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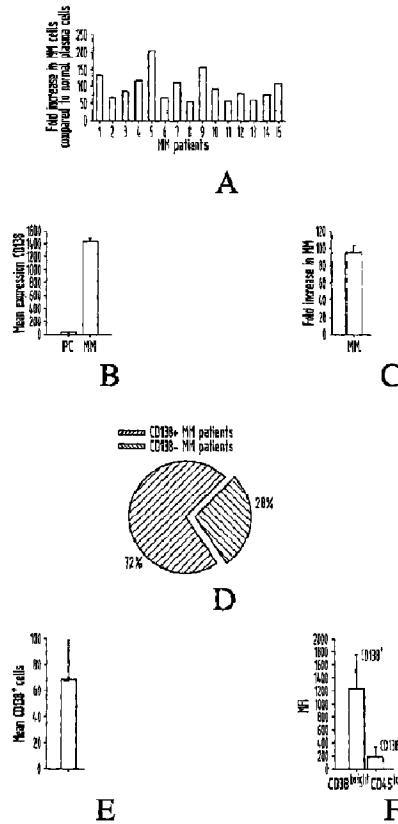
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(54) **Titre : IMMUNOCONJUGUES CIBLANT DES CELLULES A EXPRESSION DU SYNDECAN-1 ET UTILISATION DE CEUX-CI**
(54) **Title: IMMUNOCONJUGATES TARGETING SYNDECAN-1 EXPRESSING CELLS AND USE THEREOF**



(57) **Abrégé/Abstract:**

Immunoconjugates comprising a targeting agent selectively targeting cell- surface expressed syndecan-1 and at least one effector molecule as well as in vitro and in vivo methods of using those immunocomjugates are disclosed. The effector molecule may have, in its native form, high non-selective cytotoxicity, but substantially no non-selective cytotoxicity when part of said immunoconjugate. Targeting agents include the antibody B-B4 as well as other agents that bind cell- surface expressed syndecan-1.



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Certificate of Correction

**Canadian Patent No. 2,486,285
Granted: 7 March 2017 (07-03-2017)**

Les corrections suivantes
sont faites en raison de
l'article 8 de la *Loi sur les
brevets* et le document
doit être lu tel que corrigé.

The following corrections
are made pursuant to
section 8 of the *Patent Act*
and the document should
read as corrected.

In the Patent Grant:

Pages 37 to 40 of the claims should be read as attached pages 37 to 39.

CANADA 150

16 August 2017 (16-08-2017)

Date



What is claimed is:

1. An immunoconjugate comprising:

at least one targeting antibody selectively targeting cell-surface expressed syndecan-1, wherein the targeting antibody binds to a linear epitope between residues 90-95 of the core protein of human syndecan-1 (CD138), and

at least one effector molecule, wherein said effector molecule is a maytansinoid and has a molecular weight of less than 2kD, wherein said targeting antibody is functionally and releasably attached to said effector molecule to form said immunoconjugate, and wherein the immunoconjugate is configured so that the effector molecule is released from the immunoconjugate by cleavage or dissociation in cells targeted by said targeting antibody.

2. The immunoconjugate of claim 1, wherein said effector molecule has, a potency of about 10^{-11} - 10^{-8} M before being attached to the targeting antibody to form the immunoconjugate.
3. The immunoconjugate of claim 1, wherein said maytansinoid is DM1.
4. The immunoconjugate of claim 1, wherein said maytansinoid is DM3.
5. The immunoconjugate of claim 1, wherein said maytansinoid is DM4.
6. The immunoconjugate of claim 1, wherein said effector molecule has a molecular weight of between 600 and 800 Da.
7. The immunoconjugate of claim 1, wherein said targeting antibody selectively targeting cell-surface expressed syndecan-1 is an antibody fragment comprising an antigen binding region of the antibody.



