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(54) Titre : LIGNEE CELLULAIRE DE B-LYMPHOBLASTOIDE HUMAIN SECRETANT UN ANTICORPS
ANTIGANGLIOSIDE
(54) Title: HUMAN B-LYMPHOBLASTOID CELL LINE SECRETING ANTI-GANGLIOSIDE ANTIBODY

(57) **Abrégé/Abstract:**

A human B-lymphoblastoid cell line which is identified as L612. The L612 cell line is an Epstein-Barr virus transformed cell line which secretes a human monoclonal antibody (L612) which is reactive with glycoconjugates having an epitope of terminal NeuAc α 2-3 Galactose residue such as GM3 and GM4 present on a variety of human tumor tissues. The L612 antibody is useful in treating patients with the epitope containing tumors and is also useful in raising anti-id antibodies for use as surrogate antigens and diagnostic reagents.



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(21) International Application Number: PCT/US94/01469 (22) International Filing Date: 9 February 1994 (09.02.94) (30) Priority Data: 026,320 26 February 1993 (26.02.93) US (71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612-3550 (US). (72) Inventor: IRIE, Reiko, F.; 869 Gretna Green Way, Los Angeles, CA 90049 (US). (74) Agents: OLDENKAMP, David, J. et al.; Poms, Smith, Lande & Rose, Professional Corporation, 38th floor, 2029 Century Park East, Los Angeles, Ca 90067 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> 2156857
(54) Title: HUMAN B-LYMPHOBLASTOID CELL LINE SECRETING ANTI-GANGLIOSIDE ANTIBODY (57) Abstract A human B-lymphoblastoid cell line which is identified as L612. The L612 cell line is an Epstein-Barr virus transformed cell line which secretes a human monoclonal antibody (L612) which is reactive with glycoconjugates having an epitope of terminal NeuAc α 2-3 Galactose residue such as GM3 and GM4 present on a variety of human tumor tissues. The L612 antibody is useful in treating patients with the epitope containing tumors and is also useful in raising anti-id antibodies for use as surrogate antigens and diagnostic reagents.		

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HUMAN B-LYMPHOBLASTOID CELL LINE SECRETING
ANTI-GANGLIOSIDE ANTIBODY

BACKGROUND OF THE INVENTION

This invention was made with Government support
5 under Grant Nos. CA 30647, CA 42396 and CA 12582 awarded by
the National Cancer Institute.

1. Field of the Invention:

The present invention relates generally to Epstein-
Barr virus-transformed human B-lymphoblastoid cell lines.
10 More particularly, the present invention relates to such cell
lines which are capable of producing antibodies which can be
used to directly treat tumors or which can be used to raise
anti-id antibodies for use as surrogate antigens or diagnostic
reagents.

15 2. Description of Related Art:

The publications and other reference materials
referred to herein to describe the background of the invention
and to provide additional detail regarding its practice. For
convenience, the reference materials are numerically
20 referenced and grouped in the appended bibliography.

The possibility that the variable regions of
immunoglobulins could act as external antigens was first
recognized by Jerne in his idiotypic network theory (1).
According to this theory, recognition of idiotypes on the
25 antigen-combining site, or on the framework of AB1, results in
the production of anti-idiotypes (anti-ids or AB2) beta and
alpha, respectively. Such "internal

image" anti-idiotypes, by virtue of their complementarity with the original antigen binding site, mimic the original antigen and often behave in a similar biological manner. The concept of internal image refers
5 to the fact that some AB2 molecules can act as surrogate antigens and their administration can lead to the production of anti-anti-idiotypic antibodies displaying similar characteristics of AB1.

Immunization using anti-ids as surrogate antigens
10 has generated much interest among researchers, many of whom have experimented with AB2 vaccines for active specific immunization against viruses, bacteria, and other pathogens (2,3). This approach is useful when a conventional vaccine or antibodies are not available, or
15 are difficult to produce or when the corresponding antigen is not a suitable product for genetic engineering. In addition, anti-ids can be used as immunomodulators for up-regulating immunity against cancer, and as immunosuppressants to prevent rejection
20 of transplanted organs and to prevent the progression of auto-immune disease.

Gangliosides are glycosphingolipids that are fundamental membrane components on human tissues. Gangliosides undergo characteristic changes during
25 malignant transformation of normal cells and thus are desirable target antigens for immunotherapy of cancer. Melanoma synthesizes a large number of gangliosides and thus has served as a useful model to assess the potential of gangliosides as immunotherapy targets. A
30 number of tumor-associated gangliosides of human melanoma and their respective immunogenicity have been defined (12-29). In addition, a number of studies have shown that active immunization with ganglioside antigens results in prolonged survival of melanoma patients
35 (4,5). Nevertheless, this technique suffers in many areas, namely that the ganglioside antigen are many times rare or in short supply.

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Tumor-associated antigens, in most cases, are present in nature only at low levels and are relatively difficult to purify in large amounts. In contrast, anti-ids can be secreted from hybridoma cells at low
5 cost over long periods of time. Furthermore, current genetic engineering technology, while not applicable to ganglioside epitopes, can be used to synthesize the anti-id peptides. Anti-ids previously developed for active specific immunotherapy of human cancer have used
10 murine monoclonal antibodies (MuMabs) as the immunogens (6-11).

In addition to their use as surrogate antigens, murine monoclonal antibodies have also been employed to define and characterize many antigenic molecules on
15 human cancer cells. Murine monoclonal antibodies have several advantages over human monoclonal antibodies including a strong affinity for tumor antigens, higher antibody secretion by hybridoma ascites, and high antigen density on tumor cells. However, with respect
20 to therapeutic use, recent clinical trials with murine monoclonals have indicated that human monoclonal antibodies (HuMAbs) may be preferable since repeated injections of MuMabs induce anti-murine Ig antibodies in virtually all patients. This leads to formation of
25 immune complexes and immune reactions with potentially hazardous complications. In addition, HuMAbs may recognize epitopes that are overlooked by the murine immune system.

The development of HuMAbs that react with
30 ganglioside antigens on human cancer cells and the demonstration of their anti-tumor effect at the clinical level has been reported (23,12). Patients with recurrent melanoma received intratumor injections of HuMAb to ganglioside GD2 or GM2, and partial or complete
35 regression was observed in about 70% of the patients. In those melanoma patients in whom the immunotherapy was ineffective, the target antigen GD2 or GM2, was not

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expressed on the tumor cells. Two HuMAbs identified as L55 and L72 have been produced from human B-lymphoblastoid cell lines which have been transformed by Epstein-Barr virus (29). The L55 and L72 antibodies
5 were both found to be reactive with a variety of tumor cells.

Because the quantity and quality of gangliosides on human melanoma are widely heterogeneous between different cancer patients, it is desirable to avoid
10 unnecessary administration of HuMAb by examination of a pre-treatment biopsy to identify which gangliosides dominate on each patient's tumor cells.

There are three different immunological assays which have been used to detect the quality and quantity
15 of gangliosides present on a given tumor. They include: the immune adherence assay (IA); direct immunofluorescence with fluorescinated microspheres; and IA absorption, and a biochemical assay. These assays each have certain limitations and advantages. The
20 immunologic assay requires single cell suspensions from the biopsied tumor tissues. However, it is often difficult to obtain viable high yield tumor cell populations. Also, under a light microscope, tumor cells may not be readily distinguished from monocytes
25 and macrophages. The biochemical assay does not require intact cells. However, a relatively large volume of tumor is necessary for ganglioside extraction and measurement of sialic acid in the glycolipid preparation.

30 The most commonly utilized immunologic technique for defining antigen expression on biopsy specimens using murine monoclonal antibodies is immunohistochemical staining of tissue sections. However, this sensitive method is not readily applicable
35 to combinations of human monoclonal antibodies and human tissues. The indirect staining of human tumor tissues with the second antibody (anti-human Ig) usually results

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in high background from non-specific binding to abundant endogenous human Ig. Direct immunostaining using biotinylated human monoclonal antibodies may overcome this high background (30). However, this method is usually less sensitive and is most effective when a high density antigen is present on the cell surface.

There presently is a continuing need to develop additional human cell lines which are capable of producing antibody that is immunogenic with respect to the gangliosides present on tumors. The anti-ganglioside antibodies produced by the new cell lines will be useful in direct treatment of tumors and also in the production of anti-ids for use as surrogate antigens or diagnostic reagents.

15

SUMMARY OF THE INVENTION

In accordance with the present invention, a human B-lymphoblastoid cell line has been developed which secretes anti-ganglioside antibody which is reactive with a variety of tumors and which has been demonstrated to be effective in treating melanoma. The cell line of the present invention is identified as L612 and is maintained at the Division of Surgical Oncology at the University of California at Los Angeles School of Medicine. The L612 cell line has been deposited at the American Type Culture Collection (Rockville, Maryland) under ATCC accession number CRL 10724.

As a feature of the present invention, the L612 cell line is used to produce an IgM kappa antibody which is reactive with tumor antigen. The L612 antibody has been shown to be effective in treating melanoma tumors when administered by intralesional injection.

As another feature of the present invention, the L612 antibody is used to prepare murine anti-id monoclonal antibodies which are useful as surrogate antigens or which can be used in diagnostic procedures.

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According to one aspect of the present invention, there is provided a human B-lymphoblastoid cell line which is deposited at the American Type Culture Collection under ATCC accession number CRL 10724.

5 According to another aspect of the present invention, there is provided an antibody which comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2.

10 According to another aspect of the present invention, there is provided an antibody which comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 4.

15 According to another aspect of the present invention, there is provided an antibody which comprises a heavy chain variable region comprising complementary determining regions as set forth in FIG. 1.

20 According to another aspect of the present invention, there is provided an antibody which comprises a light chain variable region comprising complementary determining regions as set forth in FIG. 2.

25 According to another aspect of the present invention, there is provided a nucleotide sequence encoding at least a portion of an antibody, the nucleotide sequence encoding a sequence selected from the amino acid sequences set forth in SEQ ID NO: 2 and SEQ ID NO: 4.

30 According to another aspect of the present invention, there is provided a use of the antibody according to the invention for the treatment of tumor tissue which contain ganglioside antigens II³NeuAc-Lac-Cer(GM₃), GM4 or IV³NeuAc-nLcOse₄-Cer(SPG) that bind to an antibody secreted by

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a human β -lymphoblastoid cell line which is deposited at the American Type Culture Collection (ATCC) under ATCC accession number CRL10724 and isotypes thereof, and in the manufacture of a medicament therefore.

5

SUMMARY OF THE DRAWINGS

FIG. 1 is the nucleotide/amino acid sequence for the variable region of the L612 antibody heavy chain. The complementary determining regions are underlined.

10 FIG. 2 is the nucleotide/amino acid sequence for the variable region of the L612 antibody light chain. The complementary determining regions are underlined.

DETAILED DESCRIPTION OF THE INVENTION

The cell line in accordance with the present invention is a B-lymphoblastoid cell line which has been transformed by the Epstein-Barr virus transformation technique. The cell line is identified as L612 and is maintained at the Division of Surgical Oncology at the University of California at Los Angeles School of Medicine. The L612 cell line has been deposited on April 4, 1991 at the American Type Culture Collection with ATCC accession number CRL 10724 under the terms of the Budapest Treaty.

25 The L612 cell line was established in culture from lymphocytes by the same Epstein-Barr virus transformation technique which was used to produce two other human monoclonal anti-ganglioside antibodies, L55 (anti-GM2) and L72 (anti-GD2) (26-27 and 29). The same transformation procedure as set forth in detail in reference (29) was followed in establishing the L612 cell line. The Epstein-Barr virus transformation technique used to establish the prior L55 and L72 cell lines,

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as well as the present L612 cell line, is a conventional procedure which is known and used by researchers in this field.

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The specific procedure which was used to establish the L612 cell line is as follows.

Regional lymph nodes were received from a patient with breast cancer during mastectomy. Lymphocytes were
5 separated from these lymph nodes by first cutting the lymph nodes into small pieces and suspending them in Hank's balanced salt solution (HBSS). The suspension was then passed through a stainless mesh to separate large tissue blocks, and centrifuged on Ficoll* density
10 gradient solution to condense and separate the lymphocytes. The E-rosette formation technique was used to remove T-lymphocytes, and the B-lymphocyte fraction was washed with HBSS three times and then were incubated with Epstein-Barr virus (EBV) for 20 hours in RPMI 1640
15 containing 10% fetal calf serum. Cells were cloned by the limiting dilution technique and monitored for antibody production by the IA assay as described previously. (29).

A human breast cancer cell line, MDA-MB 436 (31)
20 and a human melanoma cell line, UCLA-SO-M12 (32) were used as targets. While clones secreting antibodies positive to 436 breast cancer cell line ceased to produce antibodies very soon after establishment, those reacting to the M12 melanoma cell line continued to
25 produce antibody and were stable. Clones secreting antibodies were adapted to RPMI 1640 containing a lower concentration of fetal calf serum gradually. The clones were then recloned 7 times in serum free medium containing growth factor (FDA approved HB series serum
30 free medium obtained from Irvine Scientific Co.) (Irvine, CA). Doubling time of L612 cell line is less than one day. The cell line secretes greater than 20 μ g/ml IgM kappa monoclonal antibody. The L612 antibody in the spent medium was purified as previously described
35 (25). In addition, the antibody produced by the L612 cell line is isolated according to any of the conventional procedures used to remove and purify

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antibodies from cell cultures.

The purified L612 antibody was tested for reactivity with a variety of human tumor tissues. The L612 antibody has a strong cytotoxic activity to antigen
5 positive human tumor cells in the presence of complement. The reactivity was tested using both the immune adherence (IA) and immune adherence absorption (IAA) assays. The results are summarized in TABLE I.

TABLE I

5	Reactivity of L612 to Human Cancer and Non-cancer Tissues.		
10		#positive/ #tested	Specimens Relative anti- genicity*
<u>Biopsied Tissues</u>			
	Melanoma	13/21**	+++
15	<u>Peripheral Blood Cells</u>		
	Lymphocytes	0/32**	-
	Erythrocytes	0/44**	-
20	<u>Cultured cell lines</u>		
	Melanoma	12/16	+++
	Lung	4/4	++
	Breast	5/7	+
	Gastro-intestine	2/7	+
	Erythroleukemia	0/1	-
25	T-Cell leukemia	1/1	+
	Myeloma	0/1	-
	Neuroblastoma	0/1	-
	B-lymphoblasts***	0/11**	-
30	* In the direct IA assay a partially purified HuMAb L612 (0.116 µg IgM/ml) was used.		
35	** These tissues were tested by the IA absorption assay.		
40	*** These were derived from Epstein-Barr virus transformed peripheral blood lymphocytes of melanoma patients.		
45	A total of 21 biopsied melanoma, peripheral blood lymphocytes (PBLs) from 32 different donor and erythrocytes from an additional 44 donors were tested. Thirteen of 21 melanoma tissues (62%) were antigen positive in contract, none of PBLs (0/32) and erythrocytes (0/44) were positive. Cultured human malignant cell lines also were investigated for the presence of the antigen (Table 1). Of 16 melanoma cell lines tested by the direct IA assay, 12 (75%) were		
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positive. Positive reactivity of other cancer cell lines were as follows: lung cancer, 4/4; breast cancer, 5/7; gastro-intestinal cancer, 2/7; other types of cancer, 1/4. However, none of eleven Epstein-Barr virus transformed B-cell lines autologous to the melanoma cell lines tested were positive even by the sensitive IA absorption technique. Though the percent positive specimens of melanoma was lower than that of lung cancer, overall reactivity per specimen was much higher in melanoma than other histologic type of cancer. Of the melanoma cell lines tested M15 and M12 showed the highest reactivity. Treatment of the cells with neuraminidase completely abolished the antigenicity of the cell lines while treatment with trypsin did not.

The reactivity of the L612 antibody with various authentic glycolipids was also determined. The glycolipids and their respective antigen titer with respect to L612 antibody are listed in Table II.

20

TABLE II

	Neutral Glycolipid	Antigen Titer
25	GbOse ₃ -Cer (CTH)	0
	GbOse ₄ -Cer (Globoside)	0
	GgOse ₄ -Cer (Asialo-GM ₁)	0
30	<u>Gangliosides</u>	
	I ³ NeuAc-Gal-Cer (GM ₄)	64
	II ³ NeuAc-Lac-Cer (GM ₃)	64
	II ³ NeuGc-Lac-Cer (GM ₃)	0
	II ³ NeuAc-GgOse ₃ -Cer (GM ₂)	0
35	II ³ NeuAc-GgOse ₄ -Cer (GM ₂) (GM _{1a})	0
	IV ³ NeuAc-nLcOse ₄ -Cer (SPG)	16
	IV ³ NeuGc-nLcOse ₄ -Cer	0
	II ³ NeuAc ₂ -Lac-Cer (GD ₃)	0
	II ³ NeuAc ₂ -GgOse ₃ -Cer (GD ₂)	0
40	IV ³ NeuAc-, II ³ NeuAc-GgAc-GgOse ₄ -Cer (GD _{1a})	0
	II ³ NeuAc ₂ -GgOse ₄ -Cer (GD _{1b})	0
	IV ³ NeuAc-, II ³ NeuAc ₂ -GgOse ₄ -Cer (GT _{1b})	0

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The authentic glycolipids (5 nmol), were tested for L612 antigen activity by the IA inhibition test. Three gangliosides, GM₄, GM₃ and PSG showed positive reactivity; however, two of these, GM₄ and GM₃ showed stronger binding (1:64) than SPG (1:16). Other gangliosides including II³NeuGc-Lac-Cer, IV³NeuGc-nLcOse₄-Cer, GM₂, GM_{1a}, GD₃, GD₂, GD_{1a}, GD_{1b} and GT_{1b}, and neutral glycolipids including GbOse₃-Cer, GbOse₄-Cer and GgOse₄-Cer showed no antigenic activity. To further confirm the above results, ELISA and enzyme immunostaining on TLC plates with authentic glycolipids were performed. The results obtained by ELISA and enzyme immunostaining were similar to those of the IA inhibition assay. The ELISA was performed using 15 authentic glycolipids bound to microtiter wells. Again, the three gangliosides, GM₃, GM₄ and SPG showed clear binding activity on solid phase ELISA. None of the remaining glycolipids showed reactivity.

Enzyme immunostaining of TLC plates with GM₄, GM₃, SPG, GD₃, GD₂, GM₂, N-glycoryl GM₃, CDH, globoside and asialo GM₁ (lug) was also performed. Strong positivity was shown with GM₃ and GM₄. Milder reactivity was observed with SPG. Very faint reactivity was observed with GM₂. Other glycolipids failed to be stained. Results of these three different types of immunologic assays demonstrate that HuMab L612 detects the terminal sugar of gangliosides, such as GM₃ and GM₄, which have a NeuAc α 2-3 Galactose residue.

Immunostaining of frozen sections with HuMab L612 indicated a strong specificity for neoplastic tissue, including melanoma, colon adenocarcinomas and lung adenocarcinomas. HuMab provides an excellent marker for identifying certain types of neoplastic tissues. It has been shown that HuMab L612 binds to renal cell carcinomas (35).

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The DNA sequence of the variable regions for both the light and heavy chains of the L612 antibody were determined by polymerase chain reaction (PCR). The guanidium thiocyanate/cesium chloride procedure was used to prepare total RNA from the L612 B lymphoblast line (34). Ten μg of RNA was mixed with 60 pmol of either μ heavy or kappa light chain 3' primers and heated at 70°C for 10 min. The mixture was then added to 50 μl reverse transcriptase reaction solution containing 10 μl 5X reverse transcriptase buffer (BRL), 4 μl 10 mM dNTP mix (200 μM of dATP, dCTP, dGTP, and dTTP final concentration) and 3 μl 600 units reverse transcriptase (Superscript, BRL). The mixture was incubated at 37°C for 1 hour.

A Standard PCR reaction was then performed with: 97 μl of PCR mixture was added to 3 μl of RNA-cDNA mixture. The PCR mixture contained 10X PCR buffer (Perkin Elmer, CA), 10 mM dNTPs mix at 60 μM final concentration of each dNTP, 5 units of Taq polymerase (Perkin Elmer), and 60 μM appropriate 5' and 3' heavy and light chain primers. The mixtures were subjected to 35 cycles of amplification at 91°C for 1 min., 52°C for 2 min., and 72°C for 1.5 min. followed by a final incubation at 72°C for 10 min. in a Perkin Elmer*/Cetus thermal cycler. An aliquot of the PCR product was on a 2% agarose gel to verify the correct size band product. The μ heavy chain and kappa light chain primers produced a 495 and 603 base pair cDNA product, respectively. When the gel showed a correct size single product the remainder of the PCR-cDNA product was subjected to PCR to obtain more product.

The gel products were isolated, pooled, subjected to phenol/chloroform extraction and ethanol precipitation. The DNA was then digested with appropriate restriction enzymes, extracted, precipitated and purified with GeneClean* (Bio101, CA). The DNA was ligated into appropriate cut restriction sites of

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Bluescript vector (Stratagene, CA). Ten independent clones of the variable region *mu* chain and four independent clones of the variable region *kappa* chain were isolated and sequenced. A J_H heavy chain probe was used for screening and verifying the heavy chain clones. Sequencing was done by the dideoxynucleotide method with T7 DNA polymerase (Sequenase, USB, Cleveland, OH) according to the manufacturer's protocol. The primers used were as follows. Heavy *mu* chain leader primers (3 primers) (29):

GGGAATTCATGGACTGGACCTGGAGG (AG) TC (CT) TCT (GT) C;
GGGAATTCATGGAG (CT) TTGGGCTGA (CG) CTGG (CG) TTT (CT) T; and
GGGAATTCATG (AG) A (AC) (AC) (AT) ACT (GT) TG (GT) (AT) (CG) C (AT) (CT) (CG) CT (CT) CTG. This contains an EcoRI (underlined) restriction site to facilitate cloning. Heavy *mu* chain J region primer was CCAAGCTTAGACGAGGGGAAAGGGTT. This contains an *Hind* III site (underlined). Light *kappa* chain leader primers were as follows (2 primers) (30). GACATCGAGCTCACCCAGTCTCCA; and GAAAT TGAGCTCACGAGTCTCCA. These primers contain an *Sac* I site (underlined). The light *kappa* chain J region primer was GCGCCGTCTAGAACTAACA CTCTCCCTGTTGAAGCTCTTTGTGACGGGCAAG. This primer contains an *Xba* site (underlined).

The cloned cDNA nucleotide sequence and the encoded amino acid sequence for the variable region of the heavy chain is set forth in SEQ ID NO:1. The heavy chain variable region amino acid sequence above is set forth in SEQ ID NO: 2. The cloned cDNA nucleotide sequence and the encoded amino acid sequence for the variable region of the light chain is set forth in SEQ ID NO:3. The light chain variable region amino acid sequence above is set forth in SEQ ID NO:4.

The nucleotide/amino acid sequence for the heavy chain variable region is also shown in FIG. 1. The complimentary determining regions (CDR's) 1, 2 and 3 are underlined. The nucleotide/amino acid sequence for the light chain variable region is also shown in FIG. 2.

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The CDR's 1, 2 and 3 are also underlined.

The L612 antibody in accordance with the present invention is administered to patients for treating tumors which contain the GM3 ganglioside or NeuAc2-3 Galactose epitope. The GM3 ganglioside includes a terminal sugar having a Neu α 2-3 Galactose residue. Any of the conventional procedures used for administering antibodies to patients for treating such tissues may be used. These procedures include intravenous or intraperitoneally injection and intralesional injection. Intralesional injection is a preferred method of administration for cutaneous recurrent tumors. The L612 antibody and the antibody producing cell line can be modified or altered by known procedures to form other immunoglobulin isotypes and cells producing such which may efficiently bind and kill tumor cells (33). The particular dosage used will vary depending upon tumor antigenicity and can be determined according to known procedures for administering antibodies such as L55 and L72. The L612 monoclonal antibody reacts strongly with human melanoma tumor biopsies. The L612 antibody also reacts less strongly with human tumor biopsies from lung cancer, breast cancer, pancreatic cancer, colon cancer and kidney cancer. The UCLASO-M12 melanoma cell line was identified as the most reactive cell line among the lines tested with the L612 monoclonal antibody. The UCLASO-M12 cell line is maintained at the Division of Surgical Oncology at the University of California at Los Angeles School of Medicine.

