but not to important normal tissues.

The distribution of reactivity in normal human tissues, human tumors and normal cynomologous monkey
15 tissues is summarized in Table I below.

TABLE I

Immunohistochemical Localization of B1, B3, B5, and BR96
in Normal Human and Monkey Tissues

NORMAL HUMAN TISSUES

	B1	B3	B5	BR96
Liver	(-)(5/5) (+ large bile duct epith)	(-)(5/5) (+ large bile duct)(2/2)	(-)(1/1)	(-)(4/4) (+ large bile duct)(1/1)
Kidney	(-)(5/5)	(-)(5/5)	(+/-apic.tub)(1/2)	(-)(3/4)(+ap.d.tub)(1/4)
Cardiac Muscle	(-)(6/6)	(10/10)	(-)(6/6)	(-)(6/6)
Lung	(++ Type II pneum.)(1/2)	(+ Type II pneum.)(5/7)	(++ Type II pneum.)(1/1)	(-)(1/1)
Cerebral Cortex	(-)(2/2)	(-)(2/2)	(-)(3/3)	(-)(2/2)
Cerebellum	(-)(2/2)	(-)(2/2)	(-)(3/3)	(-)(2/2)
Spinal Cord	(-)(2/2)	(-)(2/2)	(-)(2/2)	(-)(2/2)
Pituitary	(-)(1/1)	(-)(1/1)	(-)(1/1)	nd
Bone Marrow	(-)(2/2)	(-)(1/1)	(-)(1/1)	(-)(1/1)
Adrenal	(-)(1/1)	(-)(1/1)	(-)(1/1)	(-)(3/3)
Spleen	(-)(1/1)	(-)(1/1)	nd	(-)(1/1)
Lymph Node	(-)(1/1)	(-)(1/1)	(-)(1/1)	nd
Skin	(-)(1/1)	(-)(2/2)	(-)(2/2)	(-)(3/3)
Skeletal Muscle	(-)(1/1)	(-)(1/1)	(-)(1/1)	(-)(2/2)
Peripheral Nerve	(-),?+cap.endoth.(1/1)	(-)(?+cap. endoth)(1/2)	(-)(2/2)	(?+cap.endoth.)(1/1)
Tonsil	(+++ epith.)(2/2)	(++++ epith)(2/2)	(+++ epith.)(2/2)	nd
Esophagus	(+++ diff. epith.)(2/2)	(+++ diff. epith.)(2/2)	(+++ diff. epith)(1/1)	(+++ diff epith)(4/4)
Small Bowel	(+ mucin)(4/4)	(+ mucin)(3/3)	(+++ ap muc.gran.)(3/3)	(+++ muc.)(3/3)
Stomach	(++++ glands, muc.)(3/3)	(++++ glands, muc.)(3/3)	(+++ glands, occ het)(3/3)	(++++ glands, muc.)(4/4)
Normal Colon	(-)(7/7)	(weak het. epith)(6/6)	(++ het. muc.)(2/2)	(-;3/4)(het. +; 1/4)
Bladder	(+++ epith.)(3/3)	(+++ epith)(3/3)	(+++ epith.)(3/3)	(+++ epith.)(3/3)
Pancreas	(#1=het. + ac.; ducts; #2= + acini & ducts) (+ acini & ducts)(1/1)	(+++ het, d & ac.)(2/2)	(+++ d & ac.)(1/1)	(+++ het ac.& muc.)(2/2)
Salivary Gland	(+ acini & ducts)(1/1)	(+++ d & ac.)(1/1)	(++++ ac. & d.)(1/1)	(+++ ac. & d.)(2/2)
Mammary Gland	(-)(1/1)	(het. + ducts)(1/1)	(+++ d, het glands)(2/2)	(het. + ducts)(1/1)
Epididymis	(-)(1/1)	(-)(1/1)	(-)(1/1)	nd
Thyroid	(-)(1/1)	(+++ colloid, -epith)(1/1)	(-)(1/1)	(-)(1/1)
Parathyroid	(-)(1/1)	(-)(1/1)	(-)(1/1)	nd
Ovary	(-)(2/2)	(-)(1/1)	(-)(2/2)	nd
Fallopian Tube	(-)(1/1)	(-)(1/1)	(+ /het)(1/1)	nd
Trachea	nd	(++++ epith.)(1/1)	nd	nd
Placenta	nd	(++++ fetal endoth.)(1/1)	nd	nd

TABLE I (CONTINUED)

NORMAL CYNOMOLOGOUS MONKEY TISSUES

	B1	B3	B5	BR26
Liver	(-)(1/1)	(-)(1/1)	(-)(1/1)	(-)(1/1)
Kidney	(-)(occ. glom.)(1/1)	(+ ap dt, gl. caps.)(1/1)	(+ ap tub, gl.caps.)(1/1)	(-)(1/1)
Brain	(-)(2/2)	(-)(2/2)	(-)(1/1)	(-)(2/2)
Cerebellum	nd	nd	(-)(1/1)	nd
Spinal Cord	(-)(1/1)	(-)(1/1)	(-)(2/2)	(-)(1/1)
Periph. Nerve	nd	nd	(-)(1/1)	nd
Spleen	nd	nd	(-)(1/1)	nd
Lymph Node	nd	nd	(-)(1/1)	nd
Skin	(-)(1/1)	(-)(1/1)	(-, ex + seb. glands)(1/1)	nd
Skeletal Muscle	nd	nd	(-)(1/1)	nd
Esophagus	(+ + + diff epith)(1/1)	(+ + diff epith)(1/1)	(+ + + diff epith)(1/1)	(+ + + diff epith)(1/1)
Small Bowel	(-)(1/2)(+ muc gran)(1/2)	(-)(2/2)	(+ + ap muc. B glands)(1/1)	(-)(2/2)
Stomach	(het + glands)(1/2)	(+ + + glands)(2/2)	(+ + + glands)(1/1)	(+ + + glands, het.)(1/1)
Colon	(-)(1/1)	(-)(1/1)	nd	(-)(1/1)
Bladder	(-)(1/1)	(+/-)(1/1)	(+ + + epith)(1/1)	nd
Pancreas	(-; + muc)(2/2)	(-; + muc)(3/3)	(+ + het ac.)(2/2)	(+/- het)(2/2)
Salivary Gland	(het + + + a&d)(2/2)	(+ + het a; + + d)(2/2)	(+ + het a; + + d)(3/3)	(+ + het a; + + d)(2/2)
Mammary Glands	(het +)(1/1)	(het. + g; + + d)(1/1)	(het + + g&d)(1/1)	nd
Thyroid	(-)(1/1)	(-)(1/1)	(-)(3/3)	(-)(1/1)
Parathyroid	nd	nd	(-)(1/2)(het +)(1/2)	nd
Ovary	nd	nd	(-)(1/1)	nd
Vaginal Glands	nd	nd	(+ + + +)(1/1)	nd
Uterine Cervix	(-)(1/1)	(-)(1/1)	(+ + apic. muc.)(1/1)	nd
Uterine Endometrium	(-)(1/1)	(+ deep glands)(1/1)	(+ + deep glands)(1/1)	nd
Thymus	(-)(1/1)	(-)(1/1)	(-)(1/1)	nd
Trachea	(-)(1/1)	(+ apic epith, het gl.)(1/1)	(+ apic epith & gl.)(1/1)	nd
Tongue	(-)(1/1)	(+ diff. epith)(1/1)	(+ + diff epith)(1/1)	nd

Immunohistochemical analysis was performed on cryostat sections of fresh-frozen tissues, post-fixed in acetone and incubated with primary antibodies at 10 µg/ml except where indicated. Labeling was then performed using affinity-purified goat anti-mouse IgG conjugated to horseradish peroxidase, developed using diaminobenzidine, then treated with hematoxylin followed by osmium tetroxide. (- = no localization; + = moderate; + + strong)(x/y = x examples of this pattern seen in y samples tested)(het = heterogeneous) nd = not determined.

As shown in Table I, the B1, B3 and B5-reactive epitopes are all found in varying amounts in the mucins of the stomach and small bowel, in the differentiated cell layer in the esophagus, in the epithelia of the tonsil, trachea and urinary bladder. These epitopes or antigens, which react with the B1, B3 and B5 monoclonal antibodies, can also be found in various other epithelia in a heterogeneous distribution, such as in the pancreas, salivary gland and mammary gland. B3 has the ability to react with the fetal endothelium, suggesting that the B3 monoclonal antibody represents an antigen expressed in fetal development.

Furthermore, as shown in Table I, the overall pattern of reactivity of the B1, B3 and B5 antibodies is different from the pattern of reactivity of a previously isolated antibody termed BR96 (Hellstrom et al., Cancer Res. 50:2183-90 (1990)). BR96 demonstrates some of the same reactivity patterns as observed with B1, B3 and B5. For example, BR96 reactivity is particularly notable in distal tubules in human kidney, as is B1 and B3 reactivity. However, there are distinct differences between these four antibodies in certain sites, such as in kidneys tubules, type II pneumocytes in the lung, mucin in the colon and in pancreatic ducts. For example, BR96 reactivity is not present in mucin in normal human colon sample, as is B5 reactivity. Such information can be significant in determining whether a monoclonal antibody administered for therapeutic purposes will be toxic with respect to normal tissues.

The above four antibodies can also be evaluated in normal monkey tissues (See Table I). Tissues similar to those in the human samples are reactive; however, similar to the human tissues, there are

distinct differences between B1, B3, B5 and BR96 reactivity in kidney tubules, small bowel mucin, bladder epithelium, pancreas, cervical mucin, endometrial glands, and the epithelium of trachea and tongue. These differences indicate that each of these antibodies recognize different epitopes. Thus, the chemical structures of the epitopes are different.

Various cancer cell lines can also be examined for reactivity with B1, B3, B5 and BR96 using immunofluorescence. The results of such a study are shown in Table II presented below.

TABLE II

Immunofluorescence Localization of B1, B3, B5, and BR96
On Human Cultured Cell Lines

<u>CELL LINE</u>	<u>B1</u>	<u>B3</u>	<u>B5</u>	<u>BR96</u>
A431 (epidermoid Ca)	+++ het	++++ het	++++ het	++++
MCF-7 (breast Ca)	++++	++++	++++	++++
OVCAR-3 (ovarian Ca)	-	"	++++ het	++++
KB (cervical Ca)	-	+/- het	++++ het	-
HT-29 (colon Ca)	+	++++	++++ het	+/-
MDA-MB-468 (breast Ca)	++++	++++	nd	++++
DU145 (prostate Ca)	+ het	++ het	++++ het	++++
HTB20 (breast Ca)	+++	+++	++++ het	+++
HTB33 (cervical Ca)	+++	+++ het	++++ het	+++

Het = heterogeneous; (-) = negative; (+ = weakly positive; ++ = moderate; +++ = strong; ++++ = very strong). nd = not determined.

As clearly shown in Table II, B1, B3, B5 and BR96 react with some cell lines uniformly. However, there are differences in reactivity, especially for OVCAR-3, KB and HT-29 cells. Again such data suggests that the

- 5 epitopes recognized by four antibodies are different from a structural standpoint. Furthermore, such differences in epitope structure and therefore in reactivity with monoclonals may be an advantage in therapy in some patients.
- 10 Tumors can also be examined for the expression of antigens which react with the 4 antibodies, using peroxidase immunohistochemistry. Table III (below) shows that the B1, B3, B5 and BR96 antibodies react well with carcinomas of colon and gastric origin, and
- 15 mucinous ovarian carcinomas. Reactivity can be detected in a smaller number of breast, esophageal and other carcinomas.

TABLE III

IMMUNOHISTOCHEMICAL REACTIVITIES OF HUMAN TUMORS
WITH B1, B3, B5, AND BR96

	<u>B1</u>	<u>B3</u>	<u>B5</u>	<u>BR96</u>
Colon Ca	(++ het cells & mucin)(3/3)	(++++ cells & mucin)(9/12) (het ++)(3/12)	(+++ het)(3/3)	(+++)(3/3)
Gastric Ca	(++++ cells & mucin) (3/4)	(++++)(3/4)	(+++)(1/1)	(++++)(3/4)
Ovarian Ca	(mucinous = +++ cells & mucin) (1/1);(cystadenoCa=het+)(2/3) (het++)(6/20)	(mucinous = ++++)(2/2) (cystadenoCa:+++)(4/20) (2/2)	(mucinous = +++)(1/1) (cystadenoCa:het+++) (1/2)	(+++ mucinous)(1/1) (+ het cystadenoCa)
Breast Ca	(het +)(1/2)	(+++ het)(14/21)	(-)(2/2)	(+++)(5/7)
Esophageal Ca	nd	(het +)(4/9);(++++)(3/9)	nd	nd
Prostate Ca	nd	nd	(+++)(1/1)	(++ het)(2/2)
Cervical Ca	nd	(het +)(1/1)	(-)(1/1)	nd
Endometrial Ca	nd	(het ++)(1/1)	(+)(1/1)	nd
Lung Ca	nd	(het +)(1/3)	nd	nd

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Immunohistochemical analysis was performed on cryostat sections of fresh-frozen tissues, post-fixed in acetone and incubated with primary antibodies at 10 µg/ml except where indicated. Labeling was then performed using affinity-purified goat anti-mouse IgG conjugated to horseradish peroxidase, developed using diaminobenzidine, then treated with hematoxylin followed by osmium tetroxide. (- = no localization; + = moderate; ++ strong)(x/y = x examples of this pattern seen in y samples tested) (het = heterogeneous) nd = not determined.

5 The results of Tables I, II and III indicate that
B1, B3 and B5 react with many common tumors and appear
to react with a limited number of normal tissue sites.
In addition, these antibodies show distinct differences
in reactivity in some varying tissue samples indicating
10 that the precise epitopes they detect are different.

When MCF-7 cells bearing the B1, B3 and B5
epitopes are metabolically labelled using radioactive
amino acids, then extracted and the extracts
immunoprecipitated, the reactive species of molecules
15 that are precipitated by B1, B3 and B5 can be analyzed
by gel electrophoresis and auto radiography. B1 and
B3 specifically immunoprecipitate protein bands of a
very high molecular weight (>250,000 Daltons),
consistent with their reactivity with high molecular
20 weight mucins. Because B5 is an IgM antibody, for
technical reasons this method is unable to show
specifically reactive proteins.

Monoclonal antibodies B1, B3 and B5 can serve as
targeting agents for the construction of immunotoxins,
25 in which the monoclonal antibody is linked to a toxin,
for example, Pseudomonas exotoxin (see Table IV below).
As previously disclosed in Pastan, et al. (U.S. Pat.
No. 4,545,985) conjugates of Pseudomonas exotoxin and
monoclonal antibodies show efficacy in killing cells
30 that are targeted by the epitope-reactive site of the
antibodies. Constructions made by linking B1, B3 or B5
to such toxin would then be introduced into patients
that had tumors that were reactive with these
monoclonal antibodies, and the immunotoxins would bind
35 to and kill the tumor cells within the patient. Normal
tissues that were also reactive with these monoclonal

antibodies would be affected if the antigenic sites were accessible to the blood circulation. In the case of many of the sites of expression of antigens reactive with B1, B3, and B5, the reactive epitope appears by
5 immunohistochemistry to relatively inaccessible to the circulation, such as the reaction with mucins in the lumen of the gastrointestinal tract. Thus, it is not possible to predict with total certainty what the toxic effects of such immunotoxins would be in a human
10 patient. Tumor cells that express these surface antigens, however, are rarely in a location that would render them inaccessible to the immunotoxin, and the tumor cells should therefore, be susceptible to targeted cell killing by immunotoxins constructed with
15 B1, B3 or B5.

TABLE IV

Activity of Immunotoxin Composed of B3 and a
Pseudomonas Exotoxin Mutant in Which Lysine 57
is Converted to Arginine (B3-PE^{Arg57})

Cell Line	ID ₅₀	
	B3-PE ^{Arg57} ng/ml	MOPC-PE ^{Arg57} ng/ml
A431 Epidermoid Ca	0.2	>100
MCF-7 Breast Ca	0.3	>100

ID₅₀ is the concentration of agent that inhibits protein
synthesis by 50% in a 16 hour incubation.

In addition to bacterial or plant toxins
conjugated to monoclonal antibodies, other effector
agents may be used together with targeted monoclonal
antibodies to treat or diagnose human cancer. For
example, radionuclides conjugated to antibodies that
bind to tumors can produce cell killing based on the
high local concentration of radiation. Chemotherapeutic
drugs, for example, vinblastine or daunomycin, can be
coupled to antibodies and delivered at high
concentration to cells that react with the antibodies.
B1, B3, and B5 may provide a targeting mechanism for

such combination of effector agents that could produce successful regression of reactive human tumors when introduced into patients.

In addition to the targeting of immunotoxins to
5 tumors in a cancer patient, these antibodies also recognize materials such as surface mucins on tumor cells that would be expected to be shed into the surrounding tissues, picked up by the blood stream, and detectable in blood samples taken from distant sites.
10 Such shed antigens have proven to be useful in the diagnosis of primary and recurrent cancers using antibodies that react to these shed antigens. A currently useful example of this is the CA125 antigen that can be assayed in sera from patients with ovarian
15 cancer to predict recurrence or to confirm primary diagnosis of tumor. It is possible, therefore, that B1, B3 and B5 may be useful in the diagnosis of tumors.

Also, the selective reactivity of these antibodies with certain types of tumor cells could be exploited
20 for anatomic pathological diagnosis of tumors, clarifying the type and origin of tumors, and whether a particular group of cells represents a recurrence of a previous tumor or the development of another primary tumor elsewhere. Such a diagnostic determination can
25 be useful for the subsequent planning of anti-tumor therapy in each particular patient. In particular, immunohistochemical pathologic diagnosis in tissue sections (e.g., biopsies) or cytological preparations (e.g., Pap smears, effusions) can be performed using
30 the monoclonal antibodies of the present invention.

Another potential use of such targeting antibodies could be in the diagnosis of macroscopic foci of tumor

using antibodies B1, B3 or B5 coupled to radioisotopes that could be detected either by external body scanning (imaging diagnosis) or by localization using radiation detector probes at the time of exploratory surgery.

5 In addition to the initial clones of B1, B3 and B5 isolated as mouse monoclonal antibodies, variations of the constant regions of these antibodies incorporating constant regions of other species, such as human, could be performed, in which the resulting antibody would
10 display less immunogenicity as a foreign antigen itself when introduced into a human patient. Pharmaceutical compositions can also be made using the monoclonal antibodies.

Also, the genes responsible for the variable
15 regions of these antibodies could be isolated and targeting agents constructed using these variable region genes in tandem with genes for other proteins, such as toxin genes, or other effector proteins that could direct cell killing either directly or through
20 the activation of endogenous mechanisms, such as the immune system. The variable regions of immunoglobulin genes encode the antigen binding site which enables the chimeric antibody toxin protein to bind to and kill target cells expressing the antigen reacting with
25 antibodies B1, B3, and B5.

The present invention can be illustrated by the use of the following non-limiting examples.

EXAMPLE IProduction of the B1, B3 and B5Monoclonal Antibodies

The human tumor cell lines OVCAR-3, KB, MCF-7, HT-
5 29, MDA-MD-468, DU145, HTB20, and HTB33 have been
previously described (Hay et al., American Type Culture
Collection Catalog of Cell Lines and Hybridomes, 6th
Ed. (1988)). For antibodies B1 and B3, mice were
tolerized to normal human kidney membranes (Matthew et
10 al., J. Immunol. Methods 100:73-82 (1978) and immunized
with MCF-7 cells using methods previously described
(Willingham et al., Proc. Natl. Acad. Sci. USA 84:2474-
78 (1987)). For antibody B5, mice were not tolerized
and were immunized with A431 cells. Spleens from
15 immunized mice were removed and the suspended cells
were fused with AG8 mouse myeloma cells. The resulting
clones were screened two weeks later employing the
ScreenFast (Life Technologies, Inc. Gaithersburg, MD)
large scale screening chamber using rhodamine indirect
20 immunofluorescence on living MCF-7 and A431 cells for
B1 or B3, and B5, respectively. Selected clones were
secondarily screened using peroxidase
immunohistochemistry on cryostat sections of human
tumors and normal tissues. Clones B1, B3 and B5 were
25 selected that reacted with human colon and gastric
cancers, and not with normal human liver, kidney or
colon. After sub-cloning, the isotypes of these clones
was determined to be IgG_{1k} for clones B1 and B3, and IgM
for clone B5. Antibody was purified from the
30 supernatants of these clones using serum-free defined
culture media and ammonium sulfate precipitation.

EXAMPLE II

Determination of Distribution of Antigens
Reactive with Antibodies B1, B3 and B5 In Human Tumor-
Free Tissues, Human Tumors And Monkey Tissues

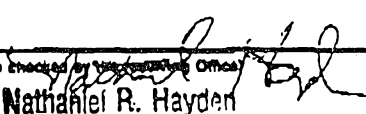
5 Samples of normal human tissues, Cynomologous
monkey tissues, and human tumors were fresh-frozen and
cryostat sections were prepared for peroxidase
immunohistochemistry as previously described
(Willingham, FOCUS 12:62-67 1990)) using B1, B3 and B5
10 as primary antibodies. Localization of antibodies was
detected by development of the peroxidase substrate
reaction using diaminobenzidines. Tissues sections
demonstrated major reactivities of B1, B3 and B5 in the
epithelium of the tonsil, stomach, esophagus, and
15 bladder, as well as in mucins of the small bowel and
colon. Similar localization was found in monkey
tissues in esophagus, small bowel, stomach, bladder,
salivary gland, and pancreas with some differences
being noted between the different antibodies (see Table
20 I). Human tumors showed strong reactivity for B1, B3
and B5 in carcinomas of colon, stomach, ovary, and
esophagus, with variable localization seen in
carcinomas from breast, cervix, prostate, endometrium
and lung (see Table III). All localizations, except as
25 noted in Table I, represented antigen reaction that
appeared to be on the surface of the cells, making
these sites potential targets for immunotherapy.

EXAMPLE IIIDetermination of the Effectiveness
of BE-PE As An Anti-Tumor Agent

B3 was coupled to Pseudomonas exotoxin as
5 previously described (Willingham et al., Proc. Natl.
Acad. Sci. USA 84:2474-78 (1987)). To do this, a
mutant form of PE in which lysine 57 of PE was mutated
to arginine (PE^{Arg57}) was used. The immunotoxin was
purified and tested in tissue culture where it was
10 shown to kill target A431 and MCF-7 cells (Table IV). A
control antibody (MOPC 21) was also coupled to PE^{Arg57}
and it had no cell killing activity. B3-PE^{Arg57} was
then given intraperitoneally to mice. The mice had
been implanted with 3 million A431 cancer cells on day
15 0, and by day 4 had small cancers which were rapidly
growing. The immunotoxin was given IP on days 4, 6,
and 8 and, as shown in Figure 1, the tumors regressed
and apparently disappeared, whereas, in the control
animals treated with diluent, the tumors grew rapidly.



International Application No: PCT/

MICROORGANISMS	
Optional Sheet in connection with the microorganism referred to on page <u>3, 4, 22</u> , line <u>33, 1, 1</u> of the description.	
A. IDENTIFICATION OF DEPOSIT:	
Further deposits are identified on an additional sheet ☐ .	
Name of depository institution:	
AMERICAN TYPE CULTURE COLLECTION	
Address of depository institution (including postal code and country):	
12301 Parklawn Drive Rockville, Maryland 20852 United States of America	
Date of deposit:	Accession Number:
12 October 1990	HB 10572
12 October 1990	HB 10673
10 October 1990	HB 10669
B. ADDITIONAL INDICATIONS: (Leave blank if not applicable). This information is continued on a separate attached sheet ☐ .	
C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE: (If the indications are not for all designated States)	
D. SEPARATE FURNISHING OF INDICATIONS: (Leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later. (Specify the general nature of the indications e.g., "Accession Number of Deposit")	
☒ This sheet was received with the international application when filed (to be checked by the receiving Office).  Nathaniel R. Hayden PCT INTERNATIONAL SERVICE DIVISION (Authorized Officer)	
☐ The date of receipt (from the applicant) by the International Bureau is:	
_____ (Authorized Officer)	

CLAIMS:

1. A hybridoma having the characteristics of a hybridoma cell lines chosen from among the group having accession numbers ATCC HB 10572, ATCC HB 10673 and/or
5 ATCC 10669 and which produces a monoclonal antibody specific for a cell surface epitope wherein said epitope is characterized by expression on normal primate tissue, malignant human cultured cell lines and human tumors.
2. A monoclonal antibody specific for a cell
10 surface epitope wherein said epitope is characterized by expression on normal primate tissue, malignant human cultured cell lines and human tumors, and is produced by a hybridoma having the characteristics of a hybridoma cell line chosen from among the group of hybridoma cell
15 lines having accession numbers ATCC HB 10572, ATCC HB 10673 and/or ATCC 10669, and the class of said monoclonal antibody is IgG or IgM.
3. The hybridoma of claim 1 wherein said malignant human cultured cell lines are selected from the group
20 consisting of A431, MCF-7, HTB 20, and HTB 33.
4. The monoclonal antibody of claim 2 wherein said malignant human cultured cell lines are selected from the group consisting of A431, MCF-7, HTB 20 and HTB 33.
5. The hybridoma of claim 1 wherein said normal
25 primate tissue is derived from esophagus, bladder or stomach.
6. The monoclonal antibody of claim 2 wherein said normal primate tissue is derived for esophagus, bladder or stomach.
- 30 7. The hybridoma of claim 1 wherein said human tumor is derived from colon, gastric or ovarian carcinomas.



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8. The monoclonal antibody of claim 2 wherein said tumor is derived from colon, gastric or ovarian carcinomas.

5 9. A hybridoma cell line having the accession number ATCC B 10572.

10. A hybridoma cell line having the accession number ATCC HB 10673.

11. A hybridoma cell line having the accession number ATCC HB 10669.

10 12. A monoclonal antibody produced by the hybridoma of claim 9 wherein said monoclonal antibody is B1.

13. A monoclonal antibody produced by the hybridoma of claim 10 wherein said monoclonal antibody is B3.

15 14. A monoclonal antibody produced by the hybridoma of claim 11 wherein said monoclonal antibody is B5.

15. A conjugate comprising a monoclonal antibody according to any one of the claims 2, 12-14 conjugated to a toxin, radionuclide or chemotherapeutic drug.

20 16. The conjugate according to claim 15 wherein the toxin is Pseudomonas exotoxin.

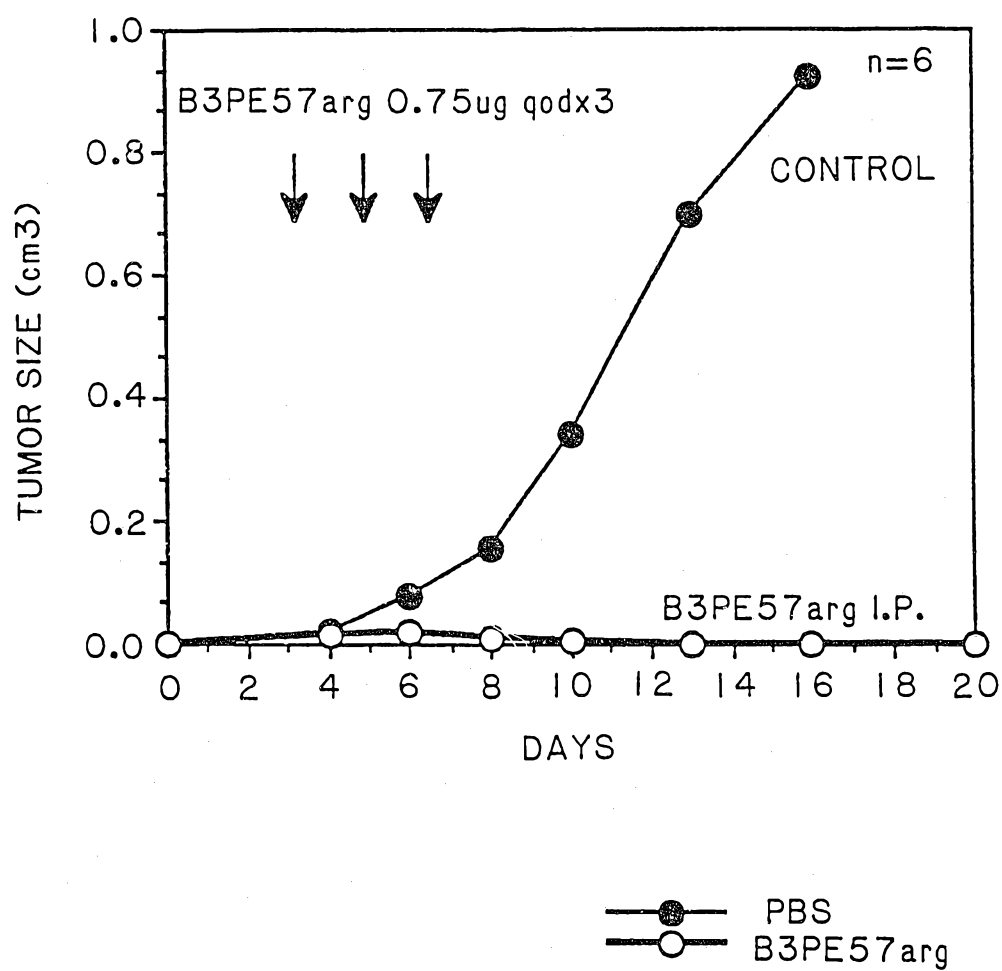
17. The conjugate according to claim 15 wherein the chemotherapeutic drug is vinblastin or daunomycin.

18. A monoclonal antibody according to any one of the claims 2, 12-14 for use in the diagnosis of cancer.



SUBSTITUTE SHEET
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SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/07226

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶

According to International Patent Classification (IPC) or to both National Classification and IPC
 IPC(5): G01N 33/574, 33/567; A61K 39/00; C07K 15/14; C12N 5/12
 U.S. Cl.: 435/7.23, 7.21, 240.27; 530/387; 424/85.8

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System	Classification Symbols
U.S.	435/7.21, 7.23, 7.9, 240.27; 530/387; 424/85.8

Documentation Searched other than Minimum Documentation
 to the Extent that such Documents are included in the Fields Searched ⁸

STN Online Databases (File Biosis, 1969-1992; File Medline, 1972-1992; File CA, 1967-1992).
 Automated Patent System (File USPAT, 1975-1992). See the Attachment.

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
<u>X</u> Y	Cancer Research, Volume 50, issued 01 April 1990, Hellstrom, et al., "Highly Tumor-reactive, Internalizing, Mouse Monoclonal Antibodies to Ley-related Cell Surface Antigens", pp. 2183-2190. see the Abstract.	<u>1,2,5,8</u> 15-21
X	Biological Abstracts, Volume 81, No. 3, issued 01 February 1986, Osborne, et al., "A Monoclonal Antibody Recognizing Desmosomes: Use in Human Pathology", the abstract no. 25179, J. Invest. Dermatol. 1985, 85(4), 385-388.	1-14, 19-20

^{*} Special categories of cited documents ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

23 JANUARY 1992

International Searching Authority

ISA/US

Date of Mailing of this International Search Report

12 FEB 1992

Signature of Authorized Officer

TONI R. SCHEINER

III DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication where appropriate, of the relevant passages	Relevant to Claim No
A	Cell, Volume 47, issued 05 December 1986. Pastan, et al., "Immunotoxins", pp. 641-648. see the Abstract.	1-21
X.P. V	Biological Abstracts, Volume 92, No.7, Issued 01 October 1991, Pastan, et al., "Characterization of Monoclonal Antibodies B1 and B3 that React with Mucinous Adenocarcinomas", the Abstract no. 77938. Cancer Res., 1991, 51(14), 3781-3787. See the Abstract.	1-16 19-21 17, 18

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	Biological Abstracts, Volume 88, No.5. Issued 01 September 1989. J. Fine et al., "Detection and Partial Characterization of a Midlamina Lucida-hemidesmosome-Associated Antigen (19-DEJ-1) Present within Human Skin", the abstract no.50184. J. Invest. Dermatol., 1989, 92(6), 825-830. See the Abstract.	1-6.9-14
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V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

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

1. ☐ Claim numbers _____ because they relate to subject matter¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out¹³, specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING¹

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

PCT/US91/07226

ATTACHMENT TO FORM PCT/TSA/210. PART II

II. FIELDS SEARCHED/SEARCH TERMS:

hybridoma or monoclonal primate

malignan? or carcinoma? or tumor?

A431 or (MCF(w)7) or (HTB(w)(20 or 33)) esophagus or bladder or
stomach colon or gastric or ovarian mucin?

Pastan. Tra H

Willingham. Mark C.

adenocarcinoma?