8. The immunoconjugate of claim 1, wherein said targeting antibody selectively targeting cell-surface expressed syndecan-1 is derived from the antibody B-B4.
9. A pharmaceutical composition comprising the immunoconjugate according to claim 1 and one or more pharmaceutically acceptable excipients.
10. A kit comprising, in separate containers, pharmaceutical compositions, wherein one container comprises the pharmaceutical composition of claim 9, and wherein, a separate container comprises a second pharmaceutical composition comprising a chemotherapeutic agent.
11. The use of the immunoconjugate of claim 1 to inhibit, delay or prevent the growth of tumor cells in a cell culture containing both syndecan-1 expressing tumor cells and non-tumor cells in an amount effective to inhibit, delay or prevent the growth of syndecan-1 expressing tumor cells.
12. The use of claim 11, wherein said effective amount induces cell death or continuous cell cycle arrest in said syndecan-1 expressing tumor cells.
13. The use of claim 12, wherein the cells in said cell culture are from a patient suffering from an hematologic malignancy or a solid tumor comprising syndecan-1 expressing cells.
14. The use of claim 13, wherein said patient is suffering from one of the following: multiple myeloma, ovarian carcinoma, kidney carcinoma, gall bladder carcinoma, breast carcinoma, prostate cancer, lung cancer, colon carcinoma, Hodgkin's and non-Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML), a solid tissue sarcoma or a colon carcinoma.
15. The use of claim 14, wherein said patient is a multiple myeloma patient.



16. The use of at least one immunoconjugate as in claim 1 to inhibit, delay, or prevent the growth of a tumor comprising syndecan-1 expressing tumor cells or the spread of syndecan-1 expressing tumor cells in a patient.

17. The use of claim 16, wherein said patient suffers from an hematologic malignancy or a solid tumor comprising syndecan-1 expressing cells.

18. The use of claim 17, wherein said patient suffers from one of the following: multiple myeloma, ovarian carcinoma, kidney carcinoma, gall bladder carcinoma, breast carcinoma, prostate cancer, lung cancer, colon carcinoma, Hodgkin's and non-Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML), solid tissue sarcoma or colon carcinoma.

19. The use of claim 18, wherein the disease is multiple myeloma.

20. The use of claim 18, wherein said effector molecule exhibits systemic cytotoxicity before being attached to the targeting antibody to form the immunoconjugate.

21. The use of the immunoconjugate as defined in claim 1 to inhibit, delay or prevent the growth of a tumor or the spread of malignant tumor cells in a patient treated with one or more cancer drugs or radiation.

22. The use of claim 21, wherein the immunoconjugate comprises a B-B4 targeting antibody.

Abstract

Immunoconjugates comprising a targeting agent selectively targeting cell-surface expressed syndecan-1 and at least one effector molecule as well as *in vitro* and *in vivo* methods of using those immunocomjugates are disclosed. The effector molecule may have, in its native form, high non-selective cytotoxicity, but substantially no non-selective cytotoxicity when part of said immunoconjugate. Targeting agents include the antibody B-B4 as well as other agents that bind cell-surface expressed syndecan-1.

initial development period of immunoconjugates, the efficiencies of conjugates having a drug as an effector molecule often were disappointing compared to the free drug.

In response, immunoconjugates with highly cytotoxic effector toxin molecules were constructed. However, while the efficiencies of this new generation of immunoconjugates were much improved, they were often immunogenic in humans, inducing neutralizing antibodies both to the toxin protein and to the mouse monoclonal antibody. In response, "humanized" antibodies conjugated to nonimmunogenic effector molecules were developed (Payne, 2003).

In the context of both highly cytotoxic drugs and toxins conjugated to a targeting agent, systemic toxicity has to be considered. If the cytotoxic drug or the toxin is highly cytotoxic, the immunoconjugate has to reach its target site without adversely affecting the host on its way. Accordingly, if the immunoconjugate circulates, for example, in the bloodstream to reach its target site, then this should occur without a substantial release of active drug. Thus, ideally, a highly cytotoxic drug or toxin of an immunoconjugate is only activated upon reaching its target.

Specificity is another factor critical for the usability of an immunoconjugate. The immunoconjugate has to be able to selectively interact with the target cells. Particularly for *in vivo* applications, it is critical that the immunoconjugate does not have substantial adverse effects on essential non-target cells. Thus, both the cellular target of the immunoconjugate and the targeting agent of the immunoconjugate have to be carefully selected to ensure specificity (Blättler and Chari, 2001).

It has also been considered important that immunoconjugates comprising targeting antibodies demonstrate pharmacokinetic and tissue distribution characteristics similar to those of corresponding antibodies (Xie, 2003).

First successes have been achieved with immunoconjugates. For example, MYLOTARG®, a conjugate of an anti-CD33 humanized monoclonal antibody and the highly cytotoxic DNA-damaging agent calicheamicin, has been recently approved by the FDA as the first drug immunoconjugate for clinical treatment of certain indications of myeloid leukemia (Bross, 2001; Hamann, 2002; Dowell, 2001).

However, there remains a need to develop effective immunoconjugates for a wide array of indications.

Summary of the Invention

The present invention pertains in one embodiment to an immunoconjugate comprising

5 at least one targeting agent selectively targeting cell-surface expressed syndecan-1,

at least one effector molecule,

wherein the effector molecule has, in its native form, high non-selective cytotoxicity,

10 wherein the targeting agent is functionally attached to said effector molecule to form the immunoconjugate, and

wherein the effector molecule has substantially no non-selective cytotoxicity when part of said immunoconjugate.

15 The cytotoxicity of the effector molecule, in its native form, on cells targeted by said targeting antibody may be higher or about the same as the cytotoxicity of the immunoconjugate on said targeted cells. The effector molecule may have, in its native form, a potency of about 10^{-14} - 10^{-7} , preferably a potency of about 10^{-13} - 10^{-7} M, of about 10^{-12} - 10^{-7} M, of about 10^{-12} - 10^{-8} M, most preferably of about 10^{-11} - 10^{-8} M, 20 which includes any narrower potency ranges encompassed by the ranges specified above, such as, but not limited to, a potency of about 10^{-11} - 10^{-10} M. The effector molecule may be a maytansinoid, in particular DM1, DM3 or DM4, a CC1065 analogue, a calicheamicin or a taxane. In certain embodiments, the effector molecule may have a molecular weight of less than 5 kDa, in particular less than 25 2kDa, more in particular less than 1kDa and in between about 600 and about 800 Da.

The targeting agent may be a targeting antibody, which includes fragments of antibodies, or non-immunoglobulin targeting molecule.

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The targeting antibody may be derived from an antibody that internalizes poorly. In certain embodiments, the targeting antibody may be derived from the antibody B-B4.

35 The present invention is also directed to a pharmaceutical composition comprising an effective amount of the immunoconjugate described above and one or more pharmaceutically acceptable excipients.

The present invention is also directed to a kit comprising, in separate containers, pharmaceutical compositions for use in combination to inhibit, delay and/or prevent the growth of tumors and/or spread of tumor cells, wherein one
5 container comprises an effective amount of above described pharmaceutical composition, and wherein, a separate container comprises a second pharmaceutical composition comprising an effective amount of an agent for the inhibition, delay and/or prevention of the growth of tumors and/or spread of tumor cells, and one or more pharmaceutically acceptable excipients. The agent in said second
10 pharmaceutical composition may be a chemotherapeutic agent or another immunoconjugate.

In one embodiment, the invention is directed to a method for treating, inhibiting, delaying and/or preventing the growth of tumor cells in a cell culture containing syndecan-1 expressing tumor cells and non-tumor cells, comprising
15 administering an effective amount of the above described immunoconjugate. The effective amount induces, in certain embodiments, cell death or continuous cell cycle arrest of said syndecan-1 expressing tumor cells. The cells in said cell culture may be obtained from a cancer patient and may, after administration of said effective amount of said immunoconjugate, be reimplanted into said cancer patient. The cells
20 in said cell culture may be isolated from a patient suffering from an hematologic malignancy and/or a solid tumor comprising syndecan-1 expressing cells, in particular from a patient suffering from one of the following: multiple myeloma, ovarian carcinoma, kidney carcinoma, gall bladder carcinoma, breast carcinoma, prostate cancer, lung cancer, colon carcinoma, Hodgkin's and non-Hodgkin's
25 lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML), a solid tissue sarcoma or a colon carcinoma.

The present invention is also directed to a method of inhibiting, delaying and/or preventing the growth of a tumor comprising syndecan-1 expressing tumor cells and/or spread of syndecan-1 expressing tumor cells in a patient in need thereof,
30 comprising administering to the patient at least one immunoconjugate in a growth of the tumor and/or spreading of the tumor cells inhibiting or reducing amount, wherein the immunoconjugate selectively inhibits, delays or prevents the growth and/or spread of syndecan-1 expressing cells. The patient may, in this embodiment
35 of the invention, suffer from an hematologic malignancy and/or a solid tumor

comprising syndecan-1 expressing cells, in particular from one of the following:
 multiple myeloma, ovarian carcinoma, kidney carcinoma, gall bladder carcinoma,
 breast carcinoma, prostate cancer, lung cