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(54) Treatment of tumors using specific anti-L1 antibody

Behandlung von Tumoren unter Verwendung eines spezifischen Anti-L1-Antikörpers

Traitement de tumeurs à l'aide d'un anticorps anti-L1 spécifique

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Description

[0001] The standard treatment of advanced cancer is often chemotherapy or radiotherapy. However, despite initial response to therapy, it is often observed that different carcinomas acquire resistance to chemotherapeutic drugs or radiotherapy leading to tumor recurrence and frequent death of the patients. Often, it is then decided to switch to another chemotherapeutic drug or to higher dosages. However, often no improvement of the clinical situation is observed.

[0002] L1 is a type I membrane glycoprotein of 200 to 230 kDa structurally belonging to the Ig superfamily (Moos M, Tacke R, Scherer H, Teplow D, Fruh K, Schachner M. Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. Nature 1988; 334:701-3). L1 plays a crucial role in axon guidance and cell migration in developing nervous system (Hortsch M. Structural and functional evolution of the L1 family: are four adhesion molecules better than one? Mol Cell Neurosci 2000; 15:1-10., Schachner M. Neural recognition molecules and synaptic plasticity. Curr Opin Cell Biol 1997; 9:627-34). Recent studies have also implicated L1 expression in the progression of human carcinomas. L1 expression was found on different tumors including lung cancer (Katayama M, Iwamatsu A, Masutani H, Furuke K, Takeda K, Wada H, et al. Expression of neural cell adhesion molecule L1 in human lung cancer cell lines. Cell Struct Funct 1997;22:511-6), gliomas (Senner V, Kismann E, Puttmann S, Hoess N, Baur I, Paulus W. L1 expressed by glioma cells promotes adhesion but not migration. Glia 2002;38:146-54), melanomas (Thies A, Schachner M, Moll I, Berger J, Schulze HJ, Brunner G, et al. Overexpression of the cell adhesion molecule L1 is associated with metastasis in cutaneous malignant melanoma. Eur J Cancer 2002;38:1708-1, Fogel M, Mechtersheimer S, Huszar M, Smirnov A, Abu DA, Tilgen W, et al. L1 adhesion molecule (CD 171) in development and progression of human malignant melanoma. Cancer Lett 2003;189:237-47), renal carcinoma (Meli ML, Carrel F, Waibel R, Amstutz H, Crompton N, Jaussi R, Moch H, Schubiger PA, Novak-Hofer I. Anti-neuroblastoma antibody chCE7 binds to an isoform of L1-CAM present in renal carcinoma cells. Int J Cancer, 1999; 83: 401-408, Allory Y, Matsuoka Y, Bazille C, Christensen EI, Ronco P, Debiec H. The L1 cell adhesion molecule is induced in renal cancer cells and correlates with metastasis in clear cell carcinomas. Clin Cancer Res 2005;11:1190-7) and colon carcinoma (Gavert N, Conacci-Sorrell M, Gast D, Schneider A, Altevogt P, Brabletz T, et al. L1, a novel target of beta-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. J Cell Biol 2005; 168:633-42). Furthermore, it is known in the art that L1 is overexpressed in ovarian and endometrial carcinomas in a stage-dependent manner (Fogel M, Gutwein P, Mechtersheimer S, Riedle S, Stoeck A, Smirnov A, et al. L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. Lancet 2003; 362:869-75).

[0003] In the art, it has been suggested to use anti-L1 antibodies for the treatment of ovarian and endometrial tumors (cf. WO 02/04952 , WO 06/013051 and Arlt MJ, Novak-Hofer I, Gast D, Gschwend V, Moldenhauer G, Grunberg J, et al. Efficient inhibition of intra-peritoneal tumor growth and dissemination of human ovarian carcinoma cells in nude mice by anti-L1-cell adhesion molecule monoclonal antibody treatment. Cancer Res 2006;66:936-43). In the art, various anti-L1 antibodies are known (e.g. mAb 14.10: Huszar M, Moldenhauer G, Gschwend V, Ben-Arie A, Altevogt P, Fogel M: Expression profile analysis in multiple human tumors identifies L1 (CD171) as a molecular marker for differential diagnosis and targeted therapy. Hum Pathol 37:1000-1008, 2006, mab chCE7: Meli ML, Carrel F, Waibel R, Amstutz H, Crompton N, Jaussi R, Moch H, Schubiger PA, Novak-Hofer I: Anti-neuroblastoma antibody chCE7 binds to an isoform of L1-CAM present in renal carcinoma cells. Int J Cancer 83:401-408, 1999, mAb UJ127.11: Patel K, Kiely F, Phimister E, Melino G, Rathjen F, Kemshead JT: The 200/220 kDa antigen recognized by monoclonal antibody (MAb) UJ127.11 on neural tissues and tumors is the human L1 adhesion molecule. Hybridoma 10:481-491, 1991, mAb 5G3: Wolff JM, Frank R, Mujoo K, Spiro RC, Reisfeld RA, Rathjen FG: A human brain glycoprotein related to the mouse cell adhesion molecule L1. J Biol Chem 263:11943-11947, 1988). Furthermore, in Sebens Müerkoster et al., Oncogene. 2007 Apr 26;26(19):2759-68, Epub 2006 Nov 6, it has been suggested to use anti-L1 antibodies for sensitizing tumor cells for the treatment with a chemotherapeutic drug or with radiotherapy.

[0004] There is always a need for improved anti-tumor agents.

[0005] The present invention relates in one aspect to a binding molecule capable of binding L1,

(a) being selected from the group consisting of single chain antibodies, scFv, multimers of scFv like diabodies, triabodies or tetrabodies, antibody fragments, Fab, tandabs, flexibodies, bispecific antibodies, and chimeric antibodies,
and/or
(b) which comprises at least one Ig domain,
and wherein the binding molecule capable of binding L1:

(i) is characterized in that its complementarity determining regions (CDRs) have the following sequences: LCDR1: RASQDISNYLN (SEQ ID No.: 24), LCDR2: YTSRLHS (SEQ ID No.: 25), LCDR3: QQGNTLPWT (SEQ ID No.: 26), HCDR1: RYWML (SEQ ID No.: 27), HCDR2: EINPRNDRTNYNEKFKT (SEQ ID No.: 28), and HCDR3: GGGYAMDY (SEQ ID No.: 29),

and which binding molecule binds L1 with an affinity (KD) of at least 10^{-10} M, or
(ii) is characterized in that its complementarity determining regions (CDRs) have the following sequences: LCDR1: QDISNY (SEQ ID No.: 30), LCDR2: YTS, LCDR3: QQGNTLPWT (SEQ ID No.: 31), HCDR1: GYTFTRYW (SEQ ID No.: 32), HCDR2: INPRNDRT (SEQ ID No.: 33), and HCDR3: ALGGGYAMDY (SEQ ID No.: 34),
and which binding molecule binds L1 with an affinity (KD) of at least 10^{-10} M.

[0006] In the context of the present invention, it has been surprisingly found that the monoclonal antibody 9.3, produced by the hybridoma cell deposited under DSMZ ACC2841, has improved anti-tumor capacities (see examples). Especially, the monoclonal antibody 9.3 has the best ability to inhibit tumor growth and invasion of tumor cells of all antibodies tested. Furthermore, the monoclonal antibody 9.3 seems to abolish chemoresistance to a greater extend than the antibody 11A tested in WO 2008/046529 (see example 13).

[0007] Monoclonal antibodies and the production of monoclonal antibodies belongs to the state of the art and is also described in the references cited in the Materials and Methods section of the examples. In general, monoclonal antibodies can, for example, be prepared in accordance with the known method of Winter & Milstein (Winter, G. & Milstein, C. (1991) Nature, 349, 293-299). An alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody directed against a polypeptide of the invention can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with the polypeptide of interest. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; WO 90/02809; Fuchs et al., 1991, Bio/Technology 9:1370-1372; Hay et al., 1992, Hum. Antibod. Hybridomas 3:81-85; Huse et al., 1989, Science 246:1275-1281; Griffiths et al., 1993, EMBO J. 12:725-734.

[0008] Since the effect of an antibody is mediated by its capacity to bind a specific epitope, the disclosure relates to all monoclonal antibodies recognizing the same epitope as the antibody 9.3. Methods for determining the epitope of a given antibody are known in the art and include the preparation of synthetic linear peptides of a given region of interest and the subsequent testing whether the antibody binds to said peptides (see Epitope Mapping, A practical approach, Oxford University Press 2001, Editors: Olwyn Westwood and Frank Hay). Alternatively, different recombinant proteins covering the region of interest can be produced and tested for the binding of the antibody (Oleszewski, M., Gutwein, P.,von der Lieth ,W., Rauch,U., Altevogt ,P. Characterization of the L1-neurocan binding site. Implications for L1-L1 homophilic binding. J.Biol.Chem. 275: 34478-34485 (2000).

[0009] Furthermore, once a specific epitope of a monoclonal antibody is known, it is within the skill of the person skilled in the art to identify or prepare other antibodies, especially monoclonal antibodies, or binding molecules as defined below which bind to the same epitope. For example, it is possible to use the peptides or proteins described above in the context of the epitope mapping also for the identification or production of said antibodies or binding molecules.

[0010] As it can be taken from the examples, the epitope of the antibody 9.3 is with the first immunoglobulin-like domain of L1. Therefore also the epitope of the monoclonal antibody is preferably within the first immunoglobulin-like domain of L1.

[0011] Disclosed is an anti-L1 monoclonal antibody, having the same capacity to inhibit tumor growth as the monoclonal antibody 9.3, produced by the hybridoma cell deposited under DSMZ ACC2841. This capacity can be tested by using the same tumor growth assay as described in the Example 1, section 1.3.9. According to the invention, "the same capacity" means that the monoclonal antibody has a tumor growth inhibiting capacity which does not differ more than 5 % from the tumor growth inhibiting capacity of the monoclonal antibody 9.3.

[0012] Preferably, this antibody also inhibits L1 dimerization, as it has been shown for the antibody 5G3 (see above).

[0013] Disclosed is an anti-L1 monoclonal antibody, characterized in that at least one of its complementarity determining regions (CDRs)

a) has one of the following sequences RASQDISNYLN, YTSRLHS, QQGNTLPWT, RYWML, EINPRNDRTNYNEKFKT, or GGGYAMDY or

b) has a sequence which, in comparison to the sequences mentioned under a) has at least one conservative amino acid exchange.

[0014] The above mentioned sequences show the CDRs of the monoclonal antibody 9.3 determined according to the method of Kabat (see Example 2). Such a monoclonal antibody can, e.g. be produced by CDR grafting or by recombinant production of the antibody. Such methods are known in the art (see e.g. Queen, U.S. Patent No. 5,585,089 and Winter, U.S: 5,225,539, Cabilly U.S. 4,816,567).

[0015] Also disclosed is an anti-L1 monoclonal antibody, characterized in that at least one of its complementarity determining regions (CDRs)

a) has one of the following sequences QDISNY, YTS, QQGNTLPWT, GYTFTRYW, INPRNDRT, or ALGGGYAMDY or

b) has a sequence which, in comparison to the sequences mentioned under a) has at least one conservative amino acid exchange.

[0016] These sequences show again the CDRs of the monoclonal antibody 9.3 (see Figure 12), but the CDRs have been determined using another method known in the art, namely according to the IMGT® method from the international ImMunoGeneTics information sytem®.

[0017] Disclosed is a monoclonal antibody, produced by the hybridoma cell deposited under DSMZ ACC2841. This hybridoma cell has been deposited with the Deutsche Sammlung fur Mikroorganismen und Zellen on April 25, 2007 under the Budapest Treaty.

[0018] Disclosed is a humanized antibody based on the monoclonal antibody as described above.

[0019] Humanized antibodies are antibody molecules from non-human species having one or more complementarily determining regions (CDRs) from the non-human species and a framework region (FR) from a human immunoglobulin molecule. (See, e.g., Queen, U.S. Patent No. 5,585,089 and Winter, U.S. 5,225,539.) Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art.

[0020] In general, in order to obtain a humanised antibody, nucleic acid sequences encoding human variable heavy chains and variable light chains may be altered by replacing one or more CDR sequences of the human (acceptor) sequence by sequence encoding the respective CDR in the mouse antibody sequence (donor sequence). The human acceptor sequence may comprise FR derived from different genes.

[0021] In a preferred embodiment, the disclosed humanized antibody has at least one non-human CDR and human framework region (FR) residues.

[0022] Sequences encoding full length antibodies can be subsequently obtained by joining the rendered variable heavy and variable light chain sequences to human constant heavy chain and constant light chain regions. Preferred human constant light chain sequences include kappa and lambda constant light chain sequences. Preferred human constant heavy chain sequences include IgG1, IgG2 and sequences encoding IgG1 mutants which have rendered immune-stimulating properties. Such mutants may have a reduced ability to activate complement and/or antibody dependent cellular cytotoxicity and are described in US 5,624,821; WO 99/58572, US 6,737,056. An especially preferred constant heavy chain is an IgG1 comprising the substitutions E233P, L234V, L235A, A327G, A330S, P331S and a deletion of residue 236.

[0023] In another embodiment, the full length antibody comprises an IgA, IgD, IgE, IgM, IgY or IgW sequence.

[0024] Suitable human donor sequences can be determined by sequence comparison of the peptide sequences encoded by the mouse donor sequences to a group of human sequences, preferably to sequences encoded by human germ line immunoglobulin genes or mature antibody genes. A human sequence with a high sequence homology, preferably with the highest homology determined may serve as the acceptor sequence for the humanization process.

[0025] In addition to the exchange of human CDRs for mouse CDRs, further manipulations in the human donor sequence may be carried out to obtain a sequence encoding a humanized antibody with optimized properties (such as affinity of the antigen).

[0026] In a preferred example, heavy chain residues 31-35, 50-58 and 95-102 and residues 6, 23, 24, and 49 in the human acceptor sequence are altered to correspond to the respective residues of the mouse sequence (Adair, U.S. 5,859,205).

[0027] Furthermore the altered human acceptor antibody variable domain sequences may also be rendered to encode one or more amino acids (according to the Kabat numbering system) of position 4, 35, 38, 43, 44, 46, 58, 62, 64, 65, 66, 67, 68, 69, 73, 85, 98 of the light variable region and 2, 4, 36, 39, 43, 45, 69, 70, 74, 75, 76, 78, 92 of the heavy variable region corresponding to the mouse donor sequence (Carter and Presta, U.S. 6,407,213)

[0028] The humanisation of a mouse L1 antibody is described in Example 2.

[0029] It is disclosed that the CDRs may be altered, preferably by exchanges leading to a conservative amino acid exchange.

[0030] In general, manipulations may result in alterations in the FR as well as the CDR regions and include exchanges, deletions and insertion of residues. The alterations may be induced by random or directed mutagenesis. An antibody phage display system, as described before, may be employed for the selection of mutants with desired and/or improved properties

[0031] Disclosed is a human antibody capable of recognizing the same epitope as the antibody 9.3. Methods for generating human antibodies are known in the art. These methods employ for example mice in which the endogenous

immunoglobulin genes have been partially or completely inactivated and human immunoglobulin loci were introduced. Upon immunization with an immunogenic epitope, these mice are capable of producing human antibodies (U.S. 5,545,807; 5,545,806; 5,569,825; 5,589,369; 5,591,669; 5,625,126; 5,633,425; 5,661,016).

[0032] In a further disclosure, the humanized antibody comprises the sequence of L1_9.3hu or L1_9.3hu3 as shown in Figure 8 a) and b).

[0033] Disclosed is a binding molecule comprising

a) at least one of the following sequences RASQDISNYLN, YTSRLHS, QQGNTLPWT, RYWML, EINPRNDRTNYNEKFKT, or GGGYAMDY or

b) at least one sequence which has in comparison to the sequences given in a) at least one conservative amino acid exchange.

[0034] As explained above, these sequences show the CDRs of the antibody 9.3 (see Example 2).

[0035] Disclosed is a binding molecule comprising

a) at least one of the following sequences QDISNY, YTS, QQGNTLPWT, GYTFTRYW, INPRNDRT, or ALGGGYAMDY or

b) at least one sequence which has in comparison to the sequences given in a) at least one conservative amino acid exchange.

[0036] As explained above, these sequences show again the CDRs of the monoclonal antibody 9.3, determined by another method known in the art.

[0037] The present invention relates in one aspect to a binding molecule capable of binding L1,

(a) being selected from the group consisting of single chain antibodies, scFv, multimers of scFv like diabodies, triabodies or tetrabodies, antibody fragments, Fab, tandabs, flexibodies, bispecific antibodies, and chimeric antibodies,
and/or
(b) which comprises at least one Ig domain,
and wherein the binding molecule capable of binding L1:

(i) is characterized in that its complementarity determining regions (CDRs) have the following sequences: LCDR1: RASQDISNYLN (SEQ ID No.: 24), LCDR2: YTSRLHS (SEQ ID No.: 25), LCDR3: QQGNTLPWT (SEQ ID No.: 26), HCDR1: RYWML (SEQ ID No.: 27), HCDR2: EINPRNDRTNYNEKFKT (SEQ ID No.: 28), and HCDR3: GGGYAMDY (SEQ ID No.: 29),
and which binding molecule binds L1 with an affinity (KD) of at least 10^{-10} M, or
(ii) is characterized in that its complementarity determining regions (CDRs) have the following sequences: LCDR1: QDISNY (SEQ ID No.: 30), LCDR2: YTS, LCDR3: QQGNTLPWT (SEQ ID No.: 31), HCDR1: GYTFTRYW (SEQ ID No.: 32), HCDR2: INPRNDRT (SEQ ID No.: 33), and HCDR3: ALGGGYAMDY (SEQ ID No.: 34),
and which binding molecule binds L1 with an affinity (KD) of at least 10^{-10} M.

[0038] According to the invention, a binding molecule is a molecule capable of binding L1. The binding molecule is an immunoglobulin comprising molecule, i.e. comprises at least one Ig domain, and/or the binding molecule of the invention is selected from the group consisting of single chain antibodies (e.g. scFv, multimers of scFv like diabodies, triabodies or tetrabodies, antibody fragments (e.g. Fab), tandabs, flexibodies, bispecific antibodies, and chimeric antibodies.

[0039] The structure of an antibody and especially the function of its CDRs is known in the art (Carter PJ. Potent antibody therapeutics by design. Nature Rev. Immunol. 6:343-357,2006).
scFv and multimers thereof, tandabs, diabodies and flexibodies are standard antibody formats known in the art, e.g. from WO 88/1649, WO 93/11161, WO 99/57150 and EP1293514B1.

[0040] In single chain Fv (scFv) the two antigen binding variable regions of the light and heavy chain (VH Fv and VL Fv) of an antibody are artificially connected by a linker peptide, designated as single chain variable fragment or single chain antibody (Bird, et al. (1988) Science 242:423-426; Orlandi, et al (1989) Proc Natl Acad Sci USA 86:3833-3837; Clarkson et al., Nature 352: 624-628 (1991)). The antigen binding site is made up of the variable domains of light and heavy chains of a monoclonal antibody. Several investigations have shown that the Fv fragment has indeed the full

intrinsic antigen binding affinity of one binding site of the whole antibody.

[0041] In the context of this invention, diabodies are scFv with two binding specificities and can either be monospecific and bivalent or bispecific and bivalent.

[0042] Tandabs and flexibodies are further antibody formats which are e.g. defined in US2007031436 and EP1293514, respectively.

[0043] Antibody fragments that contain the idiotypes of the protein can be generated by techniques known in the art. For example, such fragments include, but are not limited to, the F(ab')2 fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragment that can be generated by reducing the disulfide bridges of the F(ab')2 fragment; the Fab fragment that can be generated by treating the antibody molecular with papain and a reducing agent; and Fv fragments.

[0044] A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. (See, e.g., Cabilly et al., U.S. Patent No. 4,816,567; and Boss et al., U.S. Patent No. 4,816,397).

[0045] Bifunctional, or bispecific, antibodies have antigen binding sites of different specificities. Various forms of bispecific antibodies have been produced. These include BSIgG, which are IgG molecules comprising two distinct heavy chains and two distinct light chains that are secreted by so-called "hybrid hybridomas", and heteroantibody conjugates produced by the chemical conjugation of antibodies or antibody fragments of different specificities (Segal DM, Weiner GJ, Weiner LM. Bispecific antibodies in cancer therapy. Current Opin. Immunol. 11:558-562, 1999, Van Spriel AB, Van Ojik HH, Van de Winkel JGJ. Immunotherapeutic perspective for bispecific antibodies. Immunology Today 21:391-397, 2000).

[0046] Bispecific antibodies have been generated to deliver cells, cytotoxins, or drugs to specific sites. An important use has been to deliver host cytotoxic cells, such as natural killer or cytotoxic T cells, to specific cellular targets. (P. J. Lachmann, Clin. Exp. Immunol. 79: 315 (1990)). Another important use has been to deliver cytotoxic proteins to specific cellular targets. (V. Raso, T. Griffin, Cancer Res. 41:2073 (1981); S. Honda, Y. Ichimori, S. Iwasa, Cytotechnology 4:59 (1990)). Another important use has been to deliver anti-cancer non-protein drugs to specific cellular targets (J. Corvalan, W. Smith, V. Gore, Intl. J. Cancer Suppl. 2:22 (1988); M. Pimm et al., British J. of Cancer 61:508 (1990)). Such bispecific antibodies have been prepared by chemical cross-linking (M. Brennan et al., Science 229:81 (1985)), disulfide exchange, or the production of hybrid-hybridomas (quadromas). Quadromas are constructed by fusing hybridomas that secrete two different types of antibodies against two different antigens (Kurokawa, T. et al., Biotechnology 7.1163 (1989)).

[0047] In a preferred embodiment of the invention, the binding molecule of the invention is linked to an active substance, preferably a toxin, a nanoparticle, a cytokine, or a radionuclide. Such antibody conjugates are known in the art (Wu AM, Senter PD. Arming antibodies: prospects and challenges for immunoconjugates. Nature Biotechnol. 23:1137-1146, 2005, Pastan I, Hassan R, FitzGerald DJ, Kreitman RJ. Immunotoxin treatment of cancer. Annu. Rev. Med. 58:221-237, 2007, WO 90/12592, WO 2007/030642, WO 2004/067038, WO 2004/003183, US 2005/0074426, WO 94/04189).

[0048] The binding molecule of the invention binds L1 with an affinity (KD) of at least 10^{-10} or 10^{-11} M.

[0049] Preferably, the antibody does not significantly bind to other members of the L1-protein family as for example CHL1 (close homolog of L1, accession number NM_006614), NrCAM (Neuronal cell adhesion protein, accession number NM_001037132 or NM_005010) and/or NFASC (Neurofascin, accession number NM_015090). Preferably the antibody binds the other members of the L1-family with an at least 100-fold lower affinity, more preferably at least 1000-fold lower affinity compared to the affinity for L1. The affinity of the antibody for the different proteins can be determined for example by measuring the binding affinity to recombinant proteins as described in example 6. The binding of the antibody to the different L1 family members of the L1-family may also be determined by expressing said proteins on CHO cells and measuring the antibody binding by FACS analysis as described in Example 1.2 and Example 7.

[0050] It is disclosed that the antibody does not significantly increase the release of cytokines, e. g. tumour necrosis factor-alpha or interferon gamma. Preferably the release is not increased by more than 30%, more preferably not more than 20% and most preferably not more than 10%. The release of cytokines can be tested as described in Example 8. Alternatively the concentration of cytokines can be determined in the blood of an animal before and after the administration of the antibody. The cytokine concentration may be determined by an ELISA assay or other methods known in the art.

[0051] It is disclosed that the antibody does not significantly induce T-cell proliferation or inhibit T-cell proliferation. The effect of an antibody on T-cell proliferation can be determined as described in Example 9.

[0052] Further disclosed is a binding molecule which is capable of binding to the same L1 epitope recognized by the monoclonal antibody 9.3, produced by the hybridoma cell deposited under DSMZ ACC2841. With respect to this disclosed binding molecule, the same embodiments defined with respect to the structure of the binding molecule described above also apply to this binding molecule.

[0053] Preferably, the binding of the antibody to the epitope is not significantly increased or decreased by the glycosylation state of the L1 protein. The influence of the glycosylation state on the antibody binding can be determined as described in Example 10.

[0054] Furthermore, the disclosure relates to a hybridoma cell that produces the monoclonal antibody.

[0055] Furthermore, the disclosure relates to the hybridoma cell deposited under DSMZ ACC2841.

[0056] As explained above and as described in the example section, the disclosed monoclonal antibody or the binding molecule of the invention is especially suitable for the treatment of tumorigenic diseases.

[0057] Therefore, in another aspect, the invention relates to the binding molecule of the invention for use in a method of treatment of a tumorigenic disease.

[0058] Furthermore, the disclosure also relates to a method for treating a tumorigenic disease, wherein an antibody or binding molecule is administered to a subject in an effective amount to treat said disease.

[0059] As mentioned above, in the art it has been suggested to use anti-L1 antibodies for sensitizing tumor cells for the treatment with a chemotherapeutic drug or with radiotherapy (see Sebens Müerkoster et al., Oncogene. 2007 Apr 26;26(19):2759-68, Epub 2006 Nov 6). Consequently, in another aspect, the present invention relates to the binding molecule of the invention for use in a method of sensitizing tumor cells in a patient for the treatment with a chemotherapeutic drug or with radiotherapy.

[0060] This aspect of the present invention is especially useful in cases where the tumor cells are at least partially resistant to chemotherapy or to radiotherapy.

[0061] Therefore, in a preferred embodiment of the invention, the cells to be sensitized are at least partially resistant to the treatment with said chemotherapeutic drug or to radiotherapy.

[0062] In the context of the present invention, the term "sensitizing" is to be understood that after the treatment with the binding molecule of the invention, the tumor cells are more susceptible to the treatment with a chemotherapeutic drug or with radiotherapy than before said treatment. This can e.g. be tested by isolating tumor cells from the patient and testing in vitro whether the treatment with said binding molecule of the invention results in a sensitization of the cells. This test can be performed as described in reference (Sebens Müerkoster et al., Oncogene. 2007 Apr 26;26(19):2759-68, Epub 2006 Nov 6).

[0063] In a preferred embodiment, the cells, before the administration of the binding molecule of the invention, were not susceptible to the treatment or only susceptible to an extent that the treatment with a chemotherapeutic drug or with radiotherapy would not result in the desired therapeutic effect.

[0064] Preferably, with the help of the binding molecule of the invention, the susceptibility is increased by at least 20 %, more preferably by at least 40 % and even more preferably by at least 100 %.

[0065] An overview over chemotherapeutic drugs and radiotherapy is e.g. given in Remmington's Pharmaceutical Sciences, 5th ed., chapter 33, in particular pages 624 to 652.

[0066] Any of numerous chemotherapeutic drugs can be used in the uses of the invention. These compounds fall into several different categories, including, for example, alkylating agents, antineoplastic antibiotics, antimetabolites, and natural source derivatives.

[0067] Examples of alkylating agents that can be used in the invention include busulfan, caroplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide (i.e., cytoxan), dacarbazine, ifosfamide, lomustine, mecholarethamine, melphalan, procarbazine, streptozocin, and thiotepa.

[0068] Examples of antineoplastic antibiotics include bleomycin, dactinomycin, daunorubicin, doxorubicin, idarubicin, mitomycin (e.g., mitomycin C), mitoxantrone, pentostatin, and plicamycin.

[0069] Examples of antimetabolites include fluorodeoxyuridine, cladribine, cytarabine, floxuridine, fludarabine, flurouracil (e.g., 5-fluorouracil (5FU)), gemcitabine, hydroxyurea, mercaptopurine, methotrexate, and thioguanine.

[0070] Examples of natural source derivatives include docetaxel, etoposide, irinotecan, taxanes (e.g. paclitaxel), teniposide, topotecan, vinblastine, vincristine, vinorelbine, prednisone, and tamoxifen.

[0071] Additional examples of chemotherapeutic agents that can be used in the invention include asparaginase and mitotane.

[0072] Furthermore, also C2 ceramide can be used.

[0073] In an especially preferred embodiment, the chemotherapeutic drug is selected from the group consisting of actinomycin-D, mitomycin C, cisplatin, doxorubicin, etoposide, verapamil, podophyllotoxin, 5-FU, taxans such as paclitaxel, and carboplatin.

[0074] According to the invention, the term "radiotherapy" refers to each radiation therapy which is commonly used to treat tumors cells. In a preferred embodiment, this therapy include γ-rays, X-rays, microwaves, UV radiation as well as the direct delivery of radio-isotopes to or next to tumor cells (brachytherapy).

[0075] As mentioned above, the object of this aspect of the invention is to sensitize tumor cells for the treatment with a chemotherapeutic drug or with radiotherapy. Consequently, in a preferred embodiment, after the sensitization with the binding molecule of the invention, the patient is further treated with said chemotherapeutic drug or with said radiotherapy.

[0076] In the context of the present invention, it is envisaged to sensitize tumor cells of any cell type or to treat any tumorigenic disease. Preferably, the tumor cells or the tumorigenic disease are of a type selected from the group consisting of astrocytoma, oligodendroglioma, meningioma, neurofibroma, glioblastoma, ependymoma, Schwannoma, neurofibrosarcoma, medulloblastoma, melanoma, pancreatic cancer, prostate carcinoma, head and neck cancer, breast cancer, lung cancer, ovarian cancer, endometrial cancer, renal cancer, neuroblastomas, squamous cell carcinomas,

medulloblastomas, hepatoma, colon cancer, and mesothelioma and epidermoid carcinoma.

[0077] Furthermore, it is preferred that the tumor cells are from an epithelial tumor or the tumorigenic disease is an epithelial tumor, preferably wherein the epithelial tumor is pancreatic cancer, colon cancer, ovarian cancer or endometrial cancer.

[0078] In a disclosure, the antibody does not induce neuronal side effects when administered in a therapeutically effective amount.

[0079] As discussed above, the binding molecule is used for the preparation of a pharmaceutical composition.

[0080] In general, the pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly, in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, including but not limited to peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered orally. Saline and aqueous dextrose are preferred carriers when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions are preferably employed as liquid carriers for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0081] In a preferred embodiment, the composition is formulated, in accordance with routine procedures, as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water or saline for injection can be provided so that the ingredients may be mixed prior to administration.

[0082] The therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free carboxyl groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., those formed with free amine groups such as those derived from isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc., and those derived from sodium, potassium, ammonium, calcium, and ferric hydroxides, etc..

[0083] The amount of the therapeutic of the invention, which will be effective in the treatment of a particular disorder or condition, will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems. In general, suppositories may contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

[0084] Various delivery systems are known and can be used to administer a therapeutic of the invention, e.g., encapsulation in liposomes, microparticles, and microcapsules: use of recombinant cells capable of expressing the therapeutic, use of receptor-mediated endocytosis (e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432); construction of a therapeutic nucleic acid as part of a retroviral or other vector, etc.. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion, by bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral, rectal and intestinal mucosa, etc.), and may be administered together

with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0085] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment. This may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

[0086] In another embodiment, the therapeutic can be delivered in a vesicle, in particular a liposome (Langer, 1990, Science 249:1527-1533), more particular a cationic liposome (WO 98/40052).

[0087] In yet another embodiment, the therapeutic can be delivered via a controlled release system. In one embodiment, a pump may be used (Langer, supra). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose.

[0088] Also disclosed is a method for sensitizing tumor cells in a patient for the treatment with a chemotherapeutic drug or with radiotherapy, comprising administering to the patient an efficient amount of an anti-L1 antibody or binding molecule. All embodiments described above also apply to this method.

[0089] Throughout the invention, the term "effective amount" means that a given molecule or compound is administered in an amount sufficient to obtain a desired therapeutic effect. In case that, throughout the invention, two compounds are administered in a therapeutic effective amount, this includes that one or each of the compounds is administered in a subtherapeutic amount, i.e. that the amount of each compound on its own is not sufficient to provide a therapeutic effect, but that the combination of the compounds results in the desired therapeutic effect. However, it is also included within the present invention that each of the compounds on its own is administered in a therapeutically effective amount.

[0090] In another aspect of the invention, the invention relates to the binding molecule of the invention for use in a method of treatment of tumor cells in a patient previously treated with a chemotherapeutic drug or with radiotherapy.

[0091] As mentioned above, the treatment of tumor cells with anti-L1 antibodies has already been described in WO 02/04952 and WO 06/013051.

[0092] In the context of the present invention, the term "previously treated" may include patients which have already been treated with a chemotherapeutic drug or with radiotherapy in the course of a separated regimen which has taken place e.g. within the last six or eight months.

[0093] In the course of tumor treatment with chemotherapeutic drugs or radiotherapy it is in most cases observed that after an initial response of the tumor to such therapy (tumor mass reduction or stabilization of the disease) the tumors start to progress again. Such progression usually starts upon weeks or months after such therapy. Typically these tumors are then resistant to further treatment with the previously applied chemotherapeutic drug and other treatment modalities are wanted. As described above it has been found that such resistant tumors express L1 and therefore become a target for anti-L1 antibodies.

[0094] Therefore, according to this embodiment of the invention, the term "previously treated" preferably means that the patient previously received such treatment, such treatment showed an initial effect and - at the time of therapy with the anti-L1 antibody or the binding molecule the tumor is progressing again.

[0095] Furthermore, the term "previously treated" may also be seen in a context where the L1 anti-L1 antibody or the binding molecule and the chemotherapeutic drug or radiotherapy are used within the same regimen, meaning that the treatments are given within one treatment schedule. In this context "in one treatment schedule" means that the treatment are applied at the same time, one after another or intermittently, but - in contrast to above - time distances between the individual treatments are short (within one week or within 2-4 days) and, if a treatment success is seen, one does not wait for tumor progression before the next treatment is applied.

[0096] Preferably, in this context, the invention includes the case where a patient is treated with a chemotherapeutic drug or with radiotherapy and subsequently, preferably within one week or less and more preferably within 2-4 days, a treatment with the binding molecule of the invention is started. In a further preferred embodiment several cycles of chemotherapy or radiotherapy on one side and
treatment with the anti-L1 antibody or the binding molecule are made, with intervals of preferably one week or less and more preferably within 2-4 days.

[0097] In a preferred embodiment, the patient is at least partially resistant to the treatment with said chemotherapeutic drug or with radiotherapy, an effect often observed in the course of said treatment types (see above).

[0098] In a further aspect, the invention relates to the use of the binding molecule of the invention for use in a method of treatment of tumor cells in a patient at least partially resistant to treatment with a given chemotherapeutic drug or with radiotherapy.

[0099] In the context of the present invention, the term "resistant to treatment" means that the respective tumor cell does not react to the treatment with a chemotherapeutic drug or with radiotherapy in a complete manner. Rather, with respect to this tumor cell, treatment with said chemotherapeutic drug or radiotherapy is rather ineffective or even shows no effects.

[0100] In a further aspect of the invention, the invention relates to the binding molecule of the invention for use in a method of treatment of a tumorigenic disease, wherein the binding molecule is administered in combination with a chemotherapeutic drug or with radiotherapy, preferably wherein the chemotherapeutic drug or the radiotherapy is administered prior to the binding molecule of the invention.

[0101] According to the invention, the term "treatment of tumorigenic disease" includes both the killing of tumor cells, the reduction of the proliferation of tumor cells (e.g. by at least 30 %, at least 50 % or at least 90 %) as well as the complete inhibition of the proliferation of tumor cells. Furthermore, this term includes the prevention of a tumorigenic disease, e.g. by killing of cells that may or a prone to become a tumor cell in the future as well as the formation of metastases.

[0102] According to the invention, the term "in combination with" includes any combined administration of the binding molecule and the
chemotherapeutic drug or radiotherapy. This may include the simultaneous application of the drugs or radiotherapy or, preferably, a separate administration. In case that a separate administration is envisaged, one would preferably ensure that a significant period of time would not expire between the time of delivery, such that the binding molecule and the chemotherapeutic drug or radiotherapy would still be able to exert an advantageously combined effect on the cell. In such instances, it is preferred that one would contact the cell with both agents within about one week, preferably within about 4 days, more preferably within about 12-36 hours of each other.

[0103] The rational behind this aspect of the invention is that the administration of chemotherapeutic drugs or the treatment with radiotherapy leads to an increase of L1 expression on the surface of the tumor cells which in turn makes the tumor cells a better target for the binding molecule.

[0104] Therefore, this aspect of the invention also encompasses treatment regimens where the binding molecule is administered in combination with the chemotherapeutic drug or radiotherapy in various treatment cycles wherein each cycle may be separated by a period of time without treatment which may last e.g. for two weeks and wherein each cycle may involve the repeated administration of the binding molecule and/or the chemotherapeutic drug or radiotherapy. For example such treatment cycle may encompass the treatment with a chemotherapeutic drug or with radiotherapy, followed by e.g. the twice application of the binding molecule within 2 days.

[0105] Throughout the invention, the skilled person will understand that the individual therapy to be applied will depend on the e.g. physical conditions of the patient or on the severity of the disease and will therefore have to be adjusted on a case to case basis.

[0106] Especially in the course of such repeated treatment cycles, it is also envisaged within the present invention that the binding molecule is administered prior to the chemotherapeutic drug or the radiotherapy.

[0107] Also disclosed is a method for treating tumor cells in a patient previously treated with a chemotherapeutic drug or with radiotherapy, comprising administering to the patient a therapeutically effective amount of the anti-L1 antibody or binding molecule. Furthermore, the disclosure relates to a method for treating tumor cells in a patient at least partially resistant to treatment with a given chemotherapeutic drug or with radiotherapy, comprising administering to the patient a therapeutically effective amount of the anti-L1 antibody or binding molecule. Furthermore, the disclosure relates to a method for treating tumor cells in a patient, comprising administering to the patient a therapeutically effective amount of the anti-L1 antibody or binding molecule in combination with a chemotherapeutic drug or with radiotherapy. Furthermore, Furthermore, the disclosure relates to a method for treating tumor cells in a patient, comprising administering to the patient a therapeutically effective amount of the anti-L1 antibody or binding molecule.

[0108] The antibody may also be used in a method for a diagnostic method to determine the level of the L1 protein in body tissues or fluids.

[0109] With respect to these methods, it is disclosed that all embodiments described above for the other uses or methods also apply.

[0110] The invention also relates to the binding molecule of the invention for use in a method of treatment of a tumorigenic disease or for sensitizing of tumor cells in a patient for the treatment with a chemotherapeutic drug or with radiotherapy.

[0111] In a preferred embodiment, said use exhibits further the features as defined for the uses of the invention.

[0112] The invention also relates to pharmaceutical compositions comprising the binding molecule of the invention. With respect to said pharmaceutical composition, all embodiments described above also apply.

[0113] The invention is further illustrated by the following figures and examples.

Legends to Figures and Tables

Figure 1

[0114] (A) FACS analysis of CHO, CHO-L1, SKOV3ip and OVMz cells. Cells were stained with the indicated mAbs (10μg/ml)for 30 min at 4°C. Followed by a secondary PE-conjugated mAb. (B) Westerm blot analysis. Cell lysates from CHO wt, CHO-L1, OVMz and SKOV3ip cells were transferred on a PVDF membrane and then incubated with the indicated mAb to L1 (1μg/ml), followed by a POX-conjugated secondary mAb.

Figure 2

[0115] (A) Effect of antibodies on Erk-phosphorylation in SKOV3ip cells. Cells were incubated for 24 h at 37°C with the indicated purified antibodies to L1 (10 μg/ml) or isotype control IgG1. Cells were also treated with DMSO (vehicle), or the MEK-specific inhibitor PD59098. Cell lysates were examined for phosphorylation of Erk. (B) Effect of antibodies on Erk phosphorylation in SKOV3ip cells. Fluorescent staining of antibody treated cells with a phospho-Erk specific antibody and an Alexa488-conjugated secondary mAb.

Figure 3

[0116] Analysis of matrigel cell invasion. Antibody (10 μg/ml) treated SKOV3ip cells were seeded into a 4-well plate and allowed to invade into the matrigel for 20 h (5% CO_2; 37°C).

Figure 4

[0117] Differential gene expression in SKOV3ip cells. (A) SKOV3ip cells were transfected with L1-specific or scrambled siRNA and 72 h later mRNAs were isolated, transcribed to cDNA and used as template for qPCR (SYBRgreen analysis). (B) SKOV3ip cells were treated with the L1-9.3 mAb (10 μg/ml) or the control mAB IgG1 (10 μg/ml) and 96 h later mRNAs were isolated, transcribed to cDNA and analyzed by qPCR for the expression of the indicated genes (SYBRgreen analysis). (C) Differential gene expression of residual tumor cells. mRNAs from residual tumors were isolated from antibody treated animals, transcribed into cDNA and analyzed by qPCR for the expression of the indicated genes.

Figure 5

[0118] Tumor growth in nude mice. LacZ-tagged SKOV3ip cells were injected i. p. into nude mice and after tumor implantation animals were treated with the indicated L1 mAbs orcontrol mAb EpCAM (Hea125). After 30 days the tumor volume wasdetermined and is given as the ratio between X-Gal stained tumormass and the total situs. 6 animals were analyzed per group.

Figure 6

[0119] (A) Western blot analysis of L1-V5 constructs. Supernatant of transfected Sf9 insect cells were received from Ricardo Gouveia and analyzed by Western blot using L1-9.3 mAb and reprobed by anti-V5 mAb. (B) Western blot analysis of L1-FC constructs. L1-FC constructs were transfected into Cos-7 cells using Jet PEI™ transfection reagent as described. After 3 days supernatants were purified using SepharoseA and analyzed by Western blot using L1-9.3 mAb.

Figure 7

[0120] Homophilic cell adhesion assay. (A) The binding of J558-L1 cells was analyzed by bright field microscopy. One example of each treatment is shown here. In the red box coating with L1-Fc (10 μg/ml) is highlighted and in the black box the both controls, fibronectin (10 μg/ml) and BSA, are shown. (B) The graph shows the mean ± SD of bound cells after the indicated antibody or control treatment.

Figure 8

[0121] The antibody light chain and heavy chain DNA sequences used to construct the humanized antibodies are provided in Figures 8a and 8b respectively.

Figure 9

[0122] Amino acid sequences of the murine L1_9.3 scFv (a) and the humanized L1_9.3Hu (b) and L1_9.3Hu3 scFvs (c).

Figure 10

[0123] DNA and amino acid sequences of the expressed portions of L1_9.3 (a), L1-9.3Hu (b) and L1_9.3Hu3 scFv (c) constructs.

Figure 11

[0124] Binding of the L1_9.3, L1-9.3Hu and L1_9.3Hu3 scFvs to the human L1 cancer antigen. Rows A, B and C are coated with L1 and rows D, E and F are coated with streptavidin. The blue colour in the wells indicates binding of the individual scFv to the L1 on the plate. The lack of colour in the streptavidin coated rows shows that the single chain antibodies are specifically binding to L1

Figure 12

[0125] Genomic sequences of the variable domains of the monoclonal antibody 9.3

a) Sequence of the kappa chain variable region (dotted lines: CDR1, dashed lines: CDR2, underlined: CDR3)
b) Sequence of the heavy chain variable region (dotted lines: CDR1, dashed lines: CDR2, underlined: CDR3)

Figure 13

[0126] A) Human PBMC and L1-positive OVMZ tumor cells were incubated with L1-9.3 mAb for 24h and the amount of bound antibody was determined by FACS analysis. B) The dissociation constants K_D were estimated from the regression curves using the concentration at half-maximal binding.

Figure 14

[0127] L1-9.3 has no effect on the release of cytokines by resting and activated human PBMC. Cytokine levels of resting and OKT3-activated PBMC from three different donors were determined after an incubation for 24h in presence or absence of 20μg/ml L1-9.3. Ionomycin/PMA and LPS were used as stimulation controls. Results for IFN-γ (A) and TNF-α (B) are shown.

Figure 15

[0128] L1-9.3 does not induce T cell proliferation and has no effect on OKT3-induced T cell proliferation. Proliferation of OKT3-activated PBMC from two different donors was determined in presence or absence of 20μg/ml L1-9.3 using a BrdU incorporation assay 48h post stimulation. There was no difference, whether the antibody was added prior, in parallel or after stimulation with 75ng/ml OKT3. L1-9.3 by itself did not result in T cell activation.

Figure 16

[0129] L1-9-3 was unaffected by deglycosylation of L1. The Western blot staining of L1 in untreated and deglycosylated cell lysate is shown using several different anti-L1 mAbs. The tested antibodies can be divided into three classes in respect to their glycosylation-dependency: First class (unaffected by glycosylation): L1-9.3. Second class (binding in WB was negatively affected by deglycosylation): 11A, 14.10, OV52.24 and OV549.20. Third class (binding in WB was positively affected by deglycosylation): 35.9 and 38.12.

Figure 17

[0130] The figure shows in vivo binding of intravenously applied L1-9.3 to collecting ducts of the kidney. In vivo binding was only detectable using the amplification system CSA (Fig. 17A), while by using the conventional ABC-method, no signal was visible (Fig. 17B). Hence, L1-9.3 was detected in a range of 30-300 pmol in the tissue (L1-9.3 concentration is presumably higher than 5 ng/ml and below 50 ng/ml). Negative control did not show staining, thus, unspecific staining can be excluded (Fig. Figure 17C).The staining pattern of in vivo bound L1-9.3 (Fig. 17A) corresponds to the L1 expression

pattern in the kidney when directly staining tissue sections with L1-9.3 (Fig. 17D).

Figure 18

FACS analysis of humanized L1-9.3 mAbs

[0131] Flow cytometry analysis of SKOV3ip pcDNA3.1 luciferase cells. Cells were stained with the indicated humanized mAbs(10μg/ml) for 30min 4°C, followed by a secondary PE-conjugated mAb.

Figure 19

Mouse SKOV3ip xenograft-model

[0132] $7*10^6$ SKOV3ip pcDNA3.1 luciferase cells were injected intraperitoneal into 6 weeks old CD1 nu/nu female mice. After 24h mice were randomized in groups of 10 mice. Each group of mice was three times weekly injected with 300μg either mAb L1-chi9.3, mAbL1-hu3 or PBS intraperitoneally.

[0133] On day 33 mice were imaged (Fig. 2). Tumor volume was determined using the XENOGEN IVIS 200 System. In brief, mice were anesthetised and injected with 100μl Luciferin D (3μg/mouse) intraperitoneally. Afterwards, luciferase activity of the tumor cells was measured by detecting light emission. The tumor volume is shown as photon per second (total flux). Statistical analysis was done using the student's t-test.

Figure 20

In vivo total tumor mass

[0134] After 36 days mice were sacrificed and the tumor mass was determined.Tumor growth is given as a ratio of tumor mass to bodyweight. (*A* individual mice, *B* mean value). Statistical analysis was done using the student's t-test. Thus, the treatment of immundeficient mice with L1 9.3 antibody could be reproduced with chimarised and humanized forms of the L1 9.3 mAb.

Figure 21

[0135] PT45-P1res cells were either left untreated (w/o) or were treated with 20 μg/mL gemcitabone (A) or etoposide (B) in the absence (w/o) or presence of either 1 or 10 μg/mL anti L1CAM antibody 9.3 or 1 or 10 μg/mL isotype matched control antibody. After 24 hours, cells were analysed by caspase-3/-7 assay. Means ± SD from three independent experiments are shown. * indicates $p< 0.05$.

Figure 22

[0136] Colo357 cells were either left untreated (w/o) or were treated with 20 μg/mL gemcitabone (A) or etoposide (B) in the absence (w/o) or presence of either 1 or 10 μg/mL anti L1CAM antibody 9.3 or 1 or 10 μg/mL isotype matched control antibody. After 24 hours, cells were analysed by caspase-3/-7 assay. Means ± SD from three independent experiments are shown. * indicates $p< 0.05$.

Table 1

[0137] The table shows a summary of antibodies tested in the indicated assays.

Examples

1. Example 1

1.1 Summary of Example 1

[0138] The L1 adhesion molecule (L1-CAM) is a transmembrane cell adhesion molecule involved in cell migration and axon guidance in the developing nervous system. L1 is also over-expressed in ovarian and endometrial carcinomas. Here L1 expression is associated with poor prognosis. In carcinoma cell lines, L1 over-expression augments cell motility, tumor growth in mice and induces expression of Erk-dependent genes. Here we show that treatment with antibodies to

L1 abrogates Erk-activation, blocks cell invasion to matrigel and decreases tumor growth in nude mice. In cells treated with L1 antibodies the induction of Erk-dependent genes such as HOX A9, β3 integrin and IER 3 are reversed *in vitro* and *in vivo.* In this report, we demonstrate that the antibody L1-9.3 is the best therapeutic antibody of all tested L1 antibodies. In all cases L1-9.3 showed the best results concerning the invasive phenotype or therapeutic effect on tumor growth. We could show that L1-9.3 binds to the first Ig-like domain of L1 and can block the L1-L1 homophilic binding. The blocking of homophilic binding was only observed with L1-9.3. We conclude, that L1-9.3 is superior in therapy as it combines two functions: it blocks erk activation and interferes with the binding function of L1.

1.2 Results of Example 1

1.2.1 FACS analysis of the new L1 antibodies

[0139] Using immunization with a recombinant L1-Fc fusion protein, we generated novel L1 antibodies L1-9.3, L1-14.10, L1-35.9 and L1-38.12. To elucidate the specificity for L1 the new L1 mAbs were tested these antibodies on the endogenous L1 expressing ovarian carcinoma cell lines OVMz and SKOV3ip and the chinese hamster ovary cells CHO and stably transduced CHO-L1 cells by fluorescent staining (Fig. 1A) and Western blot analysis (Fig. 1B). All tested antibodies showed a positive staining of L1 in CHO-L1 cells (Fig. 1A). The staining pattern for the OVMz and the SKOV3ip cells was different for the antibodies. Interestingly, the L1-9.3 antibody showed bright staining of both ovarian carcinoma cell lines OVMz and SKOV3ip, whereas the L1-14.10 showed a very weak staining (Fig. 1A). The two L1 antibodies L1-35.9 and L1-38.12 could not bind to the endogenous L1 of these cells (Fig. 1A). As expected, no staining for L1 could be observed in CHO cells which we used as negative control. All new antibodies detected the fullength L1 in CHO-L1, OVMz and SKOV3ip cell lysates by Western blot analysis. The L1-negative CHO cells served again as negative control.

1.2.2 The Erk phosphorylation is decreased after antibody treatment

[0140] A recent report has shown that expression of L1 in cooperation with serum-derived growth factors lead to sustained Erk-activation and the induction of Erk-dependent genes (Silletti et al, 2004). We investigated if the suppressive effect of L1-antibodies might be due to interference with L1-mediated gene regulation. Therefore we examined the mode of action of L1 antibodies using SKOV3ip cells. The mAbs L1-11A, L1-9.3 and L1-14.10 efficiently blocked Erk-phosphorylation (Fig. 2A) *in vitro.* There was no inhibition with isotype matched control mAb, DMSO as vehicle or the L1 antibody L1-38.12 (Fig. 2A) that can bind only the neural isoform of L1. Fluorescent analysis with the phospho-specific Erk antibody confirmed a clear reduction of activated Erk. A depletion from the nucleus in L1-mAb treated cells (L1-11A, L1-9.3 and L1-14.10) could also be observed (Fig. 2B).

1.2.3 Antibody treatment with L1-antibodies reduced cell invasion

[0141] It has been demonstrated before that treatment with an antibody to L1 (L1-11A) reduced the haptotactic cell migration on fibronectin and the matrigel invasion of different cell lines (Arlt et al, 2006). We compared the invasion capacity of SKOV3ip cells treated with the different L1 antibodies. The antibodies L1-11A, L1-14.10 and especially L1-9.3 reduced the invasion of the SKOV3ip (Fig. 3). In sharp contrast, cells treated with the antibodies L1-35.9 or L1-38.12 did not show a reduction of invasion (Fig. 3).

1.2.4 Antibodies to L1 affect gene expression in vitro and in vivo

[0142] We further examined whether antibodies to L1 affect the gene expression profile in SKOV3ip cells *in vitro* in a similar fashion as observed for siRNA-mediated depletion of L1 (Fig. 4A). Indeed, qRT-PCR analysis of cells treated with L1-9.3 or L1-11A versus control antibody showed significant changes in the expression of L1-regulated genes such as β3 integrin, the transcription factors HOXA9 and the apoptosis-related genes IER 3 and STK 39 (Fig. 4A). The same set of genes was downregulated in SKOV3ip cells transduced with a L1-specific siRNA (Fig. 4B).
We tested whether mAb L1-9.3 could also influence the gene expression profile of SKOV3ip cells in vivo similar to that observed *in vitro.* To this end, mRNA from residual tumors of L1-9.3 treated mice or IgG control treated mice were isolated and subjected to qRT-PCR analysis. L1-9.3 treatment led to significant regulation of L1-dependent genes as demonstrated for HOXA9, β3 integrin and IER 3 (Fig. 4C).

1.2.5 Analysis of tumorigenicity in nude mice

[0143] Next, we investigated whether the intraperitoneal growth of SKOV3ip in mice could be inhibited by treatment with the mAbs L1-11A, L1-9.3 or L1-14.10 SKOV3ip-*lacZ* cells were injected into the peritoneal cavity of female nude

mice 2 days before the onset of therapy. Biweekly i.p. treatments were done using the 10 mg/kg antibody concentration. Control mice were treated with PBS or HEA125 (anti EpCAM) as a control antibody (biweekly 10 mg/kg i.p.). In all anti-L1 mAb treatment groups, a substantial decrease in the amount of tumor mass was visible compared with PBS or the control antibody HEA-125 (Fig. 5). Compared with the control, all anti-L1 mAbs led to a dose-dependent reduction of i.p. tumor burden [L1-11A (10 mg/kg), -40%; L1-14.10 (10 mg/kg), -30%; L1-9.3 (10 mg/kg), -60%; Fig. 5]. Tumor reduction in the group treated with the L1-9.3 (10 mg/kg) was statistically significant ($P_{\text{L1-9.3 (10 mg/kg)}} = 0.004$) compared with the PBS control. Mice treated with the HEA125 control antibody revealed no detectable reduction of SKOV3ip-*lacZ* i.p. tumor burden compared with the PBS-treated group (Fig. 5), although EpCAM is present on the SKOV3ip cells and HEA125 can bind to the tumor cells. No side effects or severe toxicity of L1-mAbs L1-11A, L1-9.3 or L1-14.10 treatment was observed during the whole course oftreatment.
Thus, treatment with antibodies to L1 reduced the tumor growth SKOV3ip cells (Fig. 5) suggesting that antibodies to L1 can regulate gene expression but also affect *in vivo* tumor growth.

1.2.6 Biacore studie of the new L1 antibodies

[0144] This study was performed by Avidex (Oxford) as described in Example 6. Table 1 summerizes these results concerning the binding kinetics of the new L1 antibodies (ka, kd and KD).

1.2.7 Epitop-mapping of L1-9.3 binding site

[0145] An important factor for the characterization of novel L1 antibodies is to examine their binding sites in L1. Therefore, we constructed a variety of L1-Fc fusion proteins covering different parts of the molecule. PCR products were amplified coding different length of L1 ectodomain regions. These constructs were cloned into the pIg vector, and expressed as Fc-fusion proteins. After purification, products were used for Western blot analysis. For comparing the results, we analyzed other recombinant L1 protein fragments (obtained from Ricardo Gouveia, Oeiras, Portugal). L1-9.3 was found to bind to first Ig domain of L1 (Fig. 6). L1-14.10 binds in the third Ig domain whereas L1-11A binds between the FN3-5 site (Fig. 6).