L612 HuMab was used as therapeutic agent for the treatment of cutaneous malignant melanoma. HuMab L612 was purified for human use as previously described (25). It was demonstrated in previous studies that intralesional injection of human anti-G_{D2} HuMab L72 could induce regression of cutaneous melanoma (23). In the present example the effect of HuMab L612 and HuMab L72 is compared.

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The 84 year old female underwent surgical excision of a rapidly growing cutaneous melanoma lesion (1.9 x 1.6 cm) on her left cheek. Pathological examination of the tissue section revealed a melanoma of Clark's level
5 IV. The ganglioside expression of the lesion biopsy was found to be moderately positive for G_{M3} and negative for G_{D2}. Two weeks after surgery, a recurrence developed at the incision site. The upper lesions were treated weekly with 1 ml HuMab L612, and the lower lesions were
10 treated with 1 ml HuMab L72 HuMab. After treatment for one month (3 treatments), necrosis and softening of lesions treated with HuMab L612 were observed. In contrast treated with HuMab L72 HuMab showed no response, and in fact, had further progressed. The
15 patient eventually expired due to extensive metastases in the liver and brain.

The L612 antibody is also useful in producing hybridomas which in turn can be used to produce anti-ids for use as surrogate antigens or diagnostic reagents.
20 The production of hybridomas and subsequent generation of anti-ids are described in the following exemplary procedure:

BALB/c mice were immunized by a subcutaneous injection of 200 µg of purified L612 monoclonal antibody
25 in complete Freund's adjuvant. After 2 weeks the animals were boosted by another subcutaneous injection of L612 in incomplete Freund's adjuvant. Eleven days following the booster, the mice were injected intraperitoneally with 200 µg of L612 in saline. After
30 three days the spleens were removed and the splenocytes fused with myeloma cell line SP2/O using the standard procedure to produce hybridomas.

After HAT medium selection, hybridoma culture wells were tested for antibody using ELISA. Hybridomas
35 secreting anti-ids (AB2) were identified by their strong binding reactivity to HuMab L612 and absent reactivity to three other control human IgMs: L55, L72, and human

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serum IgM. Unrelated proteins used as antigens included fetal bovine serum and human serum albumin. 50 μ l of IgMs or proteins (50 μ g/ml) were coated on a 96-well ELISA plate and served as antigens to detect AB2.

5 Peroxidase-conjugated goat anti-mouse IgG + IgM was used as the AB2 detection probe followed by substrate and reading absorbency at 490 nm as described previously (19).

HAT selection of approximately 2500 hybridoma

10 culture wells which were prepared as described above yielded 40 hybridomas secreting antibodies with distinct reactivity to L612 HuMAb, but no reactivity to three other control human IgMs and two unrelated serum protein antigens. To determine whether these anti-L612

15 antibodies were AB2 beta-type directed against the antigen combining site of L612, or were AB2 alpha antibodies bound to peptide regions outside the antigen-combining site of L612, the inhibitory activity of these anti-L612 antibodies against L612 binding to

20 GM3 positive target cell lines or to the purified antigen, ganglioside GM3, was tested by using three assay systems: IA inhibition, cell-ELISA inhibition, and GM3-ELISA inhibition. Of the 40 antibodies tested, seven inhibited L612 binding to an antigen positive

25 target melanoma cell line, (UCLASO-M12), and to GM3 greater than 50% in the assays, while 12 others had weak or no inhibitory activity.

Of the seven inhibitory anti-ids, one identified as 4C10 was selected for cloning as the preferred beta-type

30 anti-id for use in treating tumors. From the non-inhibitory group the anti-id identified as 18C6 was selected for cloning as the preferred alpha-type anti-id for use in immunodiagnostic assays. Both anti-ids, 4C10 and 18C6, were tested with isotype antiglobulins and

35 found to be of the IgG1 class and contain kappa light chains.

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The 4C10 and 18C6 cloned hybridoma cell lines were grown in FCS-containing RP MI 1640 medium and secreted 5-10 $\mu\text{g/ml}$ of antibody into culture supernatants. Titers of the anti-ids in these culture supernatants against L612 by ELISA ranged between 1:200 to 1:1000/ 10^6 hybridoma. Anti-id 18C6 demonstrated low binding inhibition of HuMAb L612 to target cells in the IA assay and to ganglioside GM3 in ELISA whereas 4C10 at the same antibody concentration showed strong inhibition in both the ELISA assay and the IA assay. As a control assay, 4C10 and 18C6 failed to inhibit the binding of an unrelated antigen system, HuMAb L72, to M14 target cells, or to GD2 antigen. The lack of binding inhibition of 18C6 indicates a binding location on L612 antibody outside the GM3 antigen combining site, and the specific binding inhibition of 4C10 indicates its binding location to be within or near the antigen combining site.

The hybridoma cell lines which secrete the 4C10 and 18C6 monoclonal anti-ids are being maintained at the Division of Surgical Oncology at the University of California at Los Angeles School of Medicine.

The 4C10 anti-id and other beta-type anti-ids raised against L612 can be used alone or in combination with other agents to treat tumors. They are preferred for use in treating melanoma tumors. These beta-type anti-ids may also be used as an immunomodulator to enhance anti-cancer immunity, suppress organ transplant rejection and suppress autoimmune disease.

The beta-anti-ids may be administered by any of the conventional procedures used to introduce antibodies into patients. These procedures include subcutaneous, intravenous or intratumor injection. The beta-type anti-ids are preferably conjugated with KLH and emulsified in a suitable carrier such as Freund's complete adjuvant. The particular doses used for the beta-type anti-ids will vary depending upon the tumor

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being treated and numerous other factors. The dosage levels are established by the known techniques and principles generally recognized and utilized in treating patients with antigen immunization agents or monoclonal
5 antibodies.

The immunogenic usefulness of the beta-type anti-ids to L612 antibody was demonstrated as follows:

Five syngeneic Balb/c mice were immunized with purified 4C10-KLH. As controls, four mice were
10 immunized with mouse IgG1-KLH and one mouse with KLH alone. The immunized sera were monitored by ELISA using purified GM3 as the antigen source and by the IA assay using the antigen positive M12 melanoma cell line. In the ELISA, peroxidase conjugated goat anti-mouse IgM +
15 IgG (Boeringer Mannheim) was used as a second antibody.

Measurable antibody (AB3) was produced in three of the five immunizations with 100 μ g 4C10-KLH. The immunized sera bound to GM3 but not to CDH (asialo-GM3). Sera from the five mice immunized with IgG-KLH or KLH
20 alone gave no response to either glycolipid. In further analysis to determine the Ig class of the AB3 (ELISA and TLC immunostaining), the majority of the reactivity was identified as IgM.

In order to exclude the species specific natural
25 antibodies that might react to M12 cells in the IA assay, the immunized murine sera were pre-absorbed by human red blood cells at 4°C overnight. An IA score of 4+ was obtained at 1:10 dilution of the absorbed sera. Control sera gave no reactivity even at 1:2 dilution.
30 To confirm that the positive reactivity was directed against GM3 antigen on the cell surface, IA inhibition was performed using GM3 (10 μ g), CDH (10 μ g), 4C10 (10 μ g) and unrelated IgG1 (10 μ g) purified from Balb/c hybridoma ascites. While reactivity was completely
35 inhibited by GM3 or purified 4C10, no inhibition was obtained with CDH or unrelated IgG1.

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The above example demonstrates that beta-type anti-ids produce AB3 anti-bodies which are immunoreactive with melanoma tumors. Accordingly, these beta-type anti-ids which are raised in response to the L612 antibody are effective as an immunization agent in the treatment of melanoma.

The alpha-type anti-ids produced in response to the L612 antibody may be used immunodiagnostic procedures such as a three-step cell-ELISA procedure and a three step immunoperoxidase staining of tumor tissue sections. Examples of practice are as follows:

Three-step cell-ELISA

Viable M12 cells (1×10^5) were plated onto a U-bottom 960 well microtiter plate (Immulon-1, Dynatec) after pre-blocking with 1% BSA-PBS. 50 μ l of L612 (100 μ g/ml) were added and incubated for 1 hour at room temperature. After washing the mixture to remove unbound HuMAb L612, the cells were incubated with murine monoclonal anti-id 18C6 (100 μ g/ml) for 1 hour at room temperature. After washing, 50 μ l of peroxidase-conjugated goat anti-mouse IgG antibody (1/10,000 diluted) (Jackson Immuno Research) were added and the plate was incubated for 30 minutes. After washing with PBS solution, the substrate for peroxidase was added and binding activity was determined as a function of absorbance at 490 nm with a V_{max} kinetic microplate reader.

When the antigen combining sites of L612 are occupied by GM3 expressed on tumor cells, the cell bound HuMAb L612 should have reduced its binding activity to anti-id beta, yet still retain full binding activity to anti-id alpha. The above-described procedure confirmed this process using cultured M12 melanoma cells which express a high density of the corresponding antigen.

The above-described cell binding assay represents a modified form of the ELISA technique. Several control

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assays were included to establish the specificity of the positive reaction. Control anti-ganglioside HuMAbs included L55 (IgM anti-GM2) and L72 (IgM anti-GD2), both of which exhibit strong binding ability to the GM2 and GD2 rich M14 melanoma cell line (26, 27). The anti-id 186C6 reacted strongly to M12 cells after pre-incubation with HuMaB L612, but did not react to M14 cells that were pre-incubated with L55 or L72 HuMAb. The peroxidase-conjugated anti-mouse Ig also failed to react with M12 cells in other controls including murine anti-id alone, L612, or L612 plus (anti-id beta).

The cell-ELISA assay was then applied to several other human tumor cell lines. A two-step cell binding assay (HuMAb + peroxidase-conjugated anti-human IgM) was compared with the three-step cell-binding assay to evaluate the validity of the three-step assay. The three-step assay had parallel reactivity with the two-step assay and was slightly more sensitive in almost every cell line. This data indicates that the ELISA absorbency value of the three-step assay accurately reflects differences in the density of cell surface GM3 antigens and correlates closely with the two-step in vitro assays.

25 Three-step immunoperoxidase staining of tissue sections

Tissue sections 4 μ m thick were cut from tissues freshly frozen in OTC compound and immediately fixed in cold formaldehyde buffer (12 g Tris buffer, 9 g sodium chloride, 40 ml 37% formaldehyde, pH 7.4) and air dried. Slides were dipped in Tris buffer for five minutes then treated with 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase activity.

After washing in running water for five minutes, sections were overlaid with 5% normal human serum for 20 minutes. HuMAb L612 (10 μ g IgM in 200 μ l) was then applied and incubated for 45 minutes. The slides were washed in Tris buffer for 5 minutes, the purified

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anti-id 18C6 (10 μ g IgG1 in 200 μ l) was applied and incubated for 30 minutes.

After washing the slides again, the third antibody, a biotinylated goat anti-mouse IgG (Vector Laboratories, Burlingame, CA) at 1/100 dilution was applied and incubated for 25 minutes. Peroxidase-conjugated streptavidin (1/1000 dilution) (Zymed Laboratories, San Francisco) was added after washing and incubated for 20 minutes. After washing, the slides were immersed in substrate solution containing 6 ml amino-ethyl carbazole, 50 ml of 0.02 M sodium acetate buffer (pH 5.1), and 0.4 ml of freshly prepared 3% hydrogen oxide for 5 minutes. The slides were washed once more in tap water, counterstained with hematoxylin, and cover-slips applied to the stained sections using glycerol-gelatin. The immunoperoxidase three-step assay was applied successfully to detect binding of L612 antibody to surgically biopsied tumor tissues that had been snap frozen.

Having thus described exemplary embodiments of the present invention, it should be noted by those skilled in the art that the within disclosures are exemplary only and that various other alternatives, adaptations and modifications may be made within the scope of the present invention. For example, the L612 antibody may be used to make chimeric antibodies which are also useful in a variety of treatments or as diagnostic reagents. Accordingly, the present invention is not limited to the specific embodiments as illustrated herein, but is only limited by the following claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Irie, Reiko F

(ii) TITLE OF INVENTION: HUMAN B-LYMPHOBLASTOID CELL LINE
SECRETING ANTI-GANGLIOSIDE ANTIBODY

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Poms, Smith, Lande & Rose
(B) STREET: 2121 Avenue of the Stars Suite 1400
(C) CITY: Los Angeles
(D) STATE: California
(E) COUNTRY: United States of America
(F) ZIP: 90067

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/609803
(B) FILING DATE: 05-NOV-1990

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Oldenkamp, David J
(B) REGISTRATION NUMBER: 29421
(C) REFERENCE/DOCKET NUMBER: 94268

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 3107885046
(B) TELEFAX: 3102771297

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 432 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (G) CELL TYPE: Epstein Barr Virus Transformed B cell
- (H) CELL LINE: L612

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..432
- (D) OTHER INFORMATION: /function= "Heavy Chain"
/product= "Immunoglobulin Variable Region"
/standard_name= "HuMab L612 Heavy Chain Variable
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(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 148..162
- (D) OTHER INFORMATION: /function= "Complementary
determining region 1 (CDR1)"

(ix) FEATURE:

- (A) NAME/KEY: misc-feature
- (B) LOCATION: 271..300
- (D) OTHER INFORMATION: /function= "Complementary
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(ix) FEATURE:

- (A) NAME/KEY: misc-feature
- (B) LOCATION: 397..429
- (D) OTHER INFORMATION: /function= "Complementary determining

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CCT GGG GGG TGC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT	144
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GAC TCC GTG AAG GGC CGG TTC ACC ATC TCC AGA GAC AAA TCC AAG AAC	288
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Lys Ser Lys Asn	
85 90 95	
ACG TTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACG GCC GTA	336
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(2) INFORMATION FOR SEQ ID NO:2:

2156857

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 144 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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			20					25					30			
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		35					40					45				
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Glu	Trp	Val	Ser	Ala	Ile	Ser	Gly	Ser	Gly	Gly	Ser	Thr	Tyr	Tyr	Ala	
65					70					75					80	
Asp	Ser	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Lys	Ser	Lys	Asn	
				85					90					95		
Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	
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Tyr	Tyr	Cys	Ala	Lys	Gly	Gly	Asn	Asp	Ile	Leu	Thr	Gly	Tyr	Tyr	Ala	
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(C) INDIVIDUAL ISOLATE: Epstein Barr Virus transformed B
cell

(G) CELL TYPE: B-cell

(H) CELL LINE: L612

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..360

(D) OTHER INFORMATION: /function= "Immunoglobulin light
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/product= "HuMab L612 Light Chain Variable Region"

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 58..108

(D) OTHER INFORMATION: /function= "Complementary
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(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 154..174

(D) OTHER INFORMATION: /function= "Complementary
determining region 2 (CDR2)"

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 271..297

(D) OTHER INFORMATION: /function= "Complementary
determining region 3 (CDR3)"

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Thr	Gln	Ser	Pro	Asp	Ser	Leu	Ala	Val	Ser	Leu	Gly	Glu	Arg	Ala	Thr
1					5				10					15	

48

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20 25 30	
AAC TAC TTA GCT TGG TAC CAG CAG AAA CCA GGA CAG CCT CCT AAG CTG	144
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- (A) LENGTH: 120 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu					
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					80
Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Thr					
		85		90	95
Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val					
	100		105		110
Ala Ala Pro Ser Val Phe Ile Phe					
	115		120		

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CLAIMS:

1. A human B-lymphoblastoid cell line which is deposited at the American Type Culture Collection under ATCC accession number CRL 10724.
- 5 2. An anti-ganglioside antibody which is secreted by the cell line of claim 1 or isotypes thereof.
3. An antibody which comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2.
- 10 4. An antibody which comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 4.
5. An antibody according to claim 3 wherein said antibody additionally comprises a light chain variable
15 region comprising the amino acid sequence set forth in SEQ ID NO: 4.
6. An antibody which comprises a heavy chain variable region comprising complementary determining regions as set forth in FIG. 1.
- 20 7. An antibody which comprises a light chain variable region comprising complementary determining regions as set forth in FIG. 2.
8. An antibody according to claim 6 wherein said antibody additionally comprises a light chain variable
25 region comprising complementary determining regions as set forth in FIG. 2.
9. A composition comprising the antibody according to any one of claims 2 to 8, and a pharmaceutically acceptable carrier.

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10. A nucleotide sequence encoding at least a portion of an antibody, the nucleotide sequence encoding a sequence selected from the amino acid sequences set forth in SEQ ID NO: 2 and SEQ ID NO: 4.

5 11. Use of the antibody according to any one of claims 2 to 8 in the manufacture of a medicament for the treatment of tumor tissue which contain ganglioside antigens $\text{II}^3\text{NeuAc-Lac-Cer}(\text{GM}_3)$, GM_4 or $\text{IV}^3\text{NeuAc-nLcOse}_4\text{-Cer}(\text{SPG})$ that bind to an antibody secreted by a human β -lymphoblastoid
10 cell line which is deposited at the American Type Culture Collection (ATCC) under ATCC accession number CRL10724 and isotypes thereof.

12. Use of the antibody according to any one of claims 2 to 8 for the treatment of tumor tissue which
15 contain ganglioside antigens $\text{II}^3\text{NeuAc-Lac-Cer}(\text{GM}_3)$, GM_4 or $\text{IV}^3\text{NeuAc-nLcOse}_4\text{-Cer}(\text{SPG})$ that bind to an antibody secreted by a human β -lymphoblastoid cell line which is deposited at the American Type Culture Collection (ATCC) under ATCC accession number CRL10724 and isotypes thereof.

20 13. The use according to claim 11 or 12 wherein the tumor tissue is melanoma tumor tissue.

14. The use according to claim 11 wherein the medicament for the treatment of tumor tissue is adapted for administration by intralesional injection.

SMART & BIGGAR

OTTAWA, CANADA

PATENT AGENTS

1/2

-19				-15						-10					-5
Met	Glu	Phe	Gly	Leu	Thr	Trp	Leu	Phe	Leu	Val	Ala	Asn	Leu	Lys	
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				1				5					10		
Gly	Val	Gln	Cys	Glu	Val	Gln	Leu	Leu	Asp	Ser	Gly	Gly	Gly	Leu	
GGT	GTC	CAG	TGT	GAG	GTG	CAG	CTG	TTG	GAT	TCT	GGG	GGA	GGC	TTG	
			15					20					25		
Val	Gln	Pro	Gly	Gly	Cys	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	
GTA	CAG	CCT	GGG	GGG	TGC	CTG	AGA	CTC	TCC	TGT	GCA	GCC	TCT	GGA	
			30					35					40		
Phe	Thr	Phe	Ser	<u>Ser</u>	<u>Cys</u>	<u>Ala</u>	<u>Met</u>	<u>Ser</u>	Trp	Val	Arg	Gln	Ala	Pro	
TTC	ACC	TTT	AGC	AGC	TGT	GCC	ATG	AGC	TGG	GTC	CGC	CAG	GCT	CCA	
			45					50		52	52a		55		
Gly	Lys	Gly	Leu	Glu	Trp	Val	Ser	<u>Ala</u>	<u>Ile</u>	<u>Ser</u>	<u>Gly</u>	<u>Ser</u>	<u>Gly</u>	<u>Gly</u>	
GGG	AAG	GGG	CTG	GAG	TGG	GTC	TCA	GCT	ATT	AGT	GGT	AGT	GGT	GGT	
			60					65					70		
<u>Ser</u>	<u>Thr</u>	<u>Tyr</u>	<u>Tyr</u>	<u>Ala</u>	<u>Asp</u>	<u>Ser</u>	<u>Val</u>	<u>Lys</u>	<u>Gly</u>	Arg	Phe	Thr	Ile	Ser	
AGC	ACA	TAC	TAC	GCA	GAC	TCC	GTG	AAG	GGC	CGG	TTC	ACC	ATC	TCC	
			75					80		82	82a	82b	82c		
Arg	Asp	Lys	Ser	Lys	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	
AGA	GAC	AAA	TCC	AAG	AAC	ACG	TTG	TAT	CTG	CAA	ATG	AAC	AGC	CTG	
			85					90				95			
Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Lys	<u>Gly</u>	<u>Gly</u>	<u>Asn</u>	
AGA	GCC	GAG	GAC	ACG	GCC	GTA	TAT	TAC	TGT	GCG	AAA	GGT	GGC	AAC	
			100	A	B	C				105					
<u>Asp</u>	<u>Ile</u>	<u>Leu</u>	<u>Thr</u>	<u>Gly</u>	<u>Tyr</u>	<u>Tyr</u>	<u>Ala</u>	Trp	Gly	Gln	Gly	Thr	Leu	Val	
GAT	ATT	TTG	ACT	GGT	TAT	TAT	GCT	TGG	GGC	CAG	GGA	ACC	CTG	GTC	
			110					115							
Thr	Val	Ser	Ser	Gly	Ser	Ala	Ser	Ala							
ACC	GTC	TCC	TCA	GGG	AGT	GCA	TCC	GCC							

Nucleotide and deduced amino acid sequence of the heavy chain of HuMab L612. Underlined amino acids represent the complementary determining regions CDR1, CDR2 and CDR3 in order.

FIG. 1

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1
Asp Ile Val Met

5					10					15					
Thr	Gln	Ser	Pro	Asp	Ser	Leu	Ala	Val	Ser	Leu	Gly	Glu	Arg	Ala	
ACC	CAG	TCT	CCA	GAC	TCC	CTG	GCT	GTG	TCT	CTG	GGC	GAG	AGG	GCC	

20					25		27	27a	27b	27c	27d	27e	27f		
Thr	ILe	Asn	Cys	<u>Lys</u>	Ser	Ser	Gln	Ser	Val	Leu	Tyr	Ser	Ser	Asn	
ACC	ATC	AAC	TGC	AAG	TCC	AGC	CAG	AGT	GTT	TTA	TAC	AGC	TCC	AAC	

30						35					40				
<u>Asn</u>	<u>Lys</u>	<u>Asn</u>	<u>Tyr</u>	<u>Leu</u>	<u>Ala</u>	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Pro	
AAT	AAG	AAC	TAC	TTA	GCT	TGG	TAC	CAG	CAG	AAA	CCA	GGA	CAG	CCT	

45						50				55				
Pro	Lys	Leu	Leu	Ile	Tyr	<u>Trp</u>	Ala	Ser	Thr	Arg	<u>Glu</u>	<u>Ser</u>	Gly	Val
CCT	AAG	CTG	CTC	ATT	TAC	TGG	GCA	TCT	ACC	CGG	GAA	TCC	GGG	GTC

60						65				70				
Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu
CCT	GAC	CGA	TTC	AGT	GGC	AGC	GGG	TCT	GGG	ACA	GAT	TTC	ACT	CTC

	75					80						85			
Thr	Ile	Ser	Ser	Leu	Gln	Ala	Glu	Asp	Val	Ala	Val	Tyr	Tyr	Cys	
ACC	ATC	AGC	AGC	CTG	CAG	GCT	GAA	GAT	GTG	GCA	GTT	TAT	TAC	TGT	

90					95					100				
<u>Gln</u>	<u>Gln</u>	<u>Tyr</u>	<u>Tyr</u>	<u>Ser</u>	<u>Thr</u>	<u>Pro</u>	<u>Pro</u>	<u>Thr</u>	<u>Phe</u>	<u>Gly</u>	<u>Gln</u>	<u>GLy</u>	<u>Thr</u>	<u>Lys</u>
CAG	CAA	TAT	TAT	AGT	ACT	CCT	CCG	ACG	TTC	GGC	CAA	GGG	ACC	AAG

	105					110									
Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	
GTG	GAA	ATC	AAA	CGA	ACT	GTG	GCT	GCA	CCA	TCT	GTC	TTC	ATC	TTC	

Nucleotide and deduced amino acid sequence of the light chain of HuMab L612. Underlined amino acids represent the complementary determining regions CDR1, CDR2, and CDR3 in order.

FIG. 2