1.2.8 mAB L1-9.3 blocks L1-L1 homophilic binding

[0146] We asked if the L1 antibodies could interfere with the homophilic binding function of L1. To adress this question, we used a cell adhesion assay in which L1-transfected cells are allowed to bind to immobilized L1. After initial coating of glass slides with a recombinant L1-Fc fusion protein, fibronectin for positive control (to which cells bind in an integrin dependent maner) or BSA as a negative control, we incubated J558-L1 cells with L1-11A, L1-9.3 or L1-14.10 antibody. For control, we used an IgG-control, PBS or an antibody to CD24 (SWA11). The mAb L1-9.3 could completely block the L1-L1 homophilic binding, whereas all other tested antibodies could not interfere with the homophilic binding capacity. None of the antibodies interfered with the binding to fibronectin (data not shown).

1.3 Materials and Methods

1.3.1 Cell lines and cell culture

[0147] The human ovarian carcinoma cell lines SKOV3ip (kindly provided by Ellen Vitetta, University of Texas, Dallas, TX) and OVMz were grown in DMEM (Biochrom, Berlin, Germany) with 10% FCS under cell culture conditions (5% CO_2, 95% relative humidity, 37°C). For identification and quantification of tumor mass, the SKOV3ip cells were stably transduced with a *lacZ*-encoding retroviral vector (GeneSuppressor Retroviral System, Biocarta, Hamburg, Germany). The chinese hamster ovary cell line CHO stably expressing human L1 (-hL1) were established by transfection with superfect (Stratagene, Heidelberg, Germany) and selection for L1 expression with mAb L1-11A and magnetic beads (Myltenyi Biotec, Bergisch Gladbach, Germany) or sorting with FACS Calibur. All cells were cultivated in DMEM supplemented with 10% FCS at 37°C, 5% CO_2 and 100% humidity. Human L1 encoding plasmids and J558-L1 cells were obtained from Dr. Vance Lemmon (University of Miami, Miami, FL, USA).

1.3.2 Antibodies

[0148] HEA-125, a mouse IgG1 directed against EpCAM, was described before and binds to all human adenocarcinomas (Moldenhauer et al., 1987). Monoclonal antibody L1-14.10 (Huszar et al., 2006), L1-9.3, L1-35.9 and L1-38.12 were obtained after immunization of mice with human L1-Fc protein comprising the ectodomain of L1 (Oleszewski et al., 1999). Goat anti-mouse IgG was affinity purified and absorbed to human serum proteins (Zymed Laboratories, Inc.,

San Francisco, CA).

1.3.3 Biochemical analysis

[0149] SDS-PAGE and transfer of separated proteins to Immobilon membranes using semi-dry blotting were described before (Gutwein et al., 2000). After blocking with 5% skim milk in TBS or 1% BSA in TBS/0.1% Tween-20, the blots were developed with the respective primary antibody followed by peroxidase conjugated secondary antibody and ECL detection.

1.3.4 FACS analysis

[0150] The surface staining of cells with saturating amounts of mAbs, either hybridoma supernatants or purified antibodies, and PE-conjugated goat antibodies to mouse Ig (Dianova, Hamburg, Germany) has been described elsewhere (Ebeling et al., 1996). Stained cells were analyzed with a FACScan (Becton Dickinson).

1.3.5 Immunofluorescence

[0151] For immunofluoresent staining, cells were grown on coverslips, treated for 10 min with pervanadate and fixed for 20 min with 4% paraformaldehyde/PBS at room temperature. Cells were washed in PBS and permeabilized with 0.1% NP-40 in PBS containing 5% goat serum for 15 min at room temperature. Cells were then incubated for 1 hour with first antibody (phospho-specific Erk1/2). After 3 washing steps with PBS cells were incubated 30 min in the dark to a second Alexa488-conjugated goat anti-mouse IgG. After washing the cells twice with PBS, stained cells were mounted on glass slides and examined with an epifluorescence microscope (Axioplan-2; Zeiss, Oberkochem).

1.3.6 Invasion assay

[0152] Tumor cell invasion *in vitro* was determined in a double-filter assay as described previously in Erkell et al. (1988). Briefly, a Matrigel was layered between two filters, a lower 5 μm pore nitrocellulose filter and an upper 8 μm pore polycarbonate filter. Following incubation of 10^5 cells with the filter sandwich for 20 h in 1 ml medium, the sandwich was fixed and the filters separated and stained with DAPI. Cells present in the gel on the lower filter were counted, and cell invasion was expressed as the ration of the cell number on the lower filter to the total number of cells present on both filters.

1.3.7 Quantitative PCR

[0153] For qPCR the cDNA was purified on Microspin G-50 columns (GE Healthcare, München, Germany) and quantitated by NanoDrop spectrophotometer (ND-1000. Kisker-Biotechnology, Steinfurt, Germany). Primers for qPCR were designed with the DNA Star Program and were produced by MWG (Ebersberg, Germany). β-actin was used as an internal standard. The PCR reaction was performed with the SYBRgreen mastermix (Applied biosystems, Darmstadt, Germany).

1.3.8 Cell binding assay

[0154] Cell binding assays to L1-Fc or fibronectin are described in detail in Oleszewski et al (JCB 2000).

1.3.9 Tumor model and therapy

[0155] Pathogen-free, female athymic CD1 *nu*/*nu* mice (7-9 weeks old; 20 g on average; Charles River) were inoculated with 5 x 10^6 human *lacZ*-tagged ovarian carcinoma cells (SKOV3ip-*lacZ*) into the peritoneal cavity at day 0, leading to i.p. tumor formation within 5 weeks. Anti-L1 mAbs were diluted in sterile PBS to the concentration needed for treatment. Tumor-bearing mice were treated i.p. twice weekly with a 300 μL solution of the respective dosage (10 mg/kg per application, respectively), vehicle (PBS), or Hea125 antibody control. Antibody treatments started from day 3 after tumor cell injection to give the tumor cells time to attach to the inner side of the abdominal wall and the surfaces of the i.p. organs. At autopsy (day 38), ascites was sampled from all mice and the volume was determined. All i.p. organs (including tumor mass), the abdominal wall, and the diaphragm were removed, stained with β-galactosidase substrate (X-gal; Roche-Diagnostics, Penzberg, Germany), photographed, and weighed. The indigo blue tumor mass between the organs, on the diaphragm and the inner site of the abdominal wall, was removed and weighed alone. The relative tumor burden in each mouse was calculated by dividing tumor mass weight by total *situs* weight.

2. Example 2

Humanization of the anti-L1 murine antibody L1_9.3

[0156] In order to humanize the murine anti-L1 antibody L1_9.3, the genes of human v-kappa 1 (humκ1), and variable heavy chain family III (humIII) were utilised as the acceptor sequences. The numbering system used herein for these genes is adopted from Wu and Kabat (Kabat, E. A, Wu, T. T., Perry, HM, Gottesman, KS and Foeller, C (1992) Sequences of proteins of immunological interest, Diane Books Publishing company). The murine L1_9.3 antibody light and heavy chain amino acid sequences were aligned against the amino acid sequences of the humκ1 light chain and the humIII heavy chain respectively. Two humanized L1_9.3 antibodies (L1_9.3Hu and L1_9.3Hu3) were generated by replacing the six CDRs of the human antibody with the corresponding CDRs from the murine L1_9.3 antibody.

Locations of the six Complementarity Determining Regions (CDRs)

[0157]

Loop	Kabat numbering scheme
LCDR1	L24--L34
LCDR2	L50--L56
LCDR3	L89--L97
HCDR1	H31--H35B
HCDR2	H50--H65
HCDR3	H93--H101

[0158] A number of framework residues of the murine L1_9.3 antibody were transferred to the humanized L1_9.3 antibodies:

Version 1 (L1_9.3Hu) humanized antibody - heavy chain residue numbers 6, 23, 27, 30, 43, 49, 71, 73, 76, 78 and 94, and light chain residue number 100 were transferred from the murine L1_9.3 antibody and light chain residue number 73 was replaced with the corresponding (Phe) found at this position in the human REI antibody light chain.

Version 2 (L1_9.3Hu3) humanized antibody - heavy chain residue numbers 6, 23, 27, 30, 71, 73, and 94, and light chain residue number 100 were transferred from the murine L1_9.3 antibody.

[0159] DNA sequences encoding single-chain variable fragment (scFv) analogues of the murine L1_9.3 antibody and the two humanised versions of this antibody (L1_9.3Hu, and L1_9.3Hu3) for expression in *E. coli.* were then generated. All of these scFvs contain the same linker (TSGPGDGGKGGPGKGPGGEGTKGTGPGG). The scFv genes were synthesized by GeneArt AG, Germany.

[0160] The antibody light chain and heavy chain DNA sequences used to construct the humanized antibodies are provided in Figures 8a and 8b respectively.

[0161] Figures 9a - 9c provide the amino acid sequences of the murine L1_9.3 scFv and the humanized L1_9.3Hu and L1_9.3Hu3 scFvs respectively.

3. Example 3

Cloning of DNA encoding the L1_9.3, L1-9.3Hu and L1_9.3Hu3 scFvs into E.coli periplasmic expression vectors and transformation of E. coli with these vectors.

[0162] Periplasmic expressed of scFvs is beneficial for a number of reasons. Firstly, such scFvs leak into the bacterial supernatant and from there can conveniently be assayed for binding to their cognate antigen (The L1 cancer antigen in this case).Secondly, periplasmic expression allows for purification of soluble active scFvs.

[0163] The DNA sequences encoding the L1_9.3, L1-9.3Hu and L1_9.3Hu3 scFvs as synthesized by GeneArt AG, Germany were not supplied in an *E. coli* periplasmic expression vector. Therefore, these DNA sequences where cloned into an E. *coli* periplasmic expression vector using the following methods.

[0164] The DNA encoding the synthesized scFvs were PCR rescued with the following primer pairs using standard PCR conditions and reagents:

scFv	Primer pair
L1_9.3	Yol811 and Yol812
L1-9.3Hu	Yol813 and Yol814
L1_9.3Hu3	Yol813 and Yol814

[0165] The primer sequences are shown below.

Yol811 AGCCGGCCATGGCCGATATTCAGATGACCCAGAC
Yol812 TCTATGCAGCGGCGGCACCGCCGCTGCTCACGGTAACGCTG
Yol813 AGCCGGCCATGGCCGATATTCAGATGACCCAGAG
Yol814 TCTATGCAGCGGCCGCACCGCCGCTGCTCACGGTAACCAGGGTG

[0166] The PCR products were run on a 1.6% agarose gel and bands of the correct size excised and purified. The PCR products were double digested with *Nco1* and *Not1* restriction enzymes under standard conditions followed by re-purification. The PCR products were ligated into an IPTG inducible periplasmic expression vector which contained:

- a pelB leader sequence to direct the encoded polypeptides to the periplasm where this leader sequence is then cleaved off
- *Nco1*/*Not1* cloning sites
- the human antibody kappa chain constant region

[0167] The ligated vectors were transformed into *E. coli* TG1 cells and plated on of 2xTY agar (Bacto Trypton 16g/L, yeast extract 10g/L, 15g/L bactoagar and NaCl 5g/L) supplemented with 100μg/ml ampicillin and 2% glucose. The DNA and amino acid sequences of the expressed portions of L1_9.3, L1-9.3Hu and L1_9.3Hu3 scFv constructs are shown in Figures 10a, 10b and 10c respectively.

4. Example 4

Expression of L1_9.3, and L1_9.3Hu3 single-chain antibodies in E. coli

[0168] The polypeptides expressed by these vectors include the human antibody c kappa constant region fused to the C termini of the scFvs. These c kappa constant chain containing constructs are referred to herein as single chain antibodies.

[0169] Eight *E. coli* clones for each single chain antibody construct, L1_9.3, L1_9.3Hu, and L1_9Hu3, (24 clones in total) were picked into separate wells of a 96 well plate containing 300μl of 2xTY (Bacto Trypton 16g/L, yeast extract 10g/L and NaCl 5g/L) supplemented with 100μg/ml ampicillin and 2% glucose. Each well has a 1 ml volume. The cultures were grown with shaking (200rpm) at 37°C until the cultures reached an OD_{600} of approximately 0.5. The 96 well plates were then spun down at 3200 rpm for 10 min and the supernatant was aspirated and discarded. The bacterial pellets were resuspended in fresh 2XTY 400μl supplemented with 100μg/ml ampicillin and 1mM IPTG to induce expression of the single chain antibodies. The cultures were shaken at 200rpm overnight at 25°C.

[0170] The following day the 96 well plate was spun down at 3200 rpm for 10 min to pellet the cells. The supernatant containing the expressed L1 single chain antibodies was kept for ELISA analysis.

5. Example 5

ELISA assay of binding of the L1_9.3, L1-9.3Hu and L1_9.3Hu3 scFvs to human L1 cancer antigen

[0171] This ELISA assay was carried out in order to confirm that the humanisation process had not lead to a loss of antibody binding to the L1 cancer antigen and to identify which of the clones picked correctly expressed the single chain antibody constructs.

[0172] Three rows of a 96 well plate were coated with 100μl L1 antigen comprising the extracellolar domain of the L1 protein fused to an Fc fragment (5μg/ml) in PBS for 1 hr at room temperature. A further three rows were coated with streptavidin (5μg/ml) in PBS as a control.

[0173] The wells were washed three times with 370μl of PBS and blocked with 3% milk powder in PBS for 1 hr at room temperature.

50μl of each overnight bacterial supernatant was mixed with 50μl of 6% milk powder in PBS for 1 hour.

[0174] The blocked ELISA plate was washed twice with PBS as described above and the blocked supernatants containing single chain antibody were added and incubated for 1 hp at room temperature.

[0175] The 96 well plate was washed four times with PBS 0.1% tween followed by the addition of 100μl of anti-human kappa light chains bound and free antibody HRP conjugate (Sigma A7164) 1:5000 dilution in PBS 1% BSA. The conjugate was incubated for 1 hr at room temperature followed by five washes with PBS 0.1%tween.

[0176] The ELISA was developed by the addition of TMB 2-Component Microwell Peroxidase Substrate Kit (Kirkegaard and Perry Laboratories Inc., USA) according to the manufacturer's protocol. An image of the ELISA plate is shown in Figure 4. At least four L1 binding clones have been observed for each of three single chain antibody versions. These L1 binding single chain antibody clones do not bind to streptavidin.

[0177] Figure 11 shows the binding of the L1_9.3, L1-9.3Hu and L1_9.3Hu3 scFvs to the human L1 cancer antigen. Rows A, B and C are coated with L1 and rows D, E and F are coated with streptavidin. The blue colour in the wells indicates binding of the individual scFv to the L1 on the plate. The lack of colour in the streptavidin coated rows shows that the single chain antibodies are specifically binding to L1.

6. Example 6

Determination of binding affinity

[0178] Mouse antibody L1-9.3 and humanised antibody L1-hu3 were assayed by Biacore analysis (Biacore AB, Uppsala, Sweden) to determine binding kinetics.

[0179] A BIAcore CM5 sensor chip was activated with EDC/NHS and purified recombinant L1-Fc extracellular fragment (515 μg/ml in PBS) was coupled to the CM5 sensor chip to between 200 and 3000RU. The remaining active sites were blocked by ethanolamine/HCl .Antibody binding was measured by adding antibody at concentrations from 6 to 3333nM at a flow rate of 10ul/min using the Kinject function. The chip was regenerated with 10mM Glycine pH2.0 with 500mM NaCl to remove the bound antibodies.

[0180] The binding curves were fit to a Langmuir binding model using BIAevaluation software (Biacore AB, Uppsala, Sweden). Determined KD values are shown in Table 2.

Table 2

Antibody	L1-9.3	L1-hu3
Ka [1/Ms]	**2.6×10^5**	**8.0×10^5**
Kd [1/s]	**2.2×10^{-5}**	**6.5×10^{-5}**
KD [M]	**8.5×10^{-11}**	**8.1×10^{-11}**

[0181] Table 2: The humanized variant L1-hu3 displays a similar high target affinity as the parent antibody L1-9.3.

7. Example 7

Antibody binding to PBMCs and cancer cells

[0182] PBMC were obtained by density gradient centrifugation from EDTA whole blood of healthy human donors. Cultured OVMZ tumor cells were harvested by trypsination. 1 x 10^5 cells/well (75μl) were seeded into FACS tubes. Dilutions of L1-9.3 mAb were prepared in culture medium with 10mM EDTA and 75μl/well of L1-mAb dilution were added, to PBMCs and OVMZ cells to result in final concentrations between 6.6×10^{-13} to 6.6×10^{-8} Mol. Subsequently cells were incubated over night (~24h) at 37°C / 5% CO2 in an incubator. Cells were washed directly in FACS tubes using 2ml of FACS buffer followed by centrifugation at 300g/5min/4°C. The supernatant was removed by pipetting. For staining, a PE-labelled donkey anti-mouse secondary antibody (Dako) was added at a volume of 150μl/well followed by incubation for 30min at 4°C. Washing steps were repeated as above and cells were fixed in 200μl PBS/1% formaldehyde. Sample mean fluorescence was then measured by FACS analysis.

[0183] As shown in Figure 13, L1-9.3 mAb displays a strongly reduced affinity to L1 on PBMC compared to tumor L1. L1-9.3 binding to PBMC was detected in the nanomolar range (dashed line), while binding to tumor cells could be observed at picomolar concentrations (solid line). B) The dissociation constants K_D were estimated from the regression curves using the concentration at half-maximal binding. K_D of L1-9.3 on PBMC was at least 400-fold lower than on tumor cells.

8. Example 8

Determination of cytokine release

[0184] PBMC were obtained by density gradient centrifugation from citrate whole blood of healthy human donors. Cells were resuspended in RPMI 1640/5% human serum/5ml NEAA /5ml L-Glutamin/5ml Natrium-Pyruvat. $1x10^5$ cells per 100µl were seeded in round bottom 96 well plates. In a second step, 100µl medium containing LPS (10ng/ml) L1-9.3 mAb (20µg/ml), OKT3 mAB (ebioscience) (75ng/ml) or Ionomycin/PMA (1µg/ml / 5ng/ml) were added in triplicates followed by an incubation for 24h at 37°C, 5%CO_2. As negative control, untreated PBMC were used. After 24h, levels of the cytokines interferone-gamma and tumor necrosis factor were measured by FACS analysis using the CBA-Cytokin-Flex-Sets (BD)according to manufacturers information..

[0185] The resulting cytokine levels are depicted in Figure 14. In contrast to OKT3 mAB, Ionomycin/PMA, and LPS, L1-9.3 did not significantly increase the TNF or IFN-gamma release by PBMCs.

9. Example 9

T-cell proliferation assy

[0186] PBMC were obtained by density gradient centrifugation from citrate whole blood of two healthy human donors. $1x10^5$ cells per well were seeded in flat bottom 96 well plates. In a second step, 100µl medium containing either L1-9.3 mAb (20µg/ml) and OKT3 (ebioscience, 75ng/ml) or L1-9.3 mAb (20µg/ml) or OKT3 (75ng/ml) was added in triplicates. After 1h, the latter two were supplemented with OKT3 or L1-9.3, respectively. To exclude any antibody related activation, PBMC with or without L1-9.3 were incubated in absence of OKT3. Following an incubation for 24h at 37°C, 5%CO_2 T cell proliferation was assessed using a BrdU incorporation assay (Roche) according to manufacturers information.

[0187] It can be concluded from the results shown in Figure 15, that L1-9.3 mAb does neither induce T-cell proliferation or inhibit OKT3 induced T-cell proliferation.

10. Example 10

Glycosylation dependency of antibody binding

[0188] $2x10^6$ SKOV3ip cells were seeded in a 10 cm petri dish and incubated for 24h at 37°C, 5% CO_2. After 24h, cells were washed with PBS and lysed with 500µl M-PER reagent (Pierce) according to the protocol described in the Seize Classic Mammalian Immunoprecipitation Kit (Pierce). SkOv3ip cell lysate were deglycosylated as described in the *Enzymatic CarboRelease Kit* (QA_Bio). Briefly, 2.5 µl denaturation solution was added to 35µl of cell lysate. The sample was incubated in a thermoblock at 100°C for 5min and then chilled on ice. Finally 2.5µl Triton-X and 1µl of each glycosidase contained in the *Enzymatic CarboRelease Kit* (QA_Bio) (PGNase F, O-Glycosidase, Sialidase, β-Galactosidase, Glucoaminidase) were added according to manufacturers protocol followed by an incubation at 37°C for 3h. Glycosylated and deglycosylated were subjected to SDS PAGE and subsequent Western blotting. Western blots were incubated with different L1 antibodies in dependence of their staining performance. Concentrations of 1µg/ml (9.3, 11A and 14.10), 5µg/ml (35.9) or 10µg/ml (OV52.24, OV543.18, 38.12, OV549.20) were used. L1 antibody binding to westen blot was detected with HRP-labeled anti-mouse antibody (Dianova).

[0189] As shown in Figure 16, the tested anti L1 antibodies can be divided into three classes in respect to their glycosylation-dependency: First class (unaffected by glycosylation): L1-9.3. Second class (binding in WB was negatively affected by deglycosylation): 11A, 14.10, OV52.24 and OV549.20. Third class (binding in WB was positively affected by deglycosylation): 35.9 and 38.12.

11. Example 11

Biodistribution of L1-9.3 in rabbit

[0190] A female rabbit (White Himalayan) was twice injected with L1-9.3 (0h, 24h) via the intravenous application route at a dose of 10 mg/kg. 1 control animal received a comparable volume of PBS. Animals were necropsied 72 h after the first application. Organs were fixed in 4% buffered formalin and embedded in paraffin. Histological slides were prepared and immunehistochemistry was performed. Tissue sections of the L1-9.3-treated and control animal were stained with an anti-mouse antibody to detect binding of L1-9.3 after intravenous application. Signals were visualized by DAB (Sigma). Two different detection systems, conventional Avidin/Biotin Complex method or tyramide signal amplification system CSA II method (Dako) were used, which allowed rough estimation of the amount of in vivo bound L1-9.3. The conventional

Avidin/Biotin Complex method (Vector Laboratories) is able to detect L1-9.3 concentrations of 50 ng/ml or higher, while the biotin-free tyramide signal amplification system CSA II (Dako) has a detection limit of 5ng/ml. To determine the L1 expression pattern, tissues of the control animal were incubated with primary antibody L1-9.3 and with the detection antibody. For ABC method a biotinylated anti-mouse antibody (Dianova, dilution 1:3000) was used as detection antibody, for CSA method was performed according to manufacturers protocoll.

[0191] Figure 17 shows the in vivo binding of intravenously applied L1-9.3 to collecting ducts of the kidney. In vivo binding was only detectable using the amplification system CSA (Fig. 17A), while by using the conventional ABC-method, no signal was visible (Fig. 17B). Hence, L1-9.3 was detected in a range of 30-300 pmol in the tissue (L1-9.3 concentration is presumably higher than 5 ng/ml and below 50 ng/ml). Negative control did not show staining, thus, unspecific staining can be excluded (Fig. Figure 17C).The staining pattern of in vivo bound L1-9.3 (Fig. 17A) corresponds to the L1 expression pattern in the kidney when directly staining tissue sections with L1-9.3 (Fig. 17D). It can be concluded that intravenously administered L1-9.3 antibody is able to extravasate to peripheral tissue.

12. Example 12

Function of humanized forms of L1 9.3mAb in nude mice

[0192] We investigated whether the humanized form of the mAb L1 9.3 could also inhibit the tumor growth of ovarian carcinoma in vivo. First we analysed the bindung of the two humanized forms of L1 9.3 to the selected cell line. Therefore, flow cytometry was performed on SKOV3ip pcDNA3.1 Luciferase cells. (Fig. 18). Both mAbs showed strong binding to the tumor cell line, and gave similar binding results as the native L1 9.3 mAb.

[0193] SKOV3ip pcDNA3.1Luciferase cells were injected into immundeficient mice 24h before starting the therapy. Humanized antibodies (300μg) or PBS were injected three times per week intraperitoneally. To detect the tumor growth in vivo, mice were imaged once weekly using the Xenogen IVIS 200 System. Mice were anesthetised and injected with Luciferin D, followed by detecting the light emission which is produced during luciferase activity of the tumor cells. During the time course we detected a slower tumor growth in the group of mice treated with humanized mAb compared to the control. At day 33 the last imaging data were taken. Imaging results gave a decreased tumor volume of around 80% using the hu3 mAb and approximately of 50% for chiL1 9.3. Both results were strongly significant (Fig. 19). After 36 days mice were sacrificed and tumor mass has determined. In both humanized anti-L1 mAbs treated groups a substantial decreased tumor mass was measured compared to the PBS group (Fig 20 (A, B)).

13. Example 13

[0194] Abolishment of chemoresistance by treatment with anti L1CAM monoclonal antibody 9.3 was tested as described in WO 2008/046529, Example 3 (see also Fig. 17e of WO 2008/046529). The results are shown in Figures 21 and 22. It could be demonstrated that the monoclonal antibody 9.3 abolishes chemoresistance. Its effect seems to be stronger than those of the antibody 11A tested in WO 2008/046529.

Table 1

mAb	FACS	Western blot	IP	L1-Fc	Invasion	phospho-Erk	ka (1/Ms)	kd (1/s)	KD (M)	tumor growth
L1-9.3	**+++**	**+++**	**+++**	**+++**	**-60%**	**-50%**	**2.6E+05**	**2.2E-05**	**8.5E-11**	**-60%**
L1-11A	+++	+++	+++	+++	-50%	-40%	1.0E+05	4.0E-06	4.0E-11	-40%
L1-14.10	+	++	+	+++	-40%	-40%	1.4E+04	1.0E-06	7.1E-11	-30%
L1-38.12	+	+++	+	+++	0	0	3.7E+04	2.0E-06	5.4E-11	
L1-35.9	+	+++	+	+++	0	0	4.0E+04	1.2E-05	3.0E-10	
L1-N15.17	++	-	++	++	0	0	5.3E+04	1.0E-03	1.9E-08	
L1-1D12.22	-	-	+	++	0	-20%	2.3E+04	1.0E-04	4.3E-09	
L1-1D17.3	-	-	+	++	0	0	2.3E+04	1.0E-04	4.3E-09	
L1-1D64.8	-	+++	+	+++	0	0	8.5E+04	1.5E-04	1.8E-09	
L1-1D74.8	-	+++	+	+++	-10%	0	3.0E+04	2.0E-03	6.7E-08	

[0195] Disclosed the following:

1. An anti-L1 monoclonal antibody which is capable of binding to the same L1 epitope recognized by the monoclonal antibody 9.3, produced by the hybridoma cell deposited under DSMZ ACC2841.

2. The anti-L1 monoclonal antibody according to 1, wherein the epitope is within the first immunoglobulin-like domain of L1.

3. An anti-L1 monoclonal antibody, having the same capacity to inhibit tumor growth as the monoclonal antibody 9.3, produced by the hybridoma cell deposited under DSMZ ACC2841.

4. An anti-L1 monoclonal antibody, characterized in that at least one of its complementarity determining regions (CDRs)

a) has one of the following sequences RASQDISNYLN, YTSRLHS, QQGNTLPWT, RYWML, EINPRNDRTNYNEKFKT, or GGGYAMDY or

b) has a sequence which, in comparison to the sequences mentioned under a) has at least one conservative amino acid exchange.

5. A monoclonal antibody, produced by the hybridoma cell deposited under DSMZ ACC2841.

6. A humanized antibody based on the monoclonal antibody of any of 1 to 5.

7. The humanized antibody of 6, having at least one non-human CDR and human framework region (FR) residues.

8. The humanized antibody according to any of 6 or 7, comprising the sequence of L1_9.3hu or L1_9.3hu3 as shown in Figure 8 a) and b).

9. A binding molecule comprising

a) at least one of the following sequences RASQDISNYLN, YTSRLHS, QQGNTLPWT, RYWML, EINPRNDRTNYNEKFKT, or GGGYAMDY or
b) at least one sequence which has in comparison to the sequences given in a) at least one conservative amino acid exchange.

10. The binding molecule of 9, being selected from the group consisting of single chain antibodies (e.g. scFv, multimers of scFv like diabodies, triabodies or tetrabodies, antibody fragments (e.g. Fab), tandabs, flexibodies, bispecific antibodies, and chimeric antibodies.

11. The antibody of any of 1 to 8 or the binding molecule of any of 9 or 10, linked to an active substance, preferably a toxin, a cytokine, a nanoparticle or a radionucleotide.

12. A hybridoma cell that produces the monoclonal antibody of any of 1 to 5.

13. The hybridoma cell deposited under DSMZ ACC2841.

14. Use of the antibody or the binding molecule of any of 1 to 11 for the preparation of a medicament for the treatment of a tumorigenic disease.

15. Use of the antibody or the binding molecule of any of 1 to 11 for sensitizing tumor cells in a patient for the treatment with a chemotherapeutic drug or with radiotherapy.

16. The use of 15, wherein the cells are at least partially resistant to the treatment with said chemotherapeutic drug or to radiotherapy.

17. The use of any of 15 or 16, wherein after the sensitization with the anti-L1 antibody the patient is further treated with said chemotherapeutic drug or with radiotherapy.

18. Use of the antibody or the binding molecule of any of 1 to 11 for the preparation of a medicament for the treatment of a tumorigenic disease in a patient previously treated with a chemotherapeutic drug or with radiotherapy.

19. The use of 18, wherein the patient is at least partially resistant to the treatment with said chemotherapeutic drug or with radiotherapy.

20. Use of the antibody or the binding molecule of any of 1 to 11 for the preparation of a medicament for the treatment of a tumorigenic disease in a patient at least partially resistant to treatment with a given chemotherapeutic drug or with radiotherapy.

21. Use of the antibody or the binding molecule of any of 1 to 11 for the preparation of a medicament for the treatment of a tumorigenic disease, wherein the L1 binding molecule is administered in combination with a chemotherapeutic drug or with radiotherapy.

22. The use of 21, wherein the chemotherapeutic drug or the radiotherapy is administered prior to the anti-L1 antibody.

23. The use of any of 14 to 22, wherein the tumor cells or the tumorigenic disease are of a type selected from the group consisting of astrocytoma, oligodendroglioma, meningioma, neurofibroma, glioblastoma, ependymoma, Schwannoma, neurofibrosarcoma, medulloblastoma, melanoma, pancreatic cancer, prostate carcinoma, head and neck cancer, breast cancer, lung cancer, ovarian cancer, endometrial cancer, renal cancer, neuroblastomas, squamous carcinomas, medulloblastomas, hepatoma, colon cancer and mesothelioma and epidermoid carcinoma.

24. The use of any of 14 to 22, wherein the tumor cells are from an epithelial tumor or the tumorigenic disease is an epithelial tumor, preferably wherein the epithelial tumor ispancreatic cancer, colon cancer, ovarian cancer or endometrial cancer.

25. The use of any of 15 to 24, wherein the chemotherapeutic drug is a DNA damaging agent, preferably selected from the group consisting of actinomycin-D, mitomycin C, cisplatin, doxorubicin, etoposide, verapamil, podophyllotoxin, 5-FU, taxans, preferably paclitaxel and carboplatin.

26. The use of any of 15 to 24, wherein the radiotherapy is selected from the group consisting of X-ray radiation, UV-radiation, γ-irradiation, α- or β-irradiation, and microwaves.

27. The antibody or the binding molecule of any of 1 to 11 for use as a medicament.

28. The antibody or the binding molecule of any of 1 to 11 for use as a medicament for the treatment of a tumorigenic disease or for sensitizing of tumor cells in a patient for the treatment with a chemotherapeutic drug or with radiotherapy.

29. The antibody or the binding molecule of any of 1 to 11 for use as a medicament in the treatment of tumor cells, with the features as defined in any of 16 to 26.

30. A pharmaceutical composition, comprising the antibody or the binding molecule of any of 1 to 11.

SEQUENCE LISTING

[0196]

<110> Deutsches Krebsforschungszentrum Stiftung des Öffentlichen Rechts (DKFZ)

<120> Treatment of Tumors using Specific Anti-L1 Antibody

<130> D64791PCEPT1

<160> 23

<170> PatentIn version 3.4

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Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp Ile Ser
            20                  25                  30
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Ser Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
        35                  40                  45

Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe
    50                  55                  60

Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu
65                  70                  75                  80

Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Leu
                85                  90                  95

Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
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<213> Artificial

<220>
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<400> 3

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ile Lys Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Glu Ala Ser Asn Leu Gln Ala Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Gln Ser Leu Pro Tyr
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
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<400> 4

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1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105
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<213> Artificial

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<400> 5

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
            100                 105
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<213> Artificial

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Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Ser Gly Ala
1               5                   10                  15

Ser Val Asn Leu Ser Cys Arg Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Trp Met Leu Trp Val Arg Gln Arg Pro Gly His Gly Leu Glu Trp Val
        35                  40                  45

Gly Glu Ile Asn Pro Arg Asn Asp Arg Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Thr Lys Ala Thr Leu Thr Val Asp Arg Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Thr Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
                85                  90                  95

Ala Leu Gly Gly Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
            100                 105                 110

Val Thr Val Ser Ser
        115
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<210> 7
<211> 118
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<213> Artificial

<220>
<223> HumIII antibody heavy chain amino acid sequence (Figure 8b)

<400> 7

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Lys Asp
            20                  25                  30

Tyr Ala Met Ser Ile Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
        35                  40                  45

Trp Val Ala Val Ile Ser Asn Gly Ser Asp Thr Tyr Tyr Ala Asp Ser
    50                  55                  60

Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Leu
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65 70 75 80

Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Asp Ser Arg Phe Phe Asp Val Ile Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

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<211> 117
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<223> L1_9.3hu antibody heavy chain amino acid sequence (Figure 8b)

<400> 8

Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Ser Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Arg Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
20 25 30

Trp Met Leu Trp Val Arg Gln Arg Pro Gly His Gly Leu Glu Trp Val
35 40 45

Gly Glu Ile Asn Pro Arg Asn Asp Arg Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Thr Arg Phe Thr Ile Ser Val Asp Arg Ser Lys Ser Thr Ala Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Leu Gly Gly Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 9
<211> 117
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<220>
<223> L1_9.3hu3 antibody heavy chain amino acid sequence (Figure 8b)

<400> 9

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Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Ser Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Arg Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
            20                  25                  30

Trp Met Leu Trp Val Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Glu Ile Asn Pro Arg Asn Asp Arg Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Thr Arg Phe Thr Ile Ser Val Asp Arg Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asp Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Ala Leu Gly Gly Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115
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<210> 10
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<400> 10

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1               5                  10                  15

Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Ser Gly Pro
            100                 105                 110

Gly Asp Gly Gly Lys Gly Gly Pro Gly Lys Gly Pro Gly Gly Glu Gly
        115                 120                 125

Thr Lys Gly Thr Gly Pro Gly Gly Gln Val Gln Leu Gln Gln Pro Gly
    130                 135                 140

Ala Glu Leu Val Lys Ser Gly Ala Ser Val Asn Leu Ser Cys Arg Ala
145                 150                 155                 160

Ser Gly Tyr Thr Phe Thr Arg Tyr Trp Met Leu Trp Val Arg Gln Arg
                165                 170                 175

Pro Gly His Gly Leu Glu Trp Val Gly Glu Ile Asn Pro Arg Asn Asp
            180                 185                 190

Arg Thr Asn Tyr Asn Glu Lys Phe Lys Thr Lys Ala Thr Leu Thr Val
        195                 200                 205

Asp Arg Ser Ser Ser Thr Ala Tyr Met Gln Leu Thr Ser Leu Thr Ser
    210                 215                 220

Glu Asp Ser Ala Val Tyr Phe Cys Ala Leu Gly Gly Gly Tyr Ala Met
225                 230                 235                 240

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
                245                 250
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<210> 11
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<400> 11

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
                85                  90                  95
```

```
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Ser Gly Pro
            100                 105                 110

Gly Asp Gly Gly Lys Gly Gly Pro Gly Lys Gly Pro Gly Gly Glu Gly
        115                 120                 125

Thr Lys Gly Thr Gly Pro Gly Gly Glu Val Gln Leu Val Gln Ser Gly
    130                 135                 140

Gly Gly Leu Val Gln Ser Gly Gly Ser Leu Arg Leu Ser Cys Arg Ala
145                 150                 155                 160

Ser Gly Tyr Thr Phe Thr Arg Tyr Trp Met Leu Trp Val Arg Gln Arg
                165                 170                 175

Pro Gly His Gly Leu Glu Trp Val Gly Glu Ile Asn Pro Arg Asn Asp
            180                 185                 190

Arg Thr Asn Tyr Asn Glu Lys Phe Lys Thr Arg Phe Thr Ile Ser Val
        195                 200                 205

Asp Arg Ser Lys Ser Thr Ala Tyr Leu Gln Met Asp Ser Leu Arg Ala
    210                 215                 220

Glu Asp Thr Ala Val Tyr Phe Cys Ala Leu Gly Gly Gly Tyr Ala Met
225                 230                 235                 240

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245                 250
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1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Ser Gly Pro
            100                 105                 110

Gly Asp Gly Gly Lys Gly Gly Pro Gly Lys Gly Pro Gly Gly Glu Gly
        115                 120                 125

Thr Lys Gly Thr Gly Pro Gly Gly Glu Val Gln Leu Val Gln Ser Gly
    130                 135                 140

Gly Gly Leu Val Gln Ser Gly Gly Ser Leu Arg Leu Ser Cys Arg Ala
145                 150                 155                 160

Ser Gly Tyr Thr Phe Thr Arg Tyr Trp Met Leu Trp Val Arg Gln Arg
                165                 170                 175

Pro Gly Lys Gly Leu Glu Trp Val Ala Glu Ile Asn Pro Arg Asn Asp
            180                 185                 190

Arg Thr Asn Tyr Asn Glu Lys Phe Lys Thr Arg Phe Thr Ile Ser Val
        195                 200                 205

Asp Arg Ser Lys Asn Thr Leu Tyr Leu Gln Met Asp Ser Leu Arg Ala
    210                 215                 220

Glu Asp Thr Ala Val Tyr Phe Cys Ala Leu Gly Gly Gly Tyr Ala Met
225                 230                 235                 240

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245                 250
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<400> 13

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Ala Gln Pro Ala Met Ala Asp Ile Gln Met Thr Gln Thr Thr Ser Ser
            20                  25                  30

Leu Ser Ala Phe Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser
        35                  40                  45

Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly
    50                  55                  60
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Thr Val Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr
85 90 95

Ile Ser Asn Leu Glu Gln Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln
100 105 110

Gly Asn Thr Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
115 120 125

Lys Arg Thr Ser Gly Pro Gly Asp Gly Gly Lys Gly Gly Pro Gly Lys
130 135 140

Gly Pro Gly Gly Glu Gly Thr Lys Gly Thr Gly Pro Gly Gly Gln Val
145 150 155 160

Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Ser Gly Ala Ser Val
165 170 175

Asn Leu Ser Cys Arg Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Trp Met
180 185 190

Leu Trp Val Arg Gln Arg Pro Gly His Gly Leu Glu Trp Val Gly Glu
195 200 205

Ile Asn Pro Arg Asn Asp Arg Thr Asn Tyr Asn Glu Lys Phe Lys Thr
210 215 220

Lys Ala Thr Leu Thr Val Asp Arg Ser Ser Ser Thr Ala Tyr Met Gln
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260 265 270

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305 310 315 320

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325 330 335

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
340 345 350

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His His His His His
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<212> DNA
<213> Mus musculus

<400> 15

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agcacactgg taatcgacgg cacgctcggt cctataatcg ttgatagact tgaccatagt 180
cgtctttggc ctaccgtggc actttgacga ctaaataata tggtcggcag acgtatcgcc 240
acacggctcg gcaaaatcgc cgtcgccatc gccgtggcta atatcggact ggtaaagatt 300
ggaccttgtc cttctaaaac gctggataaa aacggtcgtc ccgttgtgcg acggcacctg 360
gaaaccgcca ccgtggtttg acctttaatt tgcatgatca ccaggcccgc taccgccatt 420
tccgccaggc ccgtttccag gcccaccgct tccgtggttt ccgtgacccg ggcccccagt 480
ccaagtcgac gtcgtcggcc cacgccttga ccacttttcg ccgcgctcgc acttggactc 540
gacagcacgc tcgccgatat ggaaatgggc aataacctac gacacccacg cagtcgcagg 600
cccggtgccg gaccttaccc acccgcttta attaggcgca ttgctagcat ggttgatatt 660
gctttttaag ttttggtttc gctgggactg gcacctagca tcgtcgtcgt ggcgcatata 720
cgtcgactgc tcggactgga gacttctatc gcgccacata aagacgcgcg acccgccacc 780
gatacgctac ctaataaccc cggtcccgtg gtcgcaatgg cactcgtcgc cgccacgccg 840
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gagacaacac acggacgact tattgaagat agggtctctc cggtttcatg tcaccttcca 960
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gtcgtggatg tcggagtcgt cgtgggactg cgactcgttt cgtctgatgc tctttgtgtt 1080
tcagatgcgg acgcttcagt gggtagtccc ggactcaagc gggcagtgtt tctcgaagtt 1140
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<210> 16
<211> 406
<212> PRT
<213> Artificial

<220>
<223> amino acid sequence of expressed portion of L1_9.3Hu scFv construct (Figure 10b)

<400> 16

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1               5                  10                  15

Ala Gln Pro Ala Met Ala Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
            20                  25                  30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
        35                  40                  45

Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
    50                  55                  60
```

Ala Pro Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val
65 70 75 80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr
85 90 95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln
100 105 110

Gly Asn Thr Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
115 120 125

Lys Arg Thr Ser Gly Pro Gly Asp Gly Gly Lys Gly Gly Pro Gly Lys
130 135 140

Gly Pro Gly Gly Glu Gly Thr Lys Gly Thr Gly Pro Gly Gly Glu Val
145 150 155 160

Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Ser Gly Gly Ser Leu
165 170 175

Arg Leu Ser Cys Arg Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Trp Met
180 185 190

Leu Trp Val Arg Gln Arg Pro Gly His Gly Leu Glu Trp Val Gly Glu
195 200 205

Ile Asn Pro Arg Asn Asp Arg Thr Asn Tyr Asn Glu Lys Phe Lys Thr
210 215 220

Arg Phe Thr Ile Ser Val Asp Arg Ser Lys Ser Thr Ala Tyr Leu Gln
225 230 235 240

Met Asp Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys Ala Leu
245 250 255

Gly Gly Gly Tyr Ala Met Asp Tyr Trp Gly Ala Val Tyr Phe Cys Ala
260 265 270

Leu Gly Gly Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val
275 280 285

Thr Val Ser Ser Gly Gly Ala Ala Ala Ala Pro Ser Val Phe Ile Phe
290 295 300

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
305 310 315 320

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
325 330 335

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
340 345 350

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
355 360 365

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
370 375 380

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Ser
385 390 395 400

His His His His His His
405

<210> 17
<211> 1179
<212> DNA
<213> Artificial

<220>
<223> DNA sequence of the expressed potion of L1_9.3Hu construct (Figure 10b)

<400> 17

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ggccatggcc gatattcaga tgacccagag cccgagcagc ctgagcgcga gcgtgggtga   120
tcgtgtgacc attacctgcc gtgcgagcca ggatattagc aactatctga actggtatca   180
gcagaaaccg ggcaaagcgc cgaaactgct gatttattat accagccgtc tgcatagcgg   240
tgtgccgagc cgttttagcg gcagcggtag cggcaccgat tataccttta ccattagcag   300
cctgcagccg gaagattttg cgacctattt ttgccagcag ggcaacacgc tgccgtggac   360
ctttggcggt ggcaccaaac tggaaattaa acgtactagt ggtccgggcg atggcggtaa   420
aggcggtccg ggcaaaggtc cgggtggcga aggcaccaaa ggcactgggc ccgggggtga   480
agttcagctg gtgcagagcg gcggtggtct ggttcagagc ggtggcagcc tgcgtctgag   540
ctgtcgtgcg agcggctata ccttcacccg ttattggatg ctgtgggtgc gtcagcgtcc   600
gggccacggc ctggaatggg tgggcgaaat taatccgcgt aacgatcgta ccaactataa   660
cgaaaaattt aaaacccgct tcaccattag cgtggatcgt agcaaaagca ccgcgtatct   720
gcagatggat agcctgcgtg cggaagatac cgcggtgtat ttttgcgcgc tgggcggtgg   780
ctatgcgatg gattattggg gccagggcac cctggttacc gtgagcagcg gcggtgcggc   840
cgctgcacca tctgtcttca tcttcccgcc atctgatgag cagttgaaat ctggaactgc   900
ctctgttgtg tgcctgctga ataacttcta tcccagagag gccaaagtac agtggaaggt   960
ggataacgcc ctccaatcgg gtaactccca ggagagtgtc acagagcagg acagcaagga  1020
cagcacctac agcctcagca gcaccctgac gctgagcaaa gcagactacg agaaacacaa  1080
agtctacgcc tgcgaagtca cccatcaggg cctgagttcg cccgtcacaa agagcttcaa  1140

ccgcggagag tcacaccacc accaccacca ctagtaatt                         1179
```

<210> 18
<211> 1179
<212> DNA
<213> Artificial

<220>
<223> DNA sequence of the expressed portion of L1_9.3Hu construct (Figure 10b)

<400> 18

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ccggtaccgg ctataagtct actgggtctc gggctcgtcg gactcgcgct cgcacccact    120
agcacactgg taatggacgg cacgctcggt cctataatcg ttgatagact tgaccatagt    180
cgtctttggc ccgtttcgcg gctttgacga ctaaataata tggtcggcag acgtatcgcc    240
acacggctcg gcaaaatcgc cgtcgccatc gccgtggcta atatggaaat ggtaatcgtc    300
ggacgtcggc cttctaaaac gctggataaa aacggtcgtc ccgttgtgcg acggcacctg    360
gaaaccgcca ccgtggtttg acctttaatt tgcatgatca ccaggcccgc taccgccatt    420
tccgccaggc ccgtttccag gcccaccgct tccgtggttt ccgtgacccg ggcccccact    480
tcaagtcgac cacgtctcgc cgccaccaga ccaagtctcg ccaccgtcgg acgcagactc    540
gacagcacgc tcgccgatat ggaagtgggc aataacctac gacacccacg cagtcgcagg    600
cccggtgccg gaccttaccc acccgcttta attaggcgca ttgctagcat ggttgatatt    660
gcttttaaa ttttgggcga agtggtaatc gcacctagca tcgttttcgt ggcgcataga     720
cgtctaccta tcggacgcac gccttctatg gcgccacata aaaacgcgcg acccgccacc    780
gatacgctac ctaataaccc cggtcccgtg ggaccaatgg cactcgtcgc cgccacgccg    840
gcgacgtggt agacagaagt agaagggcgg tagactactc gtcaacttta gaccttgacg    900
gagacaacac acggacgact tattgaagat agggtctctc cggtttcatg tcaccttcca    960
cctattgcgg gaggttagcc cattgagggt cctctcacag tgtctcgtcc tgtcgttcct   1020
gtcgtggatg tcggagtcgt cgtgggactg cgactcgttt cgtctgatgc tctttgtgtt   1080
tcagatgcgg acgcttcagt gggtagtccc ggactcaagc gggcagtgtt tctcgaagtt   1140
ggcgcctctc agtgtggtgg tggtggtggt gatcattaa                          1179
```

<210> 19
<211> 389
<212> PRT
<213> Artificial

<220>
<223> amino acid sequence of expressed portion of L1_9.3Hu3 scFv construct (Figure 10c)

<400> 19

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Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
1               5                  10                  15
```

```
Ala Gln Pro Ala Met Ala Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
            20                  25                  30

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
        35                  40                  45

Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys
    50                  55                  60

Ala Pro Lys Leu Leu Ile Tyr Tyr Thr Ser Arg Leu His Ser Gly Val
65                  70                  75                  80

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr
                85                  90                  95

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln
            100                 105                 110

Gly Asn Thr Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
        115                 120                 125

Lys Arg Thr Ser Gly Pro Gly Asp Gly Gly Lys Gly Gly Pro Gly Lys
    130                 135                 140

Gly Pro Gly Gly Glu Gly Thr Lys Gly Thr Gly Pro Gly Gly Glu Val
145                 150                 155                 160

Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Ser Gly Gly Ser Leu
                165                 170                 175

Arg Leu Ser Cys Arg Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Trp Met
            180                 185                 190

Leu Trp Val Arg Gln Arg Pro Gly Lys Gly Leu Glu Trp Val Ala Glu
        195                 200                 205

Ile Asn Pro Arg Asn Asp Arg Thr Asn Tyr Asn Glu Lys Phe Lys Thr
    210                 215                 220

Arg Phe Thr Ile Ser Val Asp Arg Ser Lys Asn Thr Leu Tyr Leu Gln
225                 230                 235                 240

Met Asp Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys Ala Leu
                245                 250                 255

Gly Gly Gly Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
            260                 265                 270

Val Ser Ser Gly Gly Ala Ala Ala Pro Ser Val Phe Ile Phe Pro
        275                 280                 285
```

```
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
290                 295                 300

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
305                 310                 315                 320

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
                325                 330                 335

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
            340                 345                 350

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
        355                 360                 365

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Ser His
    370                 375                 380

His His His His His
385
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<210> 20
<211> 1179
<212> DNA
<213> Artificial

<220>
<223> DNA sequence of the expressed portion of L1_9.3Hu3 scFv construct (Figure 10c)

<400> 20

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tcgtgtgacc attacctgcc gtgcgagcca ggatattagc aactatctga actggtatca   180
gcagaaaccg ggcaaagcgc cgaaactgct gatttattat accagccgtc tgcatagcgg   240
tgtgccgagc cgttttagcg gcagcggtag cggcaccgat tataccctga ccattagcag   300
cctgcagccg gaagattttg cgacctattt ttgccagcag ggcaacacgc tgccgtggac   360
ctttggcggt ggcaccaaac tggaaattaa acgtactagt ggtccgggcg atggcggtaa   420
aggcggtccg ggcaaaggtc cgggtggcga aggcaccaaa ggcactgggc ccgggggtga   480
agttcagctg gtgcagagcg gcggtggtct ggttcagagc ggtggcagcc tgcgtctgag   540
ctgtcgtgcg agcggctata cctttacccg ttattggatg ctgtgggtgc gtcagcgtcc   600
gggtaaaggc ctggaatggg tggcggaaat taatccgcgt aacgatcgta ccaactataa   660
cgaaaaattt aaaacccgct tcaccattag cgtggatcgt agcaaaaaca ccctgtatct   720
gcagatggat agcctgcgtg cggaagatac cgcggtgtat ttttgcgcgc tgggcggtgg   780
ctatgcgatg gattattggg gccagggcac cctggttacc gtgagcagcg gcggtgcggc   840
cgctgcacca tctgtcttca tcttcccgcc atctgatgag cagttgaaat ctggaactgc   900

ctctgttgtg tgcctgctga ataacttcta tcccagagag gccaaagtac agtggaaggt   960
ggataacgcc ctccaatcgg gtaactccca ggagagtgtc acagagcagg acagcaagga  1020
cagcacctac agcctcagca gcaccctgac gctgagcaaa gcagactacg agaaacacaa  1080
agtctacgcc tgcgaagtca cccatcaggg cctgagttcg cccgtcacaa agagcttcaa  1140
ccgcggagag tcacaccacc accaccacca ctagtaatt                         1179
```

<210> 21
<211> 1179
<212> DNA
<213> Artificial

<220>
<223> DNA sequence of the expressed portion of L1_9.3Hu3 scFv construct (Figure 10c)

<400> 21

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ccggtaccgg ctataagtct actgggtctc gggctcgtcg gactcgcgct cgcacccact    120
agcacactgg taatggacgg cacgctcggt cctataatcg ttgatagact tgaccatagt    180
cgtctttggc ccgtttcgcg gctttgacga ctaaataata tggtcggcag acgtatcgcc    240
acacggctcg gcaaaatcgc cgtcgccatc gccgtggcta atatgggact ggtaatcgtc    300
ggacgtcggc cttctaaaac gctggataaa aacggtcgtc ccgttgtgcg acggcacctg    360
gaaaccgcca ccgtggtttg acctttaatt tgcatgatca ccaggcccgc taccgccatt    420
tccgccaggc ccgtttccag gcccaccgct tccgtggttt ccgtgacccg ggcccccact    480
tcaagtcgac cacgtctcgc cgccaccaga ccaagtctcg ccaccgtcgg acgcagactc    540
gacagcacgc tcgccgatat ggaaatgggc aataacctac gacacccacg cagtcgcagg    600
cccatttccg gaccttaccc accgccttta attaggcgca ttgctagcat ggttgatatt    660
gctttttaaa ttttgggcga agtggtaatc gcacctagca tcgtttttgt gggacataga    720
cgtctaccta tcggacgcac gccttctatg gcgccacata aaaacgcgcg acccgccacc    780
gatacgctac ctaataaccc cggtcccgtg ggaccaatgg cactcgtcgc cgccacgccg    840
gcgacgtggt agacagaagt agaagggcgg tagactactc gtcaacttta gaccttgacg    900
gagacaacac acggacgact tattgaagat agggtctctc cggtttcatg tcaccttcca    960
cctattgcgg gaggttagcc cattgagggt cctctcacag tgtctcgtcc tgtcgttcct   1020
gtcgtggatg tcggagtcgt cgtgggactg cgactcgttt cgtctgatgc tctttgtgtt   1080
tcagatgcgg acgcttcagt gggtagtccc ggactcaagc gggcagtgtt tctcgaagtt   1140
ggcgcctctc agtgtggtgg tggtggtggt gatcattaa                          1179
```

<210> 22
<211> 713
<212> DNA
<213> Artificial

<220>
<223> genomic sequence of the kappa chain variable region (Figure 12a)

<400> 22

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cacttcctat ctttgggtac tttttcatat accagtcaga ttgtgagcca ttgtaattga 120
agtcaagact cagcctggac atgatgtcct ctgctcagtt ccttggtctc ctgttgctct 180
gtcttcaagg taaaagttac tacaatggga attttgctgt tgcacagtga ttcttgttga 240
ctggaatttt ggaggggtcc tttcttttcc tgcttaactc tgtgggtatt tattgtgtct 300
ccactcctag gtaccagatg tgatatccag atgacacaga ctacatcctc cctgtctgcc 360
tttctgggag acagagtcac catcagttgc agggcaagtc aggacattag caattattta 420
aactggtatc agcagaaacc agatggaact gttaaactcc ttatctatta cacatcaaga 480
ttacactcag gagtcccctc aaggttcagt ggcagtgggt ctggaacaga ttattctctc 540
accattagca acctggagca agaagatttt gccacttact tttgccaaca gggtaatacg 600
cttccgtgga cattcggtgg aggcaccaag ctggaaatca aacgtaaata gaatccaaag 660
tctctttctt ccgttgtcta tgtctgtggc ttctatgtct acaaatgatg tat 713

<210> 23
<211> 839
<212> DNA
<213> Artificial

<220>
<223> genomic sequence of the heavy chain variable region (Figure 12b)

<400> 23

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ttgtctatat cacacacaga aaaacatgag atcacagttc tctctacagt tactgaacac 180
acaggacctc accatgggat ggagctatat catcctcttt ttggtagcaa cagctacagg 240
taaggggctc acagtagaag gcttgaggtc tggccatata catgggtgac agtgacatcc 300
actttgcctt tctttccaca gatgtccact cccaggtcca actgcagcag cctggggctg 360
aactggtgaa gtctggggct tcagtgaacc tgtcctgcag ggcttctggc tacaccttca 420
ccagatactg gatgctctgg gtgaggcaga ggcctggaca tggccttgag tgggttggag 480
agattaatcc tcgcaacgat cgtactaatt acaatgagaa attcaagacc aaggccacac 540
tgactgtaga ccgatcctcc agcacagcct acatgcaact caccagcctg acatctgagg 600
actctgcggt ctatttctgt gccctggggg ggggctatgc tatggactat tggggtcaag 660
gaacctcagt caccgtctcc tcaggtaaga atggcctctc caggtcttaa tttttaacct 720
ttgttatgga gttttctgag cattgcagac taatcttgga tatttgtccc tgagggagcc 780
ggctgagaga agttgggaaa taaactgtct agggatctca gagcctttag gacagatta 839

Claims

1. A binding molecule capable of binding L1,

   (a) being selected from the group consisting of single chain antibodies, scFv, multimers of scFv like diabodies, triabodies or tetrabodies, antibody fragments, Fab, tandabs, flexibodies, bispecific antibodies, and chimeric antibodies,
   and/or
   (b) which comprises at least one Ig domain,
   and wherein the binding molecule capable of binding L1:

   (i) is **characterized in that** its complementarity determining regions (CDRs) have the following sequences: LCDR1: RASQDISNYLN (SEQ ID No.: 24), LCDR2: YTSRLHS (SEQ ID No.: 25), LCDR3: QQGNTLPWT (SEQ ID No.: 26), HCDR1: RYWML (SEQ ID No.: 27), HCDR2: EINPRNDRTNYNEKFKT (SEQ ID No.: 28), and HCDR3: GGGYAMDY (SEQ ID No.: 29),
   and which binding molecule binds L1 with an affinity (KD) of at least 10^{-10} M, or
   (ii) is **characterized in that** its complementarity determining regions (CDRs) have the following sequences: LCDR1: QDISNY (SEQ ID No.: 30), LCDR2: YTS, LCDR3: QQGNTLPWT (SEQ ID No.: 31), HCDR1: GYTFTRYW (SEQ ID No.: 32), HCDR2: INPRNDRT (SEQ ID No.: 33), and HCDR3: ALGGGYAMDY (SEQ ID No.: 34),
   and which binding molecule binds L1 with an affinity (KD) of at least 10^{-10} M.

2. The binding molecule capable of binding L1 of claim 1, wherein the binding molecule binds L1 with an affinity (KD) of at least 10^{-11} M.

3. The binding molecule capable of binding L1 of claim 1 or 2, linked to an active substance, preferably a toxin, a cytokine, a nanoparticle or a radionuclide.

4. A binding molecule capable of binding L1 of any of claims 1 to 3, for use in a method of treatment of a tumorigenic disease.

5. A binding molecule capable of binding L1 of any of claims 1 to 3, for use in a method of sensitizing tumor cells in a patient for the treatment with a chemotherapeutic drug or with radiotherapy.

6. The binding molecule capable of binding L1 for use according to claim 5, wherein the cells are at least partially resistant to the treatment with said chemotherapeutic drug or to radiotherapy.

7. The binding molecule capable of binding L1 for use according to claim 5 or 6, wherein after the sensitization with the binding molecule the patient is further treated with said chemotherapeutic drug or with radiotherapy.

8. The binding molecule capable of binding L1 for use according to claim 4, in a patient previously treated with a chemotherapeutic drug or with radiotherapy.

9. The binding molecule capable of binding L1 for use according to claim 8, wherein the patient is at least partially resistant to the treatment with said chemotherapeutic drug or with radiotherapy.

10. The binding molecule capable of binding L1,

    (i) for use according to claim 4, in a patient at least partially resistant to treatment with a given chemotherapeutic drug or with radiotherapy, or
    (ii) for use according to claim 4, wherein the binding molecule is administered in combination with a chemotherapeutic drug or with radiotherapy.

11. The binding molecule capable of binding L1 for use according to claim 10, item (ii), wherein the chemotherapeutic drug or the radiotherapy is administered prior to the binding molecule.

12. The binding molecule capable of binding L1 for use of any of claims 4 to 11, wherein the tumor cells or the tumorigenic disease are of a type selected from the group consisting of astrocytoma, oligodendroglioma, meningioma, neurofi-

broma, glioblastoma, ependymoma, Schwannoma, neurofibrosarcoma, medulloblastoma, melanoma, pancreatic cancer, prostate carcinoma, head and neck cancer, breast cancer, lung cancer, ovarian cancer, endometrial cancer, renal cancer, neuroblastomas, squamous carcinomas, hepatoma, colon cancer and mesothelioma and epidermoid carcinoma, or,
wherein the tumor cells are from an epithelial tumor or the tumorigenic disease is an epithelial tumor.

13. The binding molecule capable of binding L1 for use according to claim 12, wherein the epithelial tumor is pancreatic cancer, colon cancer, ovarian cancer or endometrial cancer.

14. The binding molecule capable of binding L1 for use of any of claims 5 to 13, wherein the chemotherapeutic drug is a DNA damaging agent, preferably selected from the group consisting of actinomycin-D, mitomycin C, cisplatin, doxorubicin, etoposide, verapamil, podophyllotoxin, 5-FU, taxans, preferably paclitaxel and carboplatin, or,
wherein the radiotherapy is selected from the group consisting of X-ray radiation, UV-radiation, γ-irradiation, α- or β-irradiation, and microwaves.

15. A pharmaceutical composition, comprising the binding molecule capable of binding L1 of any of claims 1 to 3.

16. Use of a binding molecule capable of binding L1 of any of claims 1 to 3, for determining the level of the L1 protein in body tissues or fluids in vitro.

Patentansprüche

1. Bindungsmolekül, das in der Lage ist L1 zu binden,

(a) das ausgewählt ist aus der Gruppe bestehend aus Einzelketten-Antikörpern, scFv, Multimeren von scFv-ähnlichen Diabodies, Triabodies oder Tetrabodies, Antikörperfragmenten, Fab, Tandabs, Flexibodies, bispezifischen Antikörpern und chimeren Antikörpern,
und / oder
(b) das mindestens eine Ig-Domäne umfasst,
und wobei das Bindungsmolekül, das in der Lage ist L1 zu binden:

(i) dadurch charakterisiert ist, dass seine Komplementarität-bestimmenden Regionen (complementarity determining regions, CDRs) die folgenden Sequenzen haben: LCDR1: RASQDISNYLN (SEQ ID Nr.: 24), LCDR2: YTSRLHS (SEQ ID Nr.: 25), LCDR3: QQGNTLPWT (SEQ ID No.: 26), HCDR1: RYWML (SEQ ID Nr.: 27), HCDR2: EINPRNDRTNYNEKFKT (SEQ ID Nr.: 28), und HCDR3: GGGYAMDY (SEQ ID Nr.: 29), und wobei das Bindungsmolekül L1 mit einer Affinität (KD) von mindestens 10^{-10} M bindet,
oder
(ii) dadurch charakterisiert ist, dass seine Komplementarität-bestimmenden Regionen (complementarity determining regions, CDRs) die folgenden Sequenzen haben: LCDR1: QDISNY (SEQ ID Nr.: 30), LCDR2: YTS, LCDR3: QQGNTLPWT (SEQ ID Nr.: 31), HCDR1: GYTFTRYW (SEQ ID No.: 32), HCDR2: INPRNDRT (SEQ ID Nr.: 33), und HCDR3: ALGGGYAMDY (SEQ ID Nr.: 34),
und wobei das Bindungsmolekül L1 mit einer Affinität (KD) von mindestens 10^{-10} M bindet.

2. Bindungsmolekül, das in der Lage ist L1 zu binden von Anspruch 1, wobei das Bindungsmolekül L1 mit einer Affinität (KD) von mindestens 10^{-11} M bindet.

3. Bindungsmolekül, das in der Lage ist L1 zu binden von Anspruch 1 oder 2, verknüpft an eine aktive Substanz, bevorzugt ein Toxin, ein Zytokin, ein Nanopartikel, oder ein Radionuklid.

4. Bindungsmolekül, das in der Lage ist L1 zu binden von einem beliebigen der Ansprüche 1 bis 3, zur Verwendung in einem Verfahren zur Behandlung einer tumorigenen Erkrankung.

5. Bindungsmolekül, das in der Lage ist L1 zu binden von einem beliebigen der Ansprüche 1 bis 3, zur Verwendung in einem Verfahren um Tumorzellen in einem Patienten für die Behandlung mit einem chemotherapeutischen Wirkstoff oder mit Strahlentherapie zu sensibilisieren.

6. Bindungsmolekül, das in der Lage ist L1 zu binden zur Verwendung gemäß Anspruch 5, wobei die Zellen zumindest

partiell resistent sind gegenüber der Behandlung mit diesem chemotherapeutischen Wirkstoff oder gegenüber Strahlentherapie.

7. Bindungsmolekül, das in der Lage ist L1 zu binden zur Verwendung gemäß Anspruch 5 oder 6, wobei der Patient nach dem Sensibilisieren mit dem Bindungsmolekül weiter mit diesem chemotherapeutischen Wirkstoff oder mit Strahlentherapie behandelt wird.

8. Bindungsmolekül, das in der Lage ist L1 zu binden zur Verwendung gemäß Anspruch 4, in einem Patienten der zuvor mit einem chemotherapeutischen Wirkstoff oder mit Strahlentherapie behandelt wurde.

9. Bindungsmolekül, das in der Lage ist L1 zu binden zur Verwendung gemäß Anspruch 8, wobei der Patient mindestens partiell resistent gegenüber der Behandlung mit diesem chemotherapeutischen Wirkstoff oder mit Strahlentherapie ist.

10. Bindungsmolekül, das in der Lage ist L1 zu binden,

(i) zur Verwendung gemäß Anspruch 4, in einem Patienten, der mindestens partiell resistent gegenüber der Behandlung mit einem bestimmten chemotherapeutischen Wirkstoff oder mit Strahlentherapie ist, oder
(ii) zur Verwendung gemäß Anspruch 4, wobei das Bindungsmolekül in Kombination mit einem chemotherapeutischen Wirkstoff oder mit Strahlentherapie verabreicht wird.

11. Bindungsmolekül, das in der Lage ist L1 zu binden zur Verwendung gemäß Anspruch 10, Punkt (ii), wobei der chemotherapeutische Wirkstoff oder die Strahlentherapie vor dem Bindungsmolekül verabreicht wird.

12. Bindungsmolekül, das in der Lage ist L1 zu binden zur Verwendung von einem beliebigen der Ansprüche 4 bis 11, wobei die Tumorzellen oder die tumorigeneErkrankung von einem Typ sind, der ausgewählt ist aus der Gruppe bestehend aus Astrozytom, Oligodendrogliom, Meningiom, Neurofibrom, Glioblastom, Ependymom, Schwannom, Neurofribrosarkom, Medulloblastom, Melanom, Bauchspeicheldrüsenkrebs, Prostatakarzinom, Kopf- und Halskrebs, Brustkrebs, Lungenkrebs, Eierstockkrebs, Endometriumkrebs, Nierenkrebs, Neuroblastomen, Plattenepithelkarzinomen, Hepatom, Darmkrebs und Mesotheliom und epidermoidem Karzinom, oder
wobei die Tumorzellen von einem epithelialen Tumor sind oder die tumorigene Erkrankung ein epithelialer Tumor ist.

13. Bindungsmolekül, das in der Lage ist L1 zu binden zur Verwendung gemäß Anspruch 12, wobei der epitheliale Tumor Bauchspeicheldrüsenkrebs, Darmkrebs, Eierstockkrebs oder Endometriumkrebs ist.

14. Bindungsmolekül, das in der Lage ist L1 zu binden zur Verwendung von einem beliebigen der Ansprüche 5 bis 13, wobei der chemotherapeutische Wirkstoff eine DNA-schädigende Substanz ist, bevorzugt ausgewählt aus der Gruppe bestehend aus Actinomycin-D, Mitomycin C, Cisplatin, Doxorubicin, Etoposid, Verapamil, Podophyllotoxin, 5-FU, Taxanen, bevorzugt Paclitaxel und Carboplatin, oder
wobei die Strahlentherapie ausgewählt ist aus der Gruppe bestehend aus Röntgenstrahlung, UV-Strahlung, γ-Strahlung, α- oder β-Strahlung und Mikrowellen.

15. Pharmazeutische Zusammensetzung umfassend das Bindungsmolekül, das in der Lage ist L1 zu binden von einem beliebigen der Ansprüche 1 bis 3.

16. Verwendung eines Bindungsmoleküls, das in der Lage ist L1 zu binden von einem beliebigen der Ansprüche 1 bis 3, zur Bestimmung des Spiegels des L1-Proteins in Körpergeweben oder -flüssigkeiten in vitro.

Revendications

1. Molécule de liaison capable de se lier à L1,

(a) qui est choisie dans le groupe consistant en des anticorps à chaîne unique, scFv, des multimères de dianticorps, de trianticorps ou de tétra-anticorps de type scFv, des fragments d'anticorps, Fab, des tandabs, des flexibodies, des anticorps bispécifiques et des anticorps chimériques, et/ou
(b) qui comprend au moins un domaine Ig,
et la molécule de liaison capable de se lier à L1 :

(i) étant **caractérisée en ce que** ses régions de détermination de complémentarité (CDR) ont les séquences suivantes : LCDR1 : RASQDISNYLN (SEQ ID No : 24), LCDR2 : YTSRLHS (SEQ ID No : 25), LCDR3 : QQGNTLPWT (SEQ ID No : 26), HCDR1 : RYWML (SEQ ID No : 27), HCDR2 : EINPRNDRTNYNEKFKT (SEQ ID No : 28) et HCDR3 : GGGYAMDY (SEQ ID No : 29),
et laquelle molécule de liaison se liant à L1 avec une affinité (KD) d'au moins 10^{-10} M, ou
(ii) étant **caractérisée en ce que** ses régions de détermination de complémentarité (CDR) ont les séquences suivantes : LCDR1 : QDISNY (SEQ ID No : 30), LCDR2 : YTS, LCDR3 : QQGNTLPWT (SEQ ID No : 31), HCDR1: GYTFTRYW (SEQ ID No : 32), HCDR2 : INPRNDRT (SEQ ID No : 33) et HCDR3 : ALGGGYAMDY (SEQ ID No : 34),
et laquelle molécule de liaison se liant à L1 avec une affinité (KD) d'au moins 10^{-10} M.

2. Molécule de liaison capable de se lier à L1 de la revendication 1, la molécule de liaison se liant à L1 avec une affinité (KD) d'au moins 10^{-11} M.

3. Molécule de liaison capable de se lier à L1 de la revendication 1 ou 2, liée à une substance active, de préférence une toxine, une cytokine, une nanoparticule ou un radionucléide.

4. Molécule de liaison capable de se lier à L1 de l'une des revendications 1 à 3, destinée à être utilisée dans un procédé de traitement d'une maladie tumorigène.

5. Molécule de liaison capable de se lier à L1 de l'une des revendications 1 à 3, destinée à être utilisée dans un procédé de sensibilisation de cellules tumorales chez un patient pour le traitement avec un médicament chimiothérapeutique ou par radiothérapie.

6. Molécule de liaison capable de se lier à L1 destinée à être utilisée selon la revendication 5, où les cellules sont au moins partiellement résistantes au traitement avec ledit médicament chimiothérapeutique ou à la radiothérapie.

7. Molécule de liaison capable de se lier à L1 destinée à être utilisée selon la revendication 5 ou 6, où, après la sensibilisation avec la molécule de liaison, le patient est en outre traité avec ledit médicament chimiothérapeutique ou par radiothérapie.

8. Molécule de liaison capable de se lier à L1 destinée à être utilisée selon la revendication 4, chez un patient précédemment traité avec un médicament chimio-thérapeutique ou par radiothérapie.

9. Molécule de liaison capable de se lier à L1 destinée à être utilisée selon la revendication 8, où le patient est au moins partiellement résistant au traitement avec ledit médicament chimiothérapeutique ou par radio-thérapie.

10. Molécule de liaison capable de se lier à L1,

(i) destinée à être utilisée selon la revendication 4, chez un patient au moins partiellement résistant au traitement avec un médicament chimiothérapeutique donné ou par radiothérapie, ou
(ii) destinée à être utilisée selon la revendication 4, la molécule de liaison étant administrée en combinaison avec un médicament chimiothérapeutique ou avec la radio-thérapie.

11. Molécule de liaison capable de se lier à L1 destinée à être utilisée selon la revendication 10, point (ii), où le médicament chimiothérapeutique ou la radio-thérapie est administré avant la molécule de liaison.

12. Molécule de liaison capable de se lier à L1 destinée à être utilisée selon l'une des revendications 4 à 11, où les cellules tumorales ou la maladie tumorigène sont d'un type choisi dans le groupe consistant en l'astrocytome, l'oligodendrogliome, le méningiome, le neurofibrome, le glioblastome, l'épendymome, le Schwannome, le neurofibrosarcome, le médulloblastome, un mélanome, le cancer du pancréas, le cancer de la prostate, le cancer de la tête et du cou, le cancer du sein, le cancer du poumon, le cancer de l'ovaire, le cancer de l'endomètre, le cancer du rein, les neuroblastomes, les carcinomes squameux, un hépatome, le cancer du côlon et le mésothéliome et le carcinome épidermoïde, ou,
où les cellules tumorales proviennent d'une tumeur épithéliale ou la maladie tumorigène est une tumeur épithéliale.

13. Molécule de liaison capable de se lier à L1 destinée à être utilisée selon la revendication 12, où la tumeur épithéliale est le cancer du pancréas, le cancer du côlon, le cancer de l'ovaire ou le cancer de l'endomètre.

14. Molécule de liaison capable de se lier à L1 destinée à être utilisée selon l'une des revendications 5 à 13, où le médicament chimiothérapeutique est un agent endommageant l'ADN, de préférence choisi dans le groupe consistant en l'actinomycine-D, la mitomycine C, le cisplatine, la doxorubicine, l'étoposide, le vérapamil, la podophyllotoxine, le 5-FU, les taxanes, de préférence le paclitaxel et le carboplatine, ou où la radiothérapie est choisie dans le groupe consistant en le rayonnement X, le rayonnement UV, l'irradiation aux rayons γ, l'irradiation aux rayons α ou β et des micro-ondes.

15. Composition pharmaceutique, comprenant la molécule de liaison capable de se lier à L1 de l'une des revendications 1 à 3.

16. Utilisation d'une molécule de liaison capable de se lier à L1 de l'une des revendications 1 à 3, pour déterminer le niveau de la protéine L1 dans des tissus ou des fluides corporels in vitro.

Figure 1

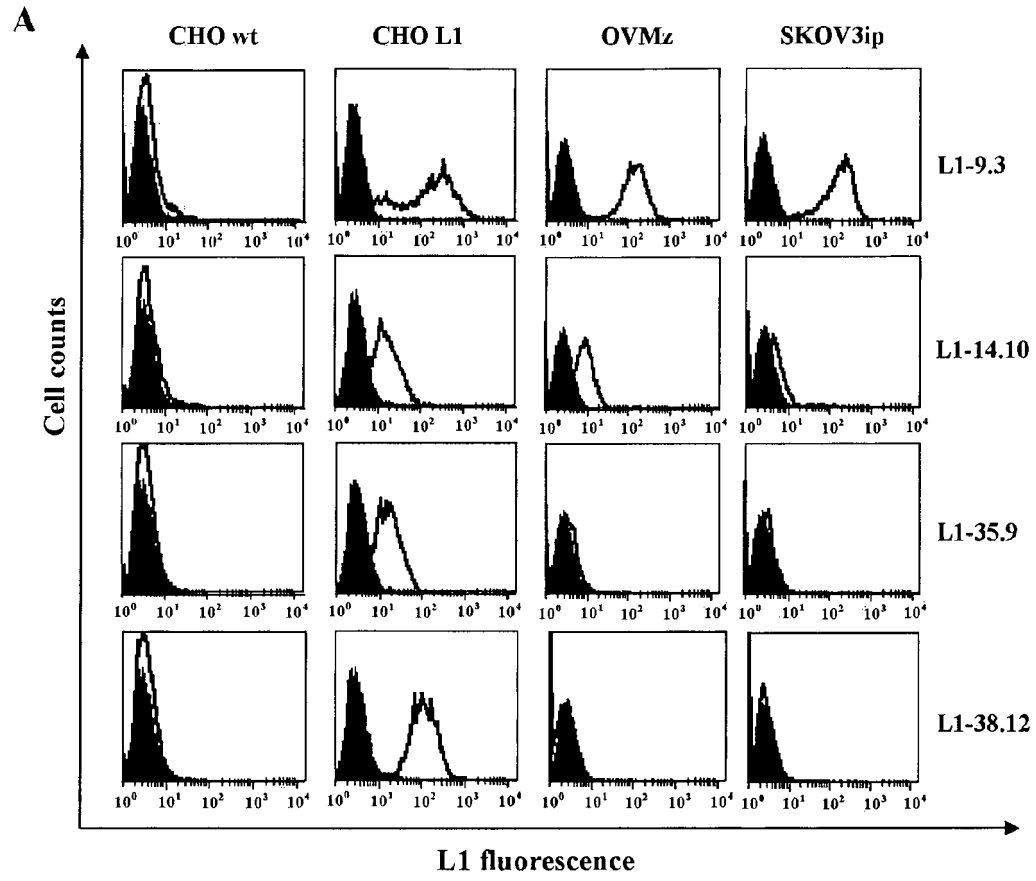

A
CHO wt
CHO L1
OVMz
SKOV3ip
L1-9.3
L1-14.10
L1-35.9
L1-38.12
Cell counts
L1 fluorescence

Figure 1

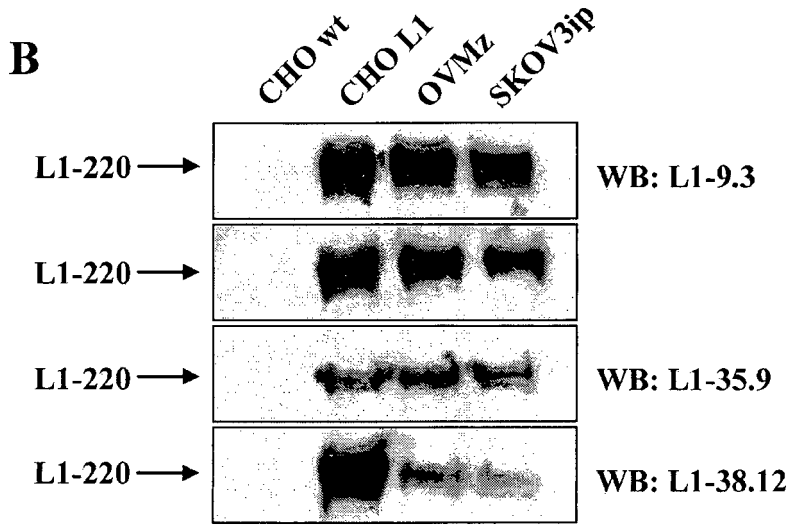

B
CHO wt
CHO L1
OVMz
SKOV3ip
L1-220
L1-220
L1-220
L1-220
WB: L1-9.3
WB: L1-35.9
WB: L1-38.12

Figure 2

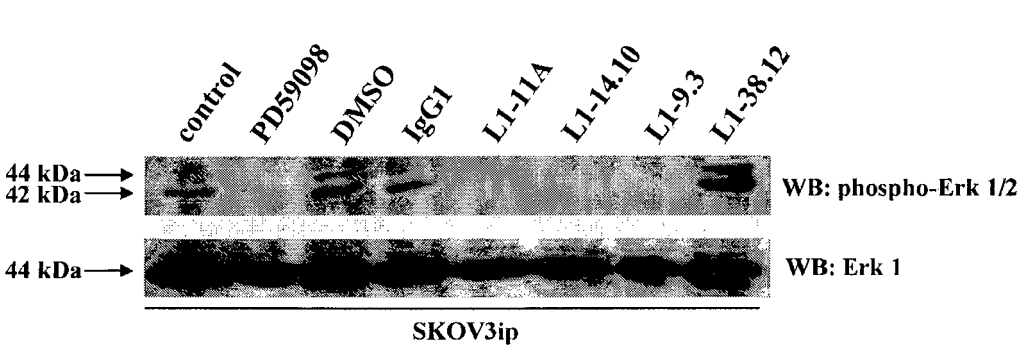

A
control
PD59098
DMSO
IgG1
L1-11A
L1-14.10
L1-9.3
L1-38.12
44 kDa
42 kDa
WB: phospho-Erk 1/2
44 kDa
WB: Erk 1
SKOV3ip
B
control
IgG1-control
DMSO
PD 98059
SKOV3ip
L1-11A
L1-9.3
L1-14.10
L1-38.12
SKOV3ip

Figure 3

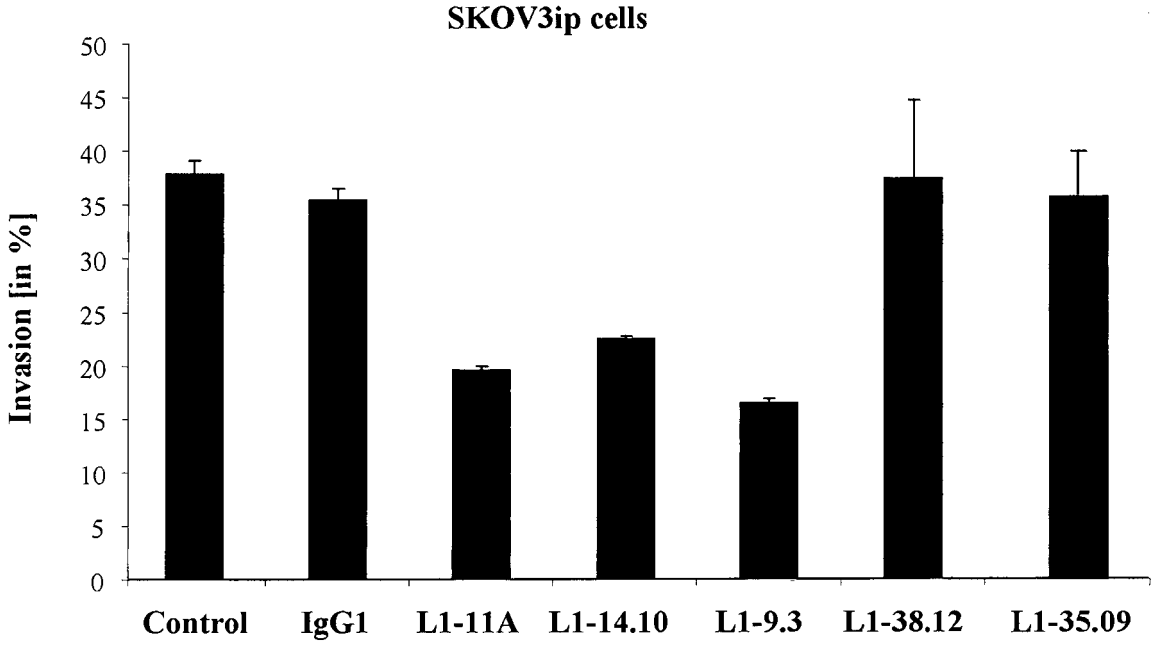

SKOV3ip cells
Invasion [in %]
50
45
40
35
30
25
20
15
10
5
0
Control
IgG1
L1-11A
L1-14.10
L1-9.3
L1-38.12
L1-35.09

Figure 4

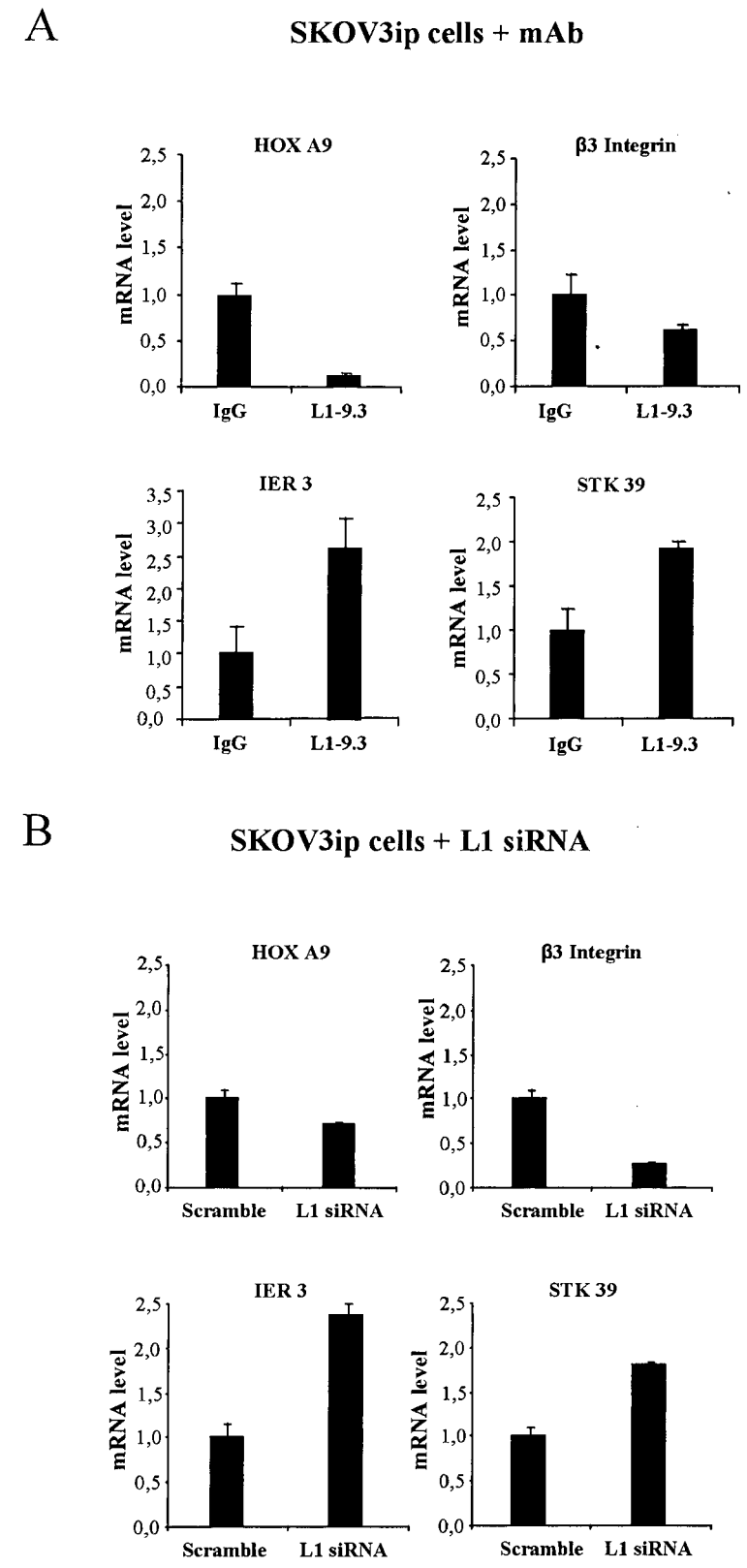

A
SKOV3ip cells + mAb
HOX A9
β3 Integrin
IER 3
STK 39
mRNA level
IgG
L1-9.3
B
SKOV3ip cells + L1 siRNA
HOX A9
β3 Integrin
IER 3
STK 39
mRNA level
Scramble
L1 siRNA

Figure 4

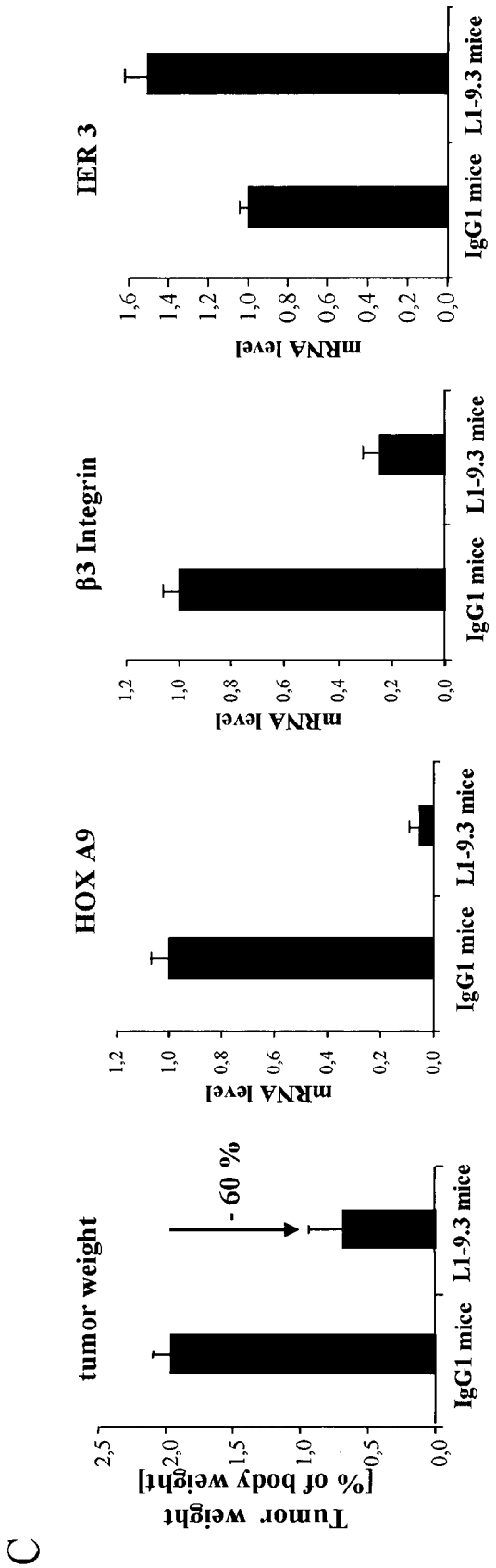

C
Tumor weight
[% of body weight]
2,5
2,0
1,5
1,0
0,5
0,0
tumor weight
- 60 %
IgG1 mice
L1-9.3 mice
mRNA level
1,2
1,0
0,8
0,6
0,4
0,2
0,0
HOX A9
IgG1 mice
L1-9.3 mice
mRNA level
1,2
1,0
0,8
0,6
0,4
0,2
0,0
β3 Integrin
IgG1 mice
L1-9.3 mice
mRNA level
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
IER 3
IgG1 mice
L1-9.3 mice

Figure 5

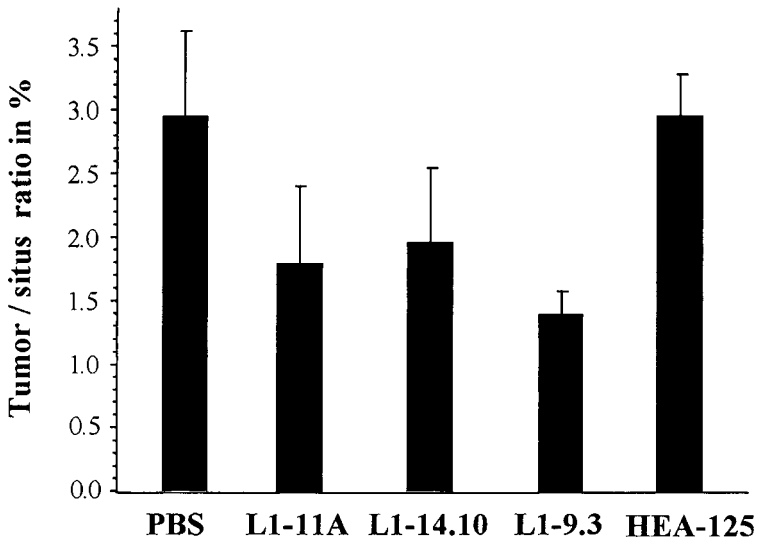
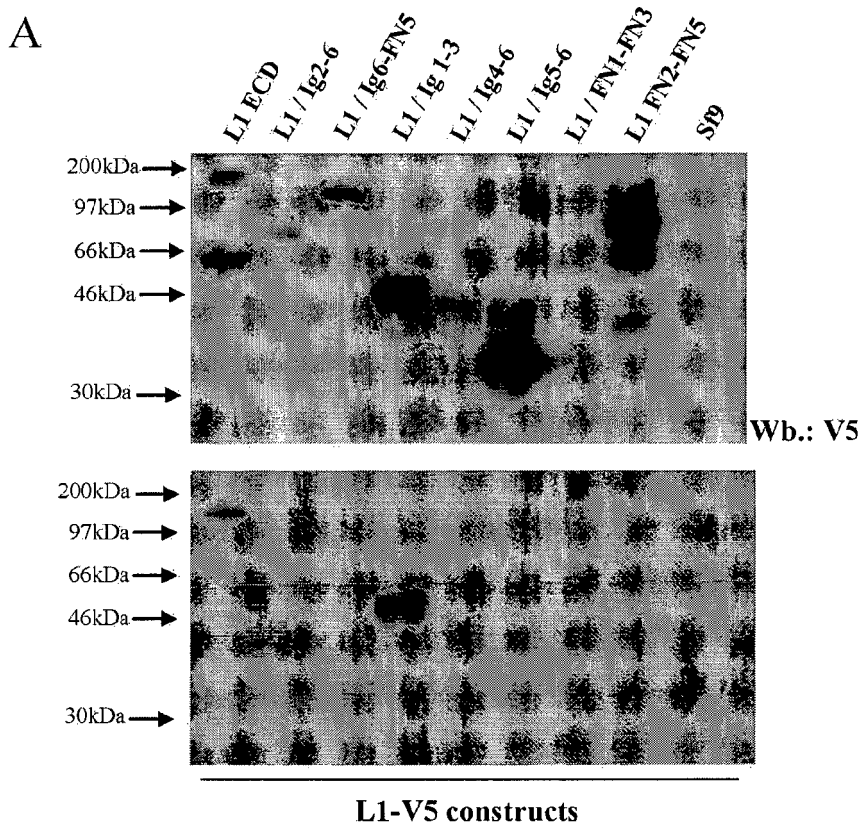

Tumor / situs ratio in %
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PBS
L1-11A
L1-14.10
L1-9.3
HEA-125

Figure 6


A
L1 ECD
L1 / Ig2-6
L1 / Ig6-FN5
L1 / Ig 1-3
L1 / Ig4-6
L1 / Ig5-6
L1 / FN1-FN3
L1 FN2-FN5
S9
200kDa
97kDa
66kDa
46kDa
30kDa
Wb.: V5
200kDa
97kDa
66kDa
46kDa
30kDa
L1-V5 constructs

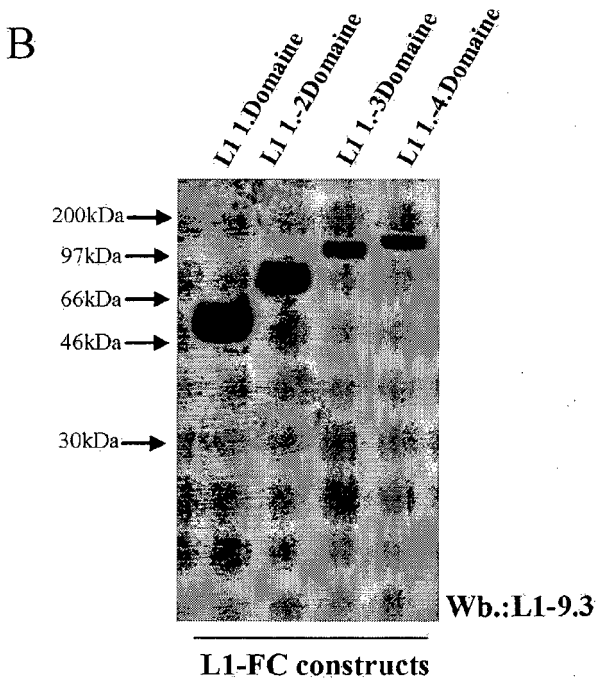
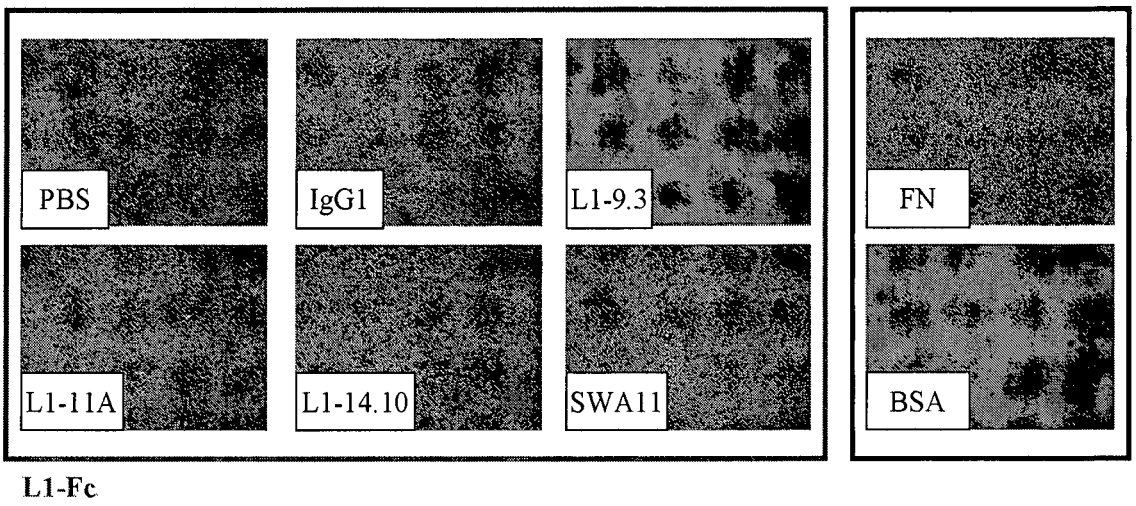

B
L1 1.Domaine
L1 1.-2Domaine
L1 1.-3Domaine
L1 1.-4.Domaine
200kDa
97kDa
66kDa
46kDa
30kDa
Wb.:L1-9.3
L1-FC constructs

Figure 7

A


PBS
IgG1
L1-9.3
FN
L1-11A
L1-14.10
SWA11
BSA
L1-Fc

B

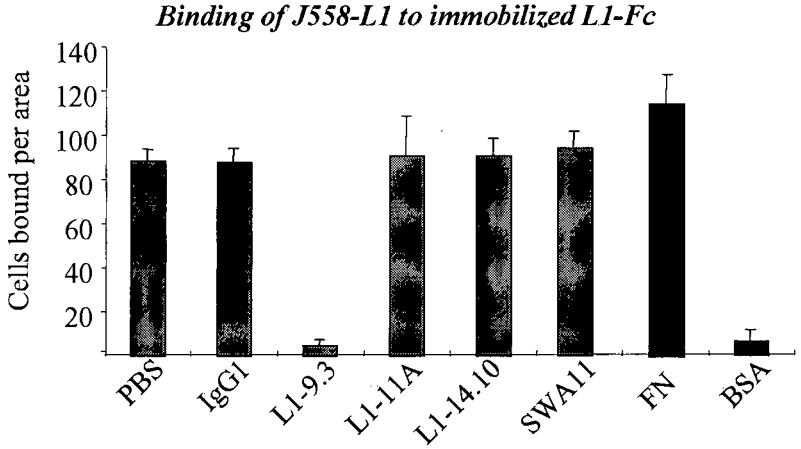

Binding of J558-L1 to immobilized L1-Fc
Cells bound per area
140
120
100
80
60
40
20
PBS
IgG1
L1-9.3
L1-11A
L1-14.10
SWA11
FN
BSA

Figure 8

a)

Antibody Light chain amino acid sequences

name		CDR1			CDR2		CDR3		
									1 1
	1 2		3	4	5	6 7 8	9		0 1
	12345678901234567890123	4567ABCDEF8901234		567890123456789	0123456	78901234567890123456789012345678	9012345ABCDEF67		8901234567890
L1_9.3	DIQMTQTTSSLSAFLGDRVTISC	RASQ	DISNYLN	WYQQKPDGTVKLLIY	YTSRLHS	GVPSRFSGSGSGTDYSLTISNLEQEDFATYFC	QQGNTLP	WT	FGGGTKLEIKR
humκ1	DIQMTQSPSSLSASVGDRVTITC	RASQSV	DISSYLN	WYQQKPGKAPKLLIY	AASSLES	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQYNSLP	YT	FGQGTKVEIK
REI	DIQMTQSPSSLSASVGDRVTITC	QASQ	DIIKYLN	WYQQTPGKAPKLLIY	EASNLQA	GVPSRFSGSGSGTDYTFTISSLQPEDIATYYC	QQYQSLP	YT	FGQGTKLQITR
L1_9.3Hu	DIQMTQSPSSLSASVGDRVTITC	RASQ	DISNYLN	WYQQKPGKAPKLLIY	YTSRLHS	GVPSRFSGSGSGTDYTFTISSLQPEDFATYFC	QQGNTLP	WT	FGGGTKLEIKR
L1_9.3Hu3	DIQMTQSPSSLSASVGDRVTITC	RASQ	DISNYLN	WYQQKPGKAPKLLIY	YTSRLHS	GVPSRFSGSGSGTDYTLTISSLQPEDFATYFC	QQGNTLP	WT	FGGGTKLEIKR

b)

Antibody Heavy chain amino acid sequences

name		CDR1		CDR2			CDR3		
							1		
	1 2 3		4	5	6	7 8 9	0		
	123456789012345678901234567890	12345AB	67890123456789	012ABC	3456789012345	678901234567890 12ABC345678901234	567890ABCDEFGHIJK12		34567890123
L1_9.3	QVQLQQPGAELVKSGASVNLSCRASGYTFT	RYWML	WVRQRPGHGLEWVG	EINP	RNDRTNYNEKFKT	KATLTVDRSSSTAYMQLTSLTSEDSAVYFCAL	GGGYAM	DY	WGQGTSVTVSS
HumIII	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	KDYAMSI	WVRQAPGKGLEWVA	VIS	NGSDTYYADSVKG	RFTISRDDSKNTLYLQMNSLRAEDTAVYYCAR	DSRFFD	VI	WGQGTLVTVSS
L1_9.3hu	EVQLVQSGGGLVQSGGSLRLSCRASGYTFT	RYWML	WVRQRPGHGLEWVG	EINP	RNDRTNYNEKFKT	RFTISVDRSKSTAYLQMDSLRAEDTAVYFCAL	GGGYAM	DY	WGQGTLVTVSS
L1_9.3hu3	EVQLVQSGGGLVQSGGSLRLSCRASGYTFT	RYWML	WVRQRPGKGLEWVA	EINP	RNDRTNYNEKFKT	RFTISVDRSKNTLYLQMDSLRAEDTAVYFCAL	GGGYAM	DY	WGQGTLVTVSS

Figure 9

a)

L1_9.3 scFv
DIQMTQTTSSLSAFLGDRVTISCRASQDISNYLNWYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTI
SNLEQEDFATYFCQQGNTLPWTFGGGTKLEIKR**TSGPGDGGKGGPGKGPGGEGTKGTGPGG**QVQLQQPGAELVKS
GASVNLSCRASGYTFTRYWMLWVRQRPGHGLEWVGEINPRNDRTNYNEKFKTKATLTVDRSSSTAYMQLTSLTSE
DSAVYFCALGGGYAMDYWGQGTSVTVSS

b)

L1_9.3Hu
DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDYT**L**TI
SSLQPEDFATYFCQQGNTLPWTFGGGTKLEIKR**TSGPGDGGKGGPGKGPGGEGTKGTGPGG**EVQLVQ**S**GGGLVQS
GGSLRLSCRASGYTFTRYWMLWVRQRPGHGLEWVGEINPRNDRTNYNEKFKTRFTISVDRSKSTAYLQMDSLRAE
DTAVYFCALGGGYAMDYWGQGTLVTVSS

c)

L1_9.3Hu3
DIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDYT**L**TI
SSLQPEDFATYFCQQGNTLPWTFGGGTKLEIKR**TSGPGDGGKGGPGKGPGGEGTKGTGPGG**EVQLVQ**S**GGGLVQS
GGSLRLSCRASGYTFTRYWMLWVRQRPG**K**GLEWV**A**EINPRNDRTNYNEKFKTRFTISVDRSK**N**T**L**YLQMDSLRAE
DTAVYFCALGGGYAMDYWGQGTLVTVSS

Figure 10

a)

L1-9.3 murine single chain antibody

```
          NdeI
             M  K   Y  L  L  P   T  A  A   A  G  L   L  L  L  A ·
     1  ACATATGAAA TACCTATTGC CTACGGCAGC CGCTGGATTG TTATTACTCG
        TGTATACTTT ATGGATAACG GATGCCGTCG GCGACCTAAC AATAATGAGC
             SfiI
                       NcoI
        · A  Q  P   A  M  A   D  I  Q  M   T  Q  T   T  S  S
    51  CGGCCCAGCC GGCCATGGCC GATATTCAGA TGACCCAGAC CACGAGCAGC
        GCCGGGTCGG CCGGTACCGG CTATAAGTCT ACTGGGTCTG GTGCTCGTCG
         L  S  A  F   L  G  D   R  V  T   I  S  C  R   A  S  Q ·
   101  CTGAGCGCGT TTCTGGGCGA TCGTGTGACC ATTAGCTGCC GTGCGAGCCA
        GACTCGCGCA AAGACCCGCT AGCACACTGG TAATCGACGG CACGCTCGGT
        · D  I  S   N  Y  L  N   W  Y  Q   Q  K  P   D  G  T  V ·
   151  GGATATTAGC AACTATCTGA ACTGGTATCA GCAGAAACCG GATGGCACCG
        CCTATAATCG TTGATAGACT TGACCATAGT CGTCTTTGGC CTACCGTGGC
        · K  L  L   I  Y  Y   T  S  R  L   H  S  G   V  P  S
   201  TGAAACTGCT GATTTATTAT ACCAGCCGTC TGCATAGCGG TGTGCCGAGC
        ACTTTGACGA CTAAATAATA TGGTCGGCAG ACGTATCGCC ACACGGCTCG
         R  F  S  G   S  G  S   G  T  D   Y  S  L  T   I  S  N ·
   251  CGTTTTAGCG GCAGCGGTAG CGGCACCGAT TATAGCCTGA CCATTTCTAA
        GCAAAATCGC CGTCGCCATC GCCGTGGCTA ATATCGGACT GGTAAAGATT
        · L  E  Q   E  D  F  A   T  Y  F   C  Q  Q   G  N  T  L ·
   301  CCTGGAACAG GAAGATTTTG CGACCTATTT TTGCCAGCAG GGCAACACGC
        GGACCTTGTC CTTCTAAAAC GCTGGATAAA AACGGTCGTC CCGTTGTGCG
        · P  W  T   F  G  G   G  T  K  L   E  I  K   R  T  S
   351  TGCCGTGGAC CTTTGGCGGT GGCACCAAAC TGGAAATTAA ACGTACTAGT
        ACGGCACCTG GAAACCGCCA CCGTGGTTTG ACCTTTAATT TGCATGATCA
         G  P  G  D   G  G  K   G  G  P   G  K  G  P   G  G  E ·
   401  GGTCCGGGCG ATGGCGGTAA AGGCGGTCCG GGCAAAGGTC CGGGTGGCGA
        CCAGGCCCGC TACCGCCATT TCCGCCAGGC CCGTTTCCAG GCCCACCGCT
                            SmaI
                            XmaI
                            AvaI                PstI
        · G  T  K   G  T  G  P   G  G  Q   V  Q  L   Q  Q  P  G ·
   451  AGGCACCAAA GGCACTGGGC CCGGGGGTCA GGTTCAGCTG CAGCAGCCGG
        TCCGTGGTTT CCGTGACCCG GGCCCCCAGT CCAAGTCGAC GTCGTCGGCC
        · A  E  L   V  K  S   G  A  S  V   N  L  S   C  R  A
   501  GTGCGGAACT GGTGAAAAGC GGCGCCAGCG TGAACCTGAG CTGTCGTGCG
        CACGCCTTGA CCACTTTTCG CCGCGCTCGC ACTTGGACTC GACAGCACGC
         S  G  Y  T   F  T  R   Y  W  M   L  W  V  R   Q  R  P ·
   551  AGCGGCTATA CCTTTACCCG TTATTGGATG CTGTGGGTGC GTCAGCGTCC
        TCGCCGATAT GGAAATGGGC AATAACCTAC GACACCCACG CAGTCGCAGG
        · G  H  G   L  E  W  V   G  E  I   N  P  R   N  D  R  T ·
   601  GGGCCACGGC CTGGAATGGG TGGGCGAAAT TAATCCGCGT AACGATCGTA
        CCCGGTGCCG GACCTTACCC ACCCGCTTTA ATTAGGCGCA TTGCTAGCAT
        · N  Y  N   E  K  F   K  T  K  A   T  L  T   V  D  R
   651  CCAACTATAA CGAAAAATTC AAAACCAAAG CGACCCTGAC CGTGGATCGT
        GGTTGATATT GCTTTTTAAG TTTTGGTTTC GCTGGGACTG GCACCTAGCA
         S  S  S  T   A  Y  M   Q  L  T   S  L  T  S   E  D  S ·
   701  AGCAGCAGCA CCGCGTATAT GCAGCTGACG AGCCTGACCT CTGAAGATAG
        TCGTCGTCGT GGCGCATATA CGTCGACTGC TCGGACTGGA GACTTCTATC
                       BssHII
        · A  V  Y   F  C  A  L   G  G  G   Y  A  M   D  Y  W  G ·
   751  CGCGGTGTAT TTCTGCGCGC TGGGCGGTGG CTATGCGATG GATTATTGGG
        GCGCCACATA AAGACGCGCG ACCCGCCACC GATACGCTAC CTAATAACCC
                                                 NotI
        · Q  G  T   S  V  T   V  S  S  G   G  A  A   A  A  P
   801  GCCAGGGCAC CAGCGTTACC GTGAGCAGCG GCGGTGCGGC CGCTGCACCA
        CGGTCCCGTG GTCGCAATGG CACTCGTCGC CGCCACGCCG GCGACGTGGT
         S  V  F  I   F  P  P   S  D  E   Q  L  K  S   G  T  A ·
   851  TCTGTCTTCA TCTTCCCGCC ATCTGATGAG CAGTTGAAAT CTGGAACTGC
        AGACAGAAGT AGAAGGGCGG TAGACTACTC GTCAACTTTA GACCTTGACG
        · S  V  V   C  L  L  N   N  F  Y   P  R  E   A  K  V  Q ·
   901  CTCTGTTGTG TGCCTGCTGA ATAACTTCTA TCCCAGAGAG GCCAAAGTAC
        GAGACAACAC ACGGACGACT TATTGAAGAT AGGGTCTCTC CGGTTTCATG
        · W  K  V   D  N  A   L  Q  S  G   N  S  Q   E  S  V
```

Figure 10 a) cont.

```
   951  AGTGGAAGGT GGATAACGCC CTCCAATCGG GTAACTCCCA GGAGAGTGTC
        TCACCTTCCA CCTATTGCGG GAGGTTAGCC CATTGAGGGT CCTCTCACAG
```

```
       T  E  Q  D   S  K  D   S  T  Y   S  L  S  S   T  L  T ·
1001   ACAGAGCAGG ACAGCAAGGA CAGCACCTAC AGCCTCAGCA GCACCCTGAC
       TGTCTCGTCC TGTCGTTCCT GTCGTGGATG TCGGAGTCGT CGTGGGACTG
       · L  S  K   A  D  Y  E   K  H  K   V  Y  A   C  E  V  T ·
1051   GCTGAGCAAA GCAGACTACG AGAAACACAA AGTCTACGCC TGCGAAGTCA
       CGACTCGTTT CGTCTGATGC TCTTTGTGTT TCAGATGCGG ACGCTTCAGT
       · H  Q  G   L  S  S   P  V  T  K   S  F  N   R  G  E
1101   CCCATCAGGG CCTGAGTTCG CCCGTCACAA AGAGCTTCAA CCGCGGAGAG
       GGGTAGTCCC GGACTCAAGC GGGCAGTGTT TCTCGAAGTT GGCGCCTCTC
        S  H  H  H   H  H  H   *  *
1151   TCACACCACC ACCACCACCA CTAGTAAT
       AGTGTGGTGG TGGTGGTGGT GATCATTA
```

Figure 10

b)

L1-9.3Hu humanized single chain antibody

```
          NdeI
            M  K   Y  L  L  P   T  A  A   A  G  L   L  L  L  A ·
      1   ACATATGAAA TACCTATTGC CTACGGCAGC CGCTGGATTG TTATTACTCG
          TGTATACTTT ATGGATAACG GATGCCGTCG GCGACCTAAC AATAATGAGC
              SfiI

                        NcoI                                AvaI
          ·  A  Q  P   A  M  A   D  I  Q  M   T  Q  S   P  S  S
     51   CGGCCCAGCC GGCCATGGCC GATATTCAGA TGACCCAGAG CCCGAGCAGC
          GCCGGGTCGG CCGGTACCGG CTATAAGTCT ACTGGGTCTC GGGCTCGTCG
           L  S  A  S   V  G  D   R  V  T   I  T  C  R   A  S  Q ·
    101   CTGAGCGCGA GCGTGGGTGA TCGTGTGACC ATTACCTGCC GTGCGAGCCA
          GACTCGCGCT CGCACCCACT AGCACACTGG TAATGGACGG CACGCTCGGT
          ·  D  I  S   N  Y  L  N   W  Y  Q   Q  K  P   G  K  A  P ·
    151   GGATATTAGC AACTATCTGA ACTGGTATCA GCAGAAACCG GGCAAAGCGC
          CCTATAATCG TTGATAGACT TGACCATAGT CGTCTTTGGC CCGTTTCGCG
          ·  K  L  L   I  Y  Y   T  S  R  L   H  S  G   V  P  S
    201   CGAAACTGCT GATTTATTAT ACCAGCCGTC TGCATAGCGG TGTGCCGAGC
          GCTTTGACGA CTAAATAATA TGGTCGGCAG ACGTATCGCC ACACGGCTCG
           R  F  S  G   S  G  S   G  T  D   Y  T  F  T   I  S  S ·
    251   CGTTTTAGCG GCAGCGGTAG CGGCACCGAT TATACCTTTA CCATTAGCAG
          GCAAAATCGC CGTCGCCATC GCCGTGGCTA ATATGGAAAT GGTAATCGTC
            PstI
          ·  L  Q  P   E  D  F  A   T  Y  F   C  Q  Q   G  N  T  L ·
    301   CCTGCAGCCG GAAGATTTTG CGACCTATTT TTGCCAGCAG GGCAACACGC
          GGACGTCGGC CTTCTAAAAC GCTGGATAAA AACGGTCGTC CCGTTGTGCG
          ·  P  W  T   F  G  G   G  T  K  L   E  I  K   R  T  S
    351   TGCCGTGGAC CTTTGGCGGT GGCACCAAAC TGGAAATTAA ACGTACTAGT
          ACGGCACCTG GAAACCGCCA CCGTGGTTTG ACCTTTAATT TGCATGATCA
           G  P  G  D   G  G  K   G  G  P   G  K  G  P   G  G  E ·
    401   GGTCCGGGCG ATGGCGGTAA AGGCGGTCCG GGCAAAGGTC CGGGTGGCGA
          CCAGGCCCGC TACCGCCATT TCCGCCAGGC CCGTTTCCAG GCCCACCGCT
                              SmaI
                              XmaI
                              AvaI
          ·  G  T  K   G  T  G  P   G  G  E   V  Q  L   V  Q  S  G ·
    451   AGGCACCAAA GGCACTGGGC CCGGGGGTGA AGTTCAGCTG GTGCAGAGCG
          TCCGTGGTTT CCGTGACCCG GGCCCCCACT TCAAGTCGAC CACGTCTCGC
           ·  G  G  L   V  Q  S   G  G  S  L   R  L  S   C  R  A
    501   GCGGTGGTCT GGTTCAGAGC GGTGGCAGCC TGCGTCTGAG CTGTCGTGCG
          CGCCACCAGA CCAAGTCTCG CCACCGTCGG ACGCAGACTC GACAGCACGC
           S  G  Y  T   F  T  R   Y  W  M   L  W  V  R   Q  R  P ·
    551   AGCGGCTATA CCTTCACCCG TTATTGGATG CTGTGGGTGC GTCAGCGTCC
          TCGCCGATAT GGAAGTGGGC AATAACCTAC GACACCCACG CAGTCGCAGG
          ·  G  H  G   L  E  W  V   G  E  I   N  P  R   N  D  R  T ·
    601   GGGCCACGGC CTGGAATGGG TGGGCGAAAT TAATCCGCGT AACGATCGTA
          CCCGGTGCCG GACCTTACCC ACCCGCTTTA ATTAGGCGCA TTGCTAGCAT
           ·  N  Y  N   E  K  F   K  T  R  F   T  I  S   V  D  R
    651   CCAACTATAA CGAAAAATTT AAAACCCGCT TCACCATTAG CGTGGATCGT
          GGTTGATATT GCTTTTTAAA TTTTGGGCGA AGTGGTAATC GCACCTAGCA
                             PstI
           S  K  S  T   A  Y  L   Q  M  D   S  L  R  A   E  D  T ·
    701   AGCAAAAGCA CCGCGTATCT GCAGATGGAT AGCCTGCGTG CGGAAGATAC
          TCGTTTTCGT GGCGCATAGA CGTCTACCTA TCGGACGCAC GCCTTCTATG
                       BssHII
          ·  A  V  Y   F  C  A  L   G  G  G   Y  A  M   D  Y  W  G ·
    751   CGCGGTGTAT TTTTGCGCGC TGGGCGGTGG CTATGCGATG GATTATTGGG
          GCGCCACATA AAAACGCGCG ACCCGCCACC GATACGCTAC CTAATAACCC
                                                 NotI
          ·  Q  G  T   L  V  T   V  S  S  G   G  A  A   A  A  P
    801   GCCAGGGCAC CCTGGTTACC GTGAGCAGCG GCGGTGCGGC CGCTGCACCA
          CGGTCCCGTG GGACCAATGG CACTCGTCGC CGCCACGCCG GCGACGTGGT
           S  V  F  I   F  P  P   S  D  E   Q  L  K  S   G  T  A ·
    851   TCTGTCTTCA TCTTCCCGCC ATCTGATGAG CAGTTGAAAT CTGGAACTGC
```

Figure 10 b) cont.

```
          AGACAGAAGT AGAAGGGCGG TAGACTACTC GTCAACTTTA GACCTTGACG
          ·  S  V  V   C  L  L  N   N  F  Y   P  R  E   A  K  V  Q ·
```

```
   901  CTCTGTTGTG TGCCTGCTGA ATAACTTCTA TCCCAGAGAG GCCAAAGTAC
        GAGACAACAC ACGGACGACT TATTGAAGAT AGGGTCTCTC CGGTTTCATG
        · W  K  V    D  N  A    L  Q  S  G    N  S  Q    E  S  V
 951    AGTGGAAGGT GGATAACGCC CTCCAATCGG GTAACTCCCA GGAGAGTGTC
        TCACCTTCCA CCTATTGCGG GAGGTTAGCC CATTGAGGGT CCTCTCACAG
         T  E  Q  D    S  K  D    S  T  Y    S  L  S  S    T  L  T  ·
  1001  ACAGAGCAGG ACAGCAAGGA CAGCACCTAC AGCCTCAGCA GCACCCTGAC
        TGTCTCGTCC TGTCGTTCCT GTCGTGGATG TCGGAGTCGT CGTGGGACTG
        · L  S  K    A  D  Y  E    K  H  K    V  Y  A    C  E  V  T  ·
  1051  GCTGAGCAAA GCAGACTACG AGAAACACAA AGTCTACGCC TGCGAAGTCA
        CGACTCGTTT CGTCTGATGC TCTTTGTGTT TCAGATGCGG ACGCTTCAGT
        · H  Q  G    L  S  S    P  V  T  K    S  F  N    R  G  E
  1101  CCCATCAGGG CCTGAGTTCG CCCGTCACAA AGAGCTTCAA CCGCGGAGAG
        GGGTAGTCCC GGACTCAAGC GGGCAGTGTT TCTCGAAGTT GGCGCCTCTC
         S  H  H  H    H  H  H    *  *
  1151  TCACACCACC ACCACCACCA CTAGTAATT
        AGTGTGGTGG TGGTGGTGGT GATCATTAA
```

Figure 10

c)

L1-9.3Hu3 humanized single chain antibody

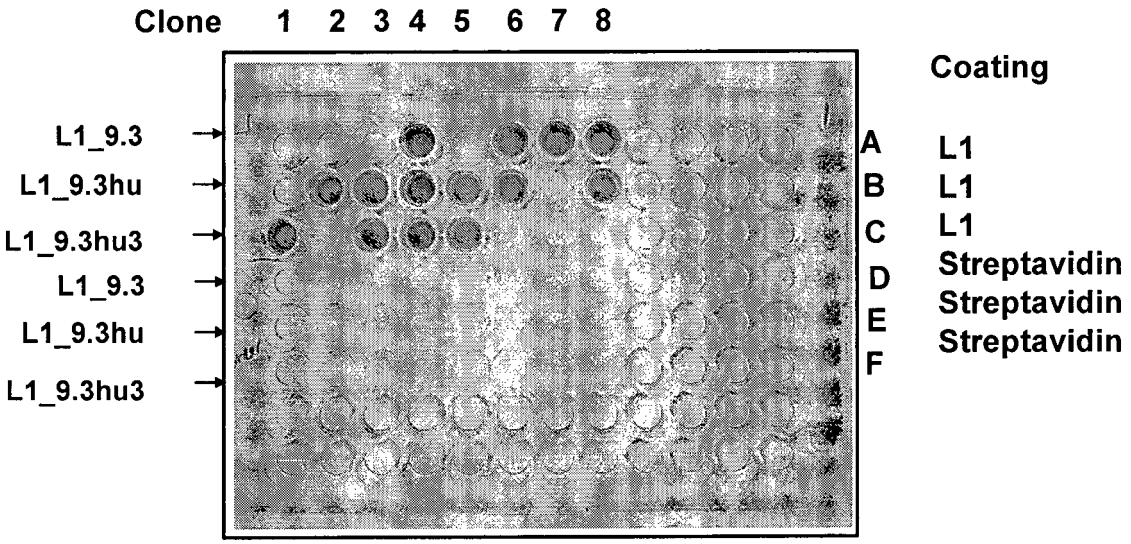
```
        NdeI
          M  K   Y  L  L  P   T  A  A   A  G  L   L  L  L  A ·
    1 ACATATGAAA TACCTATTGC CTACGGCAGC CGCTGGATTG TTATTACTCG
      TGTATACTTT ATGGATAACG GATGCCGTCG GCGACCTAAC AATAATGAGC
          SfiI
                     NcoI                              AvaI
      · A  Q  P   A  M  A   D  I  Q  M   T  Q  S   P  S  S
   51 CGGCCCAGCC GGCCATGGCC GATATTCAGA TGACCCAGAG CCCGAGCAGC
      GCCGGGTCGG CCGGTACCGG CTATAAGTCT ACTGGGTCTC GGGCTCGTCG
       L  S  A  S   V  G  D   R  V  T   I  T  C  R   A  S  Q ·
  101 CTGAGCGCGA GCGTGGGTGA TCGTGTGACC ATTACCTGCC GTGCGAGCCA
      GACTCGCGCT CGCACCCACT AGCACACTGG TAATGGACGG CACGCTCGGT
      · D  I  S   N  Y  L  N   W  Y  Q   Q  K  P   G  K  A  P ·
  151 GGATATTAGC AACTATCTGA ACTGGTATCA GCAGAAACCG GGCAAAGCGC
      CCTATAATCG TTGATAGACT TGACCATAGT CGTCTTTGGC CCGTTTCGCG
      · K  L  L   I  Y  Y   T  S  R  L   H  S  G   V  P  S
  201 CGAAACTGCT GATTTATTAT ACCAGCCGTC TGCATAGCGG TGTGCCGAGC
      GCTTTGACGA CTAAATAATA TGGTCGGCAG ACGTATCGCC ACACGGCTCG
       R  F  S  G   S  G  S   G  T  D   Y  T  L  T   I  S  S ·
  251 CGTTTTAGCG GCAGCGGTAG CGGCACCGAT TATACCCTGA CCATTAGCAG
      GCAAAATCGC CGTCGCCATC GCCGTGGCTA ATATGGGACT GGTAATCGTC
        PstI
        ~~~~~~
      · L  Q  P   E  D  F  A   T  Y  F   C  Q  Q   G  N  T  L ·
  301 CCTGCAGCCG GAAGATTTTG CGACCTATTT TTGCCAGCAG GGCAACACGC
      GGACGTCGGC CTTCTAAAAC GCTGGATAAA AACGGTCGTC CCGTTGTGCG
      · P  W  T   F  G  G   G  T  K  L   E  I  K   R  T  S
  351 TGCCGTGGAC CTTTGGCGGT GGCACCAAAC TGGAAATTAA ACGTACTAGT
      ACGGCACCTG GAAACCGCCA CCGTGGTTTG ACCTTTAATT TGCATGATCA
       G  P  G  D   G  G  K   G  G  P   G  K  G  P   G  G  E ·
  401 GGTCCGGGCG ATGGCGGTAA AGGCGGTCCG GGCAAAGGTC CGGGTGGCGA
      CCAGGCCCGC TACCGCCATT TCCGCCAGGC CCGTTTCCAG GCCCACCGCT
                         SmaI
                         XmaI
                         AvaI
      · G  T  K   G  T  G  P   G  G  E   V  Q  L   V  Q  S  G ·
  451 AGGCACCAAA GGCACTGGGC CCGGGGGTGA AGTTCAGCTG GTGCAGAGCG
      TCCGTGGTTT CCGTGACCCG GGCCCCCACT TCAAGTCGAC CACGTCTCGC
      · G  G  L   V  Q  S   G  G  S  L   R  L  S   C  R  A
  501 GCGGTGGTCT GGTTCAGAGC GGTGGCAGCC TGCGTCTGAG CTGTCGTGCG
      CGCCACCAGA CCAAGTCTCG CCACCGTCGG ACGCAGACTC GACAGCACGC
       S  G  Y  T   F  T  R   Y  W  M   L  W  V  R   Q  R  P ·
  551 AGCGGCTATA CCTTTACCCG TTATTGGATG CTGTGGGTGC GTCAGCGTCC
      TCGCCGATAT GGAAATGGGC AATAACCTAC GACACCCACG CAGTCGCAGG
      · G  K  G   L  E  W  V   A  E  I   N  P  R   N  D  R  T ·
  601 GGGTAAAGGC CTGGAATGGG TGGCGGAAAT TAATCCGCGT AACGATCGTA
      CCCATTTCCG GACCTTACCC ACCGCCTTTA ATTAGGCGCA TTGCTAGCAT
      · N  Y  N   E  K  F   K  T  R  F   T  I  S   V  D  R
  651 CCAACTATAA CGAAAAATTT AAAACCCGCT TCACCATTAG CGTGGATCGT
      GGTTGATATT GCTTTTTAAA TTTTGGGCGA AGTGGTAATC GCACCTAGCA
                        PstI
       S  K  N  T   L  Y  L   Q  M  D   S  L  R  A   E  D  T ·
  701 AGCAAAAACA CCCTGTATCT GCAGATGGAT AGCCTGCGTG CGGAAGATAC
      TCGTTTTTGT GGGACATAGA CGTCTACCTA TCGGACGCAC GCCTTCTATG
                   BssHII
      · A  V  Y   F  C  A  L   G  G  G   Y  A  M   D  Y  W  G ·
  751 CGCGGTGTAT TTTTGCGCGC TGGGCGGTGG CTATGCGATG GATTATTGGG
      GCGCCACATA AAAACGCGCG ACCCGCCACC GATACGCTAC CTAATAACCC
                                                 NotI
      · Q  G  T   L  V  T   V  S  S  G   G  A  A   A  A  P
  801 GCCAGGGCAC CCTGGTTACC GTGAGCAGCG GCGGTGCGGC CGCTGCACCA
```

Figure 10 c) cont.

```
      CGGTCCCGTG GGACCAATGG CACTCGTCGC CGCCACGCCG GCGACGTGGT
       S  V  F  I   F  P  P   S  D  E   Q  L  K  S   G  T  A ·
```

```
 851  TCTGTCTTCA TCTTCCCGCC ATCTGATGAG CAGTTGAAAT CTGGAACTGC
      AGACAGAAGT AGAAGGGCGG TAGACTACTC GTCAACTTTA GACCTTGACG
      · S  V  V   C  L  L  N   N  F  Y   P  R  E   A  K  V  Q ·
 901  CTCTGTTGTG TGCCTGCTGA ATAACTTCTA TCCCAGAGAG GCCAAAGTAC
      GAGACAACAC ACGGACGACT TATTGAAGAT AGGGTCTCTC CGGTTTCATG
      · W  K  V   D  N  A   L  Q  S  G   N  S  Q   E  S  V
 951  AGTGGAAGGT GGATAACGCC CTCCAATCGG GTAACTCCCA GGAGAGTGTC
      TCACCTTCCA CCTATTGCGG GAGGTTAGCC CATTGAGGGT CCTCTCACAG
       T  E  Q  D   S  K  D   S  T  Y   S  L  S  S   T  L  T ·
1001  ACAGAGCAGG ACAGCAAGGA CAGCACCTAC AGCCTCAGCA GCACCCTGAC
      TGTCTCGTCC TGTCGTTCCT GTCGTGGATG TCGGAGTCGT CGTGGGACTG
      · L  S  K   A  D  Y  E   K  H  K   V  Y  A   C  E  V  T ·
1051  GCTGAGCAAA GCAGACTACG AGAAACACAA AGTCTACGCC TGCGAAGTCA
      CGACTCGTTT CGTCTGATGC TCTTTGTGTT TCAGATGCGG ACGCTTCAGT
      · H  Q  G   L  S  S   P  V  T  K   S  F  N   R  G  E
1101  CCCATCAGGG CCTGAGTTCG CCCGTCACAA AGAGCTTCAA CCGCGGAGAG
      GGGTAGTCCC GGACTCAAGC GGGCAGTGTT TCTCGAAGTT GGCGCCTCTC
       S  H  H  H   H  H  H   *  *
1151  TCACACCACC ACCACCACCA CTAGTAATT
      AGTGTGGTGG TGGTGGTGGT GATCATTAA
```

Figure 11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L1_9.3hu
L1_9.3hu3
L1_9.3
L1_9.3hu
L1_9.3hu3
Coating
A L1
B L1
C L1
D Streptavidin
E Streptavidin
F Streptavidin

Figure 12

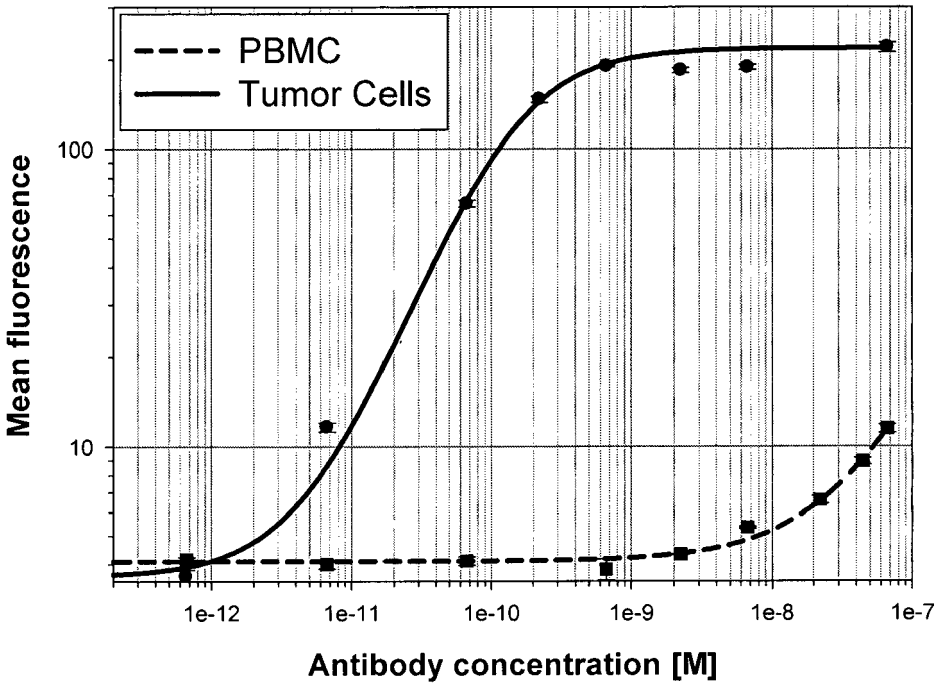
a)

GAAGAGTTAGCCTTGCAGCTGTGCTCAGCCCTAAATAGTTCCCAAAAATTTGCATGCTCTCACTTC
CTATCTTTGGGTACTTTTTCATATACCAGTCAGATTGTGAGCCATTGTAATTGAAGTCAAGACTCA
GCCTGGAC***ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTCTTCAA***GGTAAAAGT
TACTACAATGGGAATTTTGCTGTTGCACAGTGATTCTTGTTGACTGGAATTTTGGAGGGGTCCTTT
CTTTTCCTGCTTAACTCTGTGGGTATTTATTGTGTCTCCACTCCTA**GGTACCAGATGTGATATCCA**
GATGACACAGACTACATCCTCCCTGTCTGCCTTTCTGGGAGACAGAGTCACCATCAGTTGCAG
GGCAAGTCAGGACATTAGCAATTATTTAAACTGGTATCAGCAGAAACCAGATGGAACTGTTAAA
CTCCTTATCTATTACACATCAAGATTACACTCAGGAGTCCCCTCAAGGTTCAGTGGCAGTGGGT
CTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATTTTGCCACTTACTTTTGC
CAACAGGGTAATACGCTTCCGTGGACATTCGGTGGAGGCACCAAGCTGGAAATCAAACGTAAA
TAGAATCCAAAGTCTCTTTCTTCCGTTGTCTATGTCTGTGGCTTCTATGTCTACAAATGATGTAT

b)

TTCAGCATCCTGATTCCTGACCCAGGTGTCCCTTCTTCTCCAGCAGGAGTAGGTGCTCATCTAAT
ATGTATCCTGCTCATGAATATGCAAATCCTCTGAATCTACATGGTAAATGTAGGTTTGTCTATATCA
CACACAGAAAAACATGAGATCACAGTTCTCTCTACAGTTACTGAACACACAGGACCTCACC***ATGG***
GATGGAGCTATATCATCCTCTTTTTGGTAGCAACAGCTACAGGTAAGGGGCTCACAGTAGAAGG
CTTGAGGTCTGGCCATATACATGGGTGACAGTGACATCCACTTTGCCTTTCTTTCCACA**GATGTC**
CACTCCCAGGTCCAACTGCAGCAGCCTGGGGCTGAACTGGTGAAGTCTGGGGCTTCAGTGAAC
CTGTCCTGCAGGGCTTCTGGCTACACCTTCACCAGATACTGGATGCTCTGGGTGAGGCAGAGG
CCTGGACATGGCCTTGAGTGGGTTGGAGAGAATTAATCCTCGCAACGATCGTACTAATTACAATG
AGAAATTCAAGACCAAGGCCACACTGACTGTAGACCGATCCTCCAGCACAGCCTACATGCAAC
TCACCAGCCTGACATCTGAGGACTCTGCGGTCTATTTCTGTGCCCTGGGGGGGGGCTATGCTAT
GGACTATTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGGTAAGAATGGCCTCTCCAGGTCT
TAATTTTTAACCTTTGTTATGGAGTTTTCTGAGCATTGCAGACTAATCTTGGATATTTGTCCCTGAG
GGAGCCGGCTGAGAGAAGTTGGGAAATAAACTGTCTAGGGATCTCAGAGCCTTTAGGACAGATT
A

Figure 13



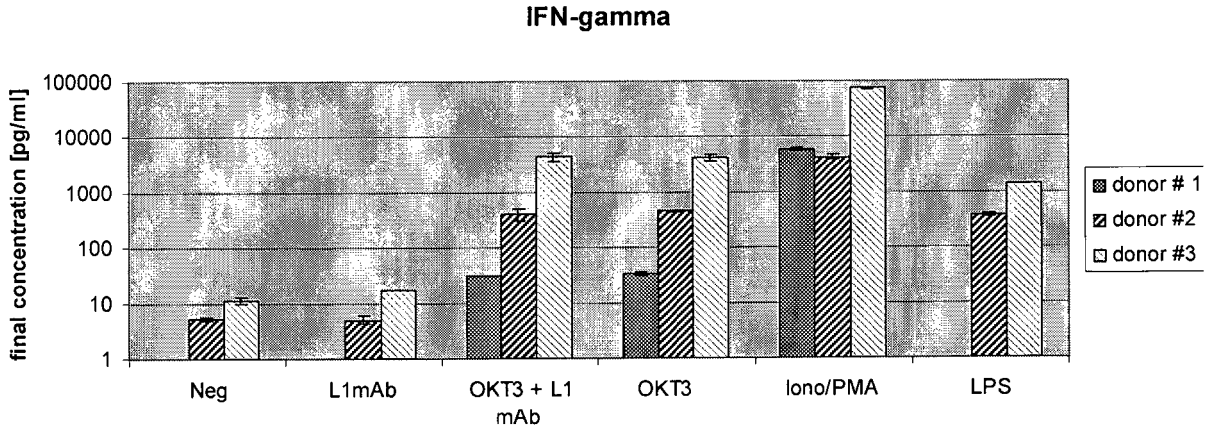
A)

B)

	K_D [M]
Tumor Cells	1×10^{-10}
PBMC (estimated)	$>4 \times 10^{-8}$

Figure 14

A)


IFN-gamma
final concentration [pg/ml]
100000
10000
1000
100
10
1
Neg
L1mAb
OKT3 + L1 mAb
OKT3
Iono/PMA
LPS
donor # 1
donor #2
donor #3

B)

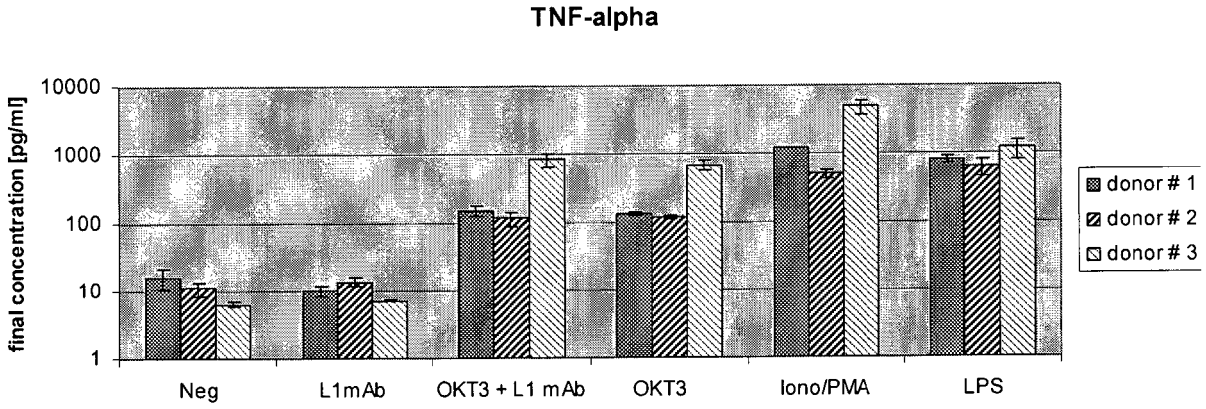

TNF-alpha
final concentration [pg/ml]
10000
1000
100
10
1
Neg
L1mAb
OKT3 + L1 mAb
OKT3
Iono/PMA
LPS
donor # 1
donor # 2
donor # 3

Figure 15

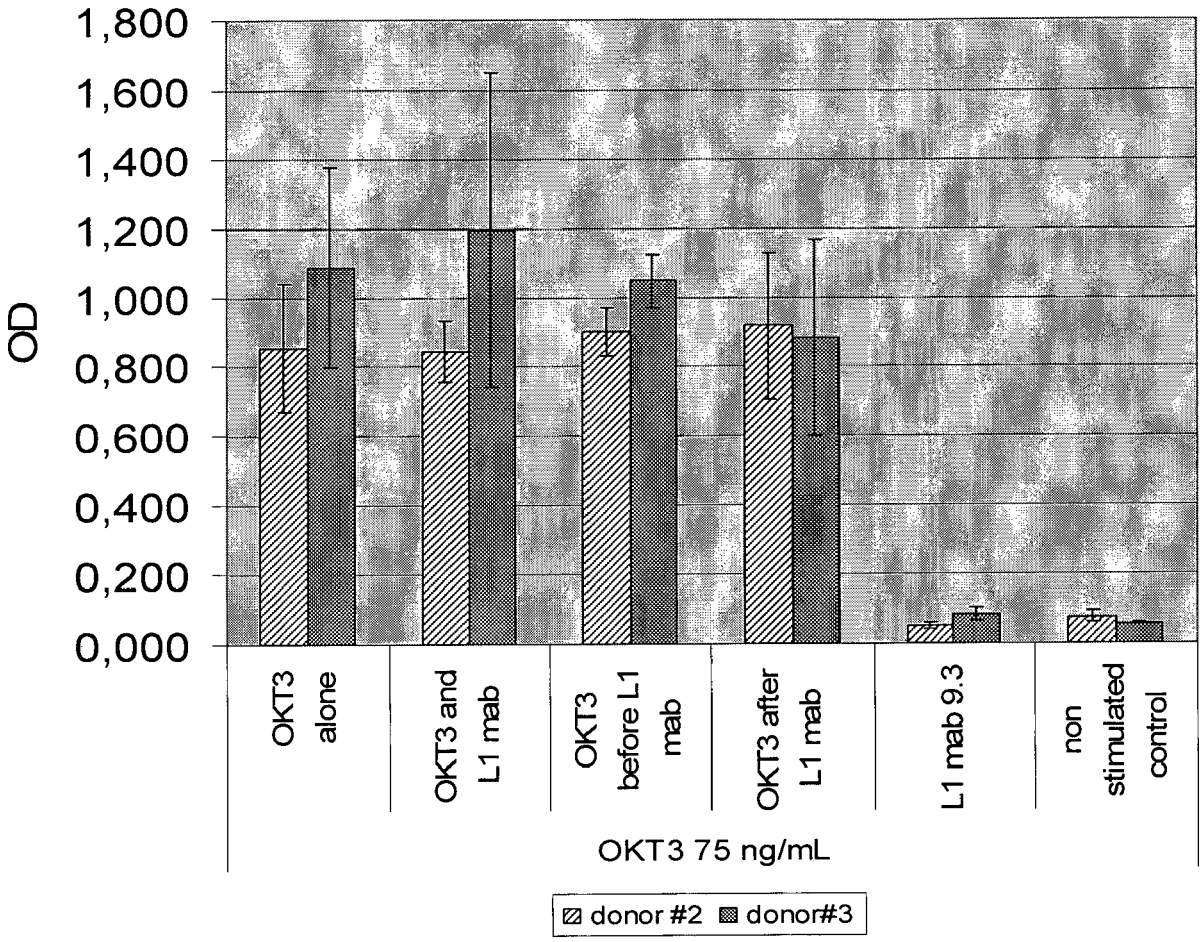

1,800
1,600
1,400
1,200
1,000
0,800
0,600
0,400
0,200
0,000
OD
OKT3 alone
OKT3 and L1 mab
OKT3 before L1 mab
OKT3 after L1 mab
L1 mab 9.3
non stimulated control
OKT3 75 ng/mL
donor #2
donor#3

Figure 16

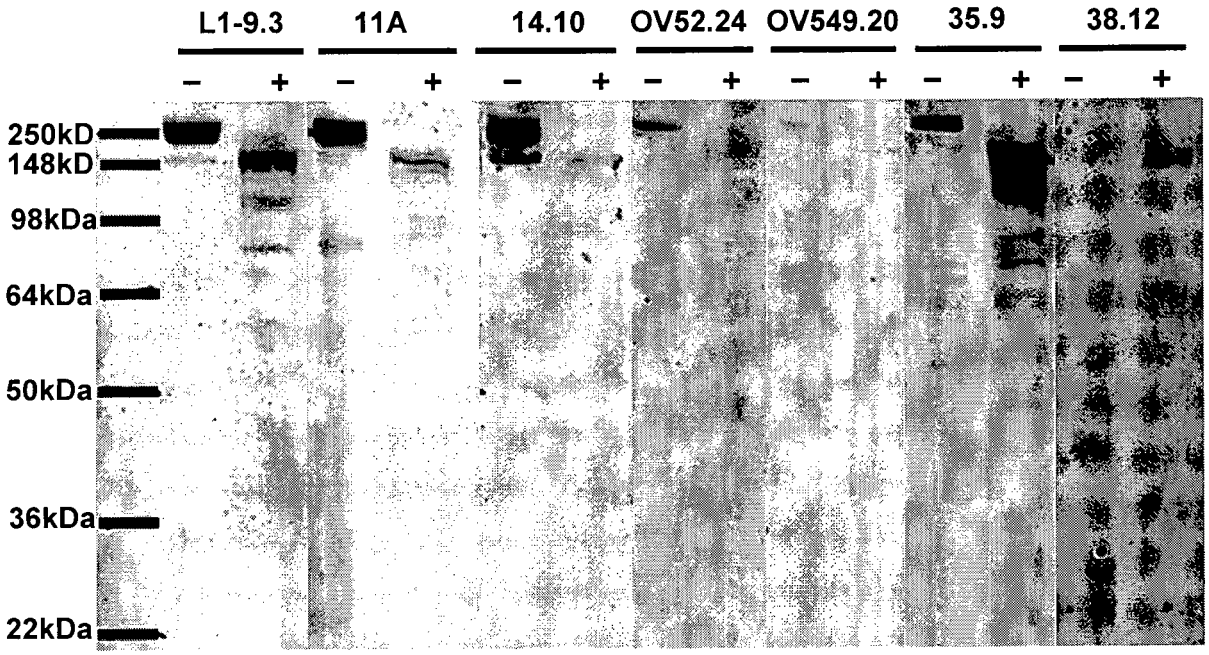
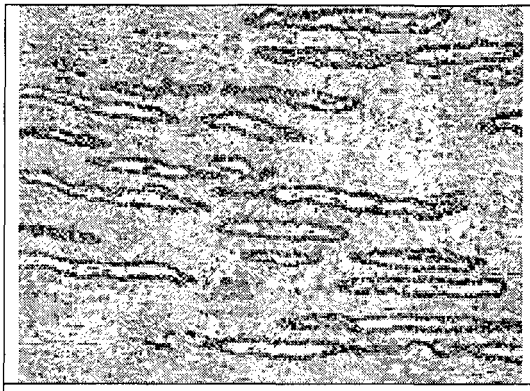
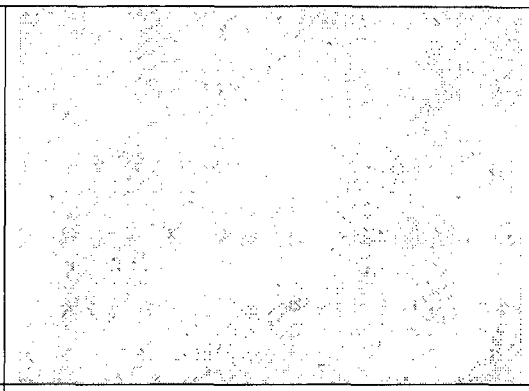

L1-9.3
11A
14.10
OV52.24
OV549.20
35.9
38.12
– + – + – + – + – + – + – +
250kD
148kD
98kDa
64kDa
50kDa
36kDa
22kDa

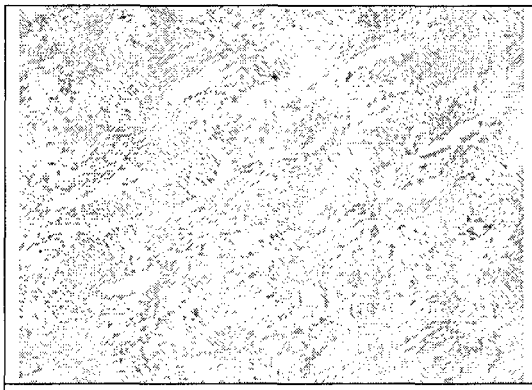
Figure 17



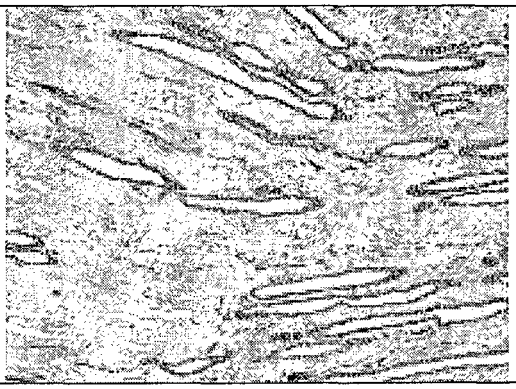
A) Collecting ducts of a L1-9.3-treated rabbit, stained with anti-mouse antibody
amplification system CSA



B) Collecting ducts of a L1-9.3-treated rabbit, stained with anti-mouse antibody
conventional ABC method

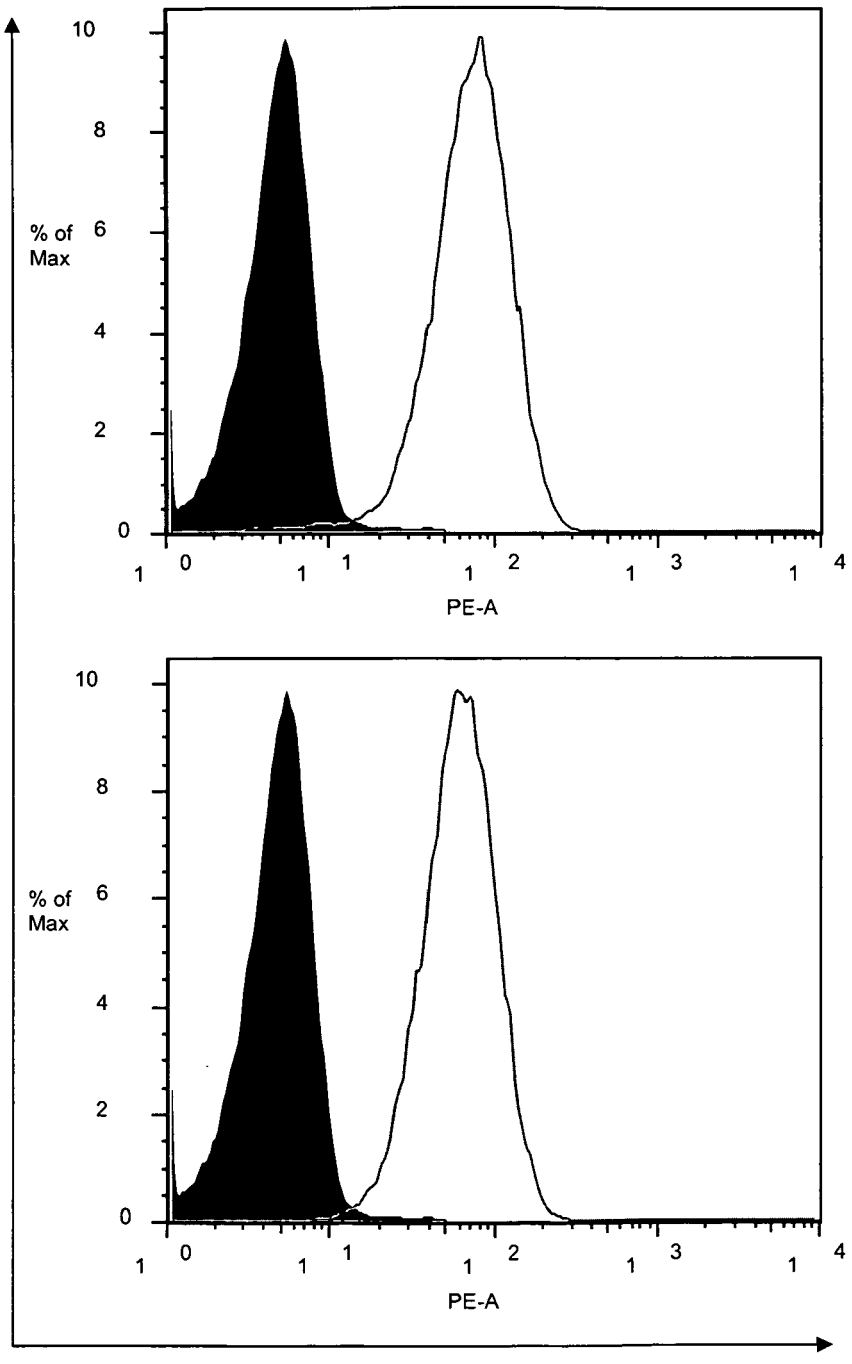


C) Collecting ducts of a PBS-treated control rabbit, stained with anti-mouse antibody
Negative control (amplification system CSA)



D) Collecting ducts of a PBS-treated control rabbit, stained with L1-9.3 and anti-mouse antibody
amplification system CSA

Figure 18



SKOV3ip pcDNA 3.1 Luciferase

Figure 19

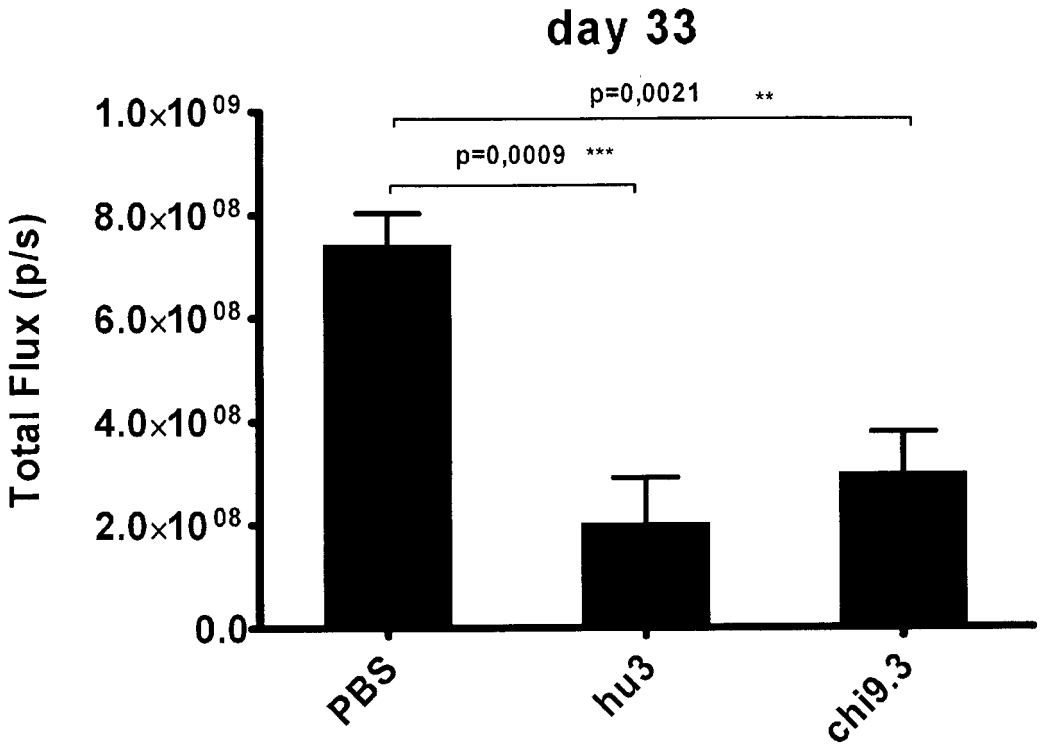

day 33
p=0,0021 **
p=0,0009 ***
Total Flux (p/s)
1.0×10 09
8.0×10 08
6.0×10 08
4.0×10 08
2.0×10 08
0.0
PBS
hu3
chi9.3

Figure 20

A

B

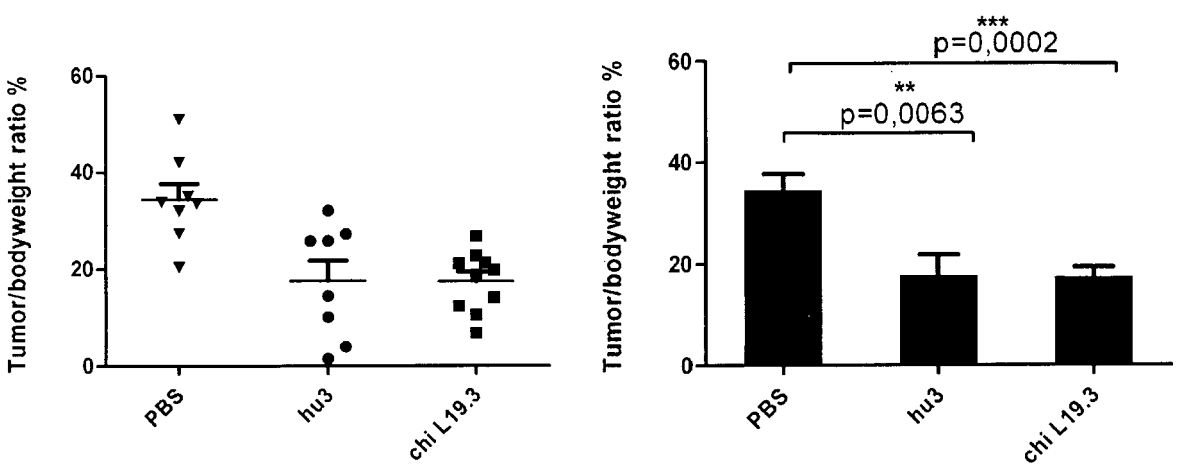

day 36
Tumor/bodyweight ratio %
60
40
20
0
PBS
hu3
chi L19.3
***
p=0,0002
**
p=0,0063

Figure 21 A

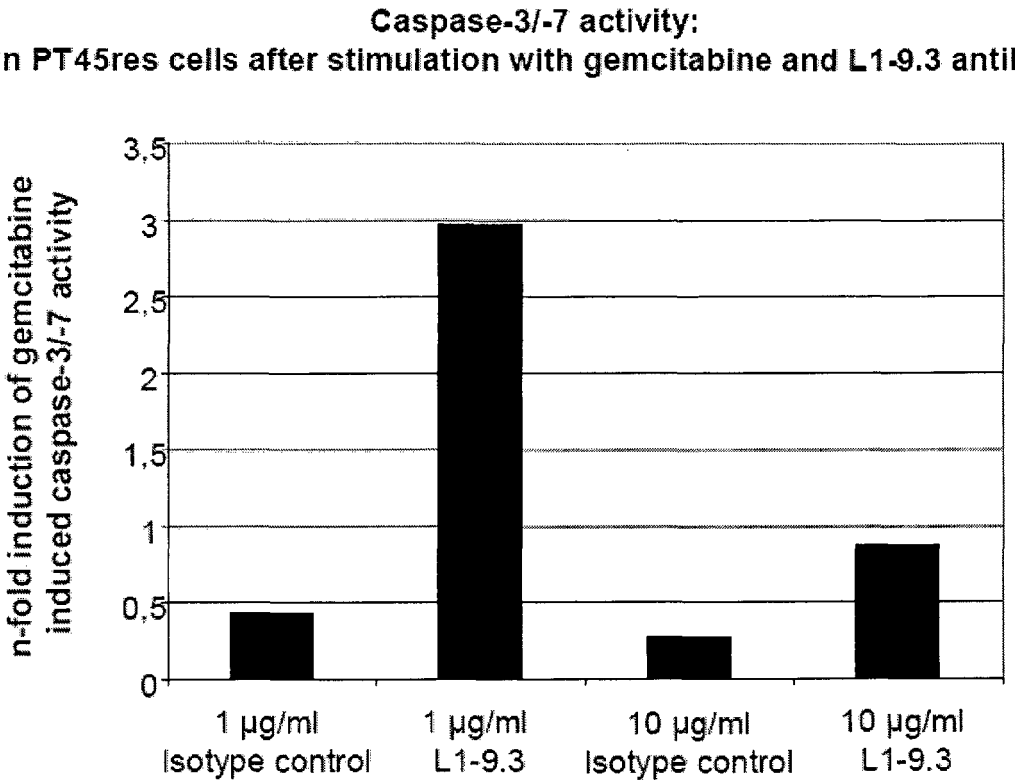


Figure 21 B

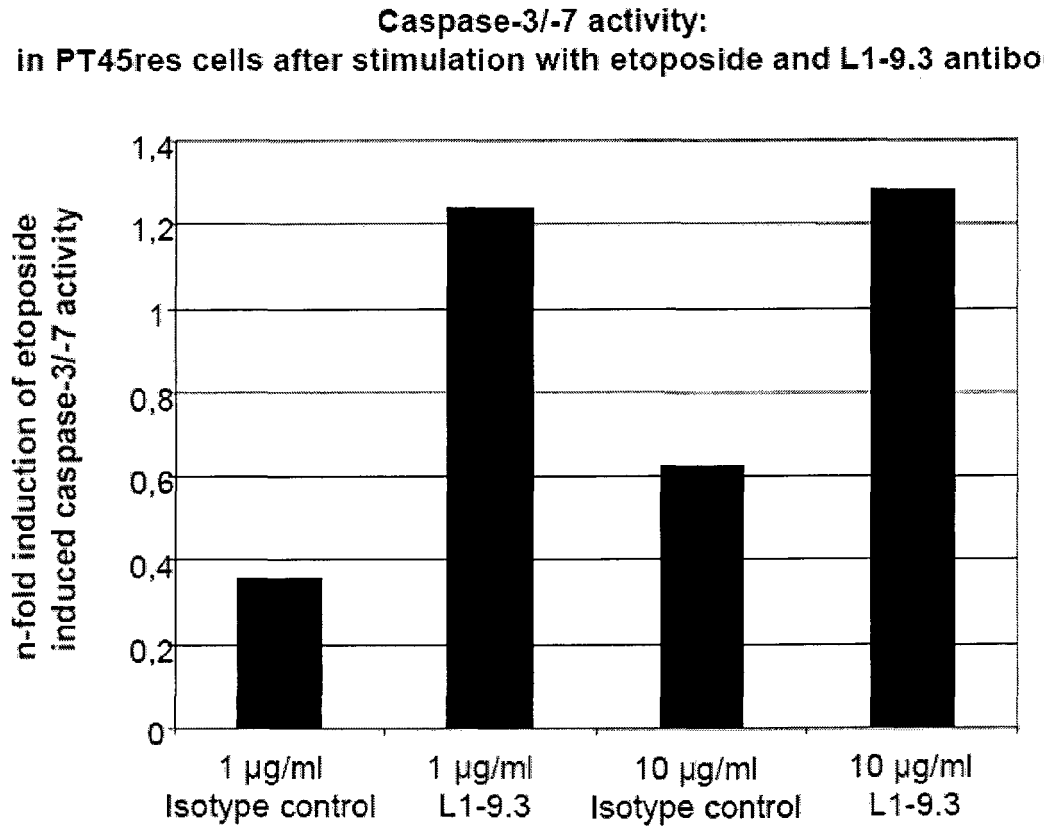


Figure 22 A

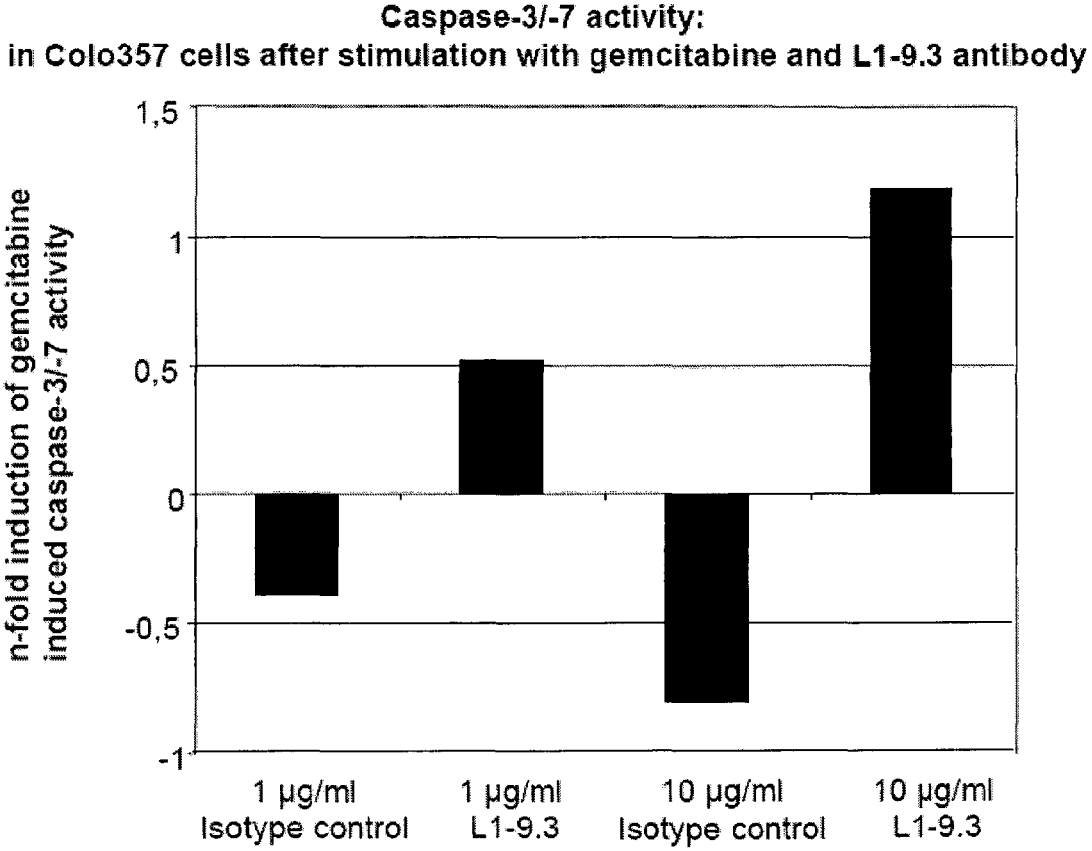
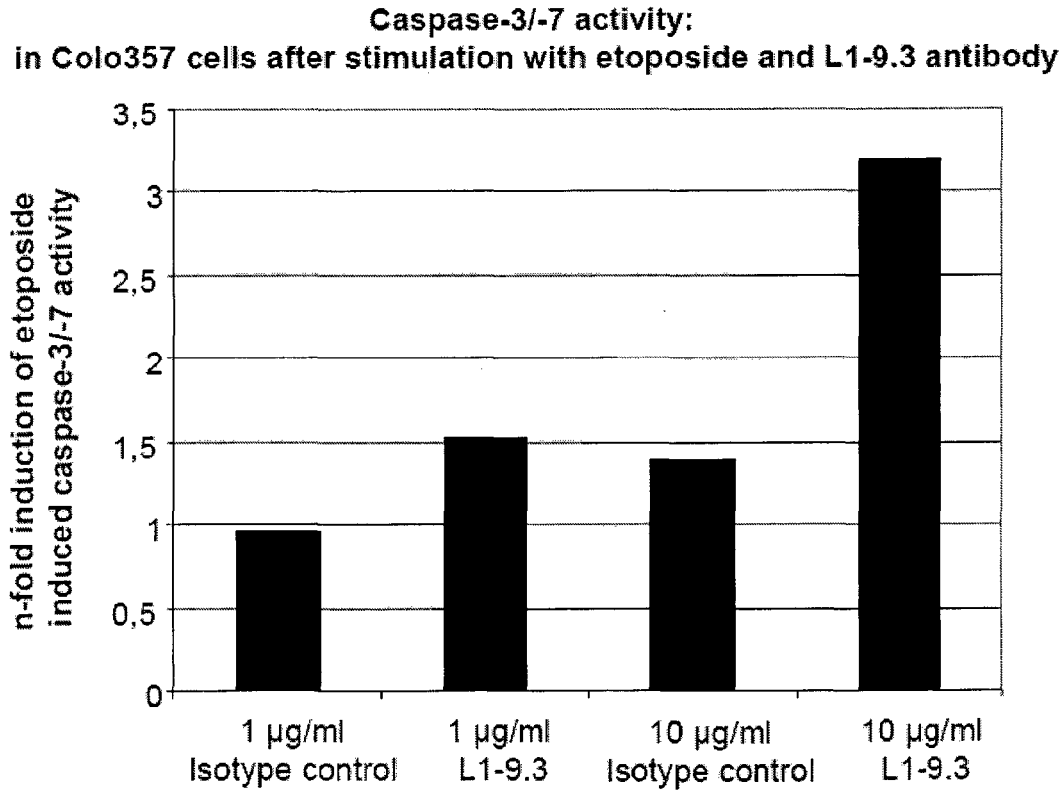


Figure 22 B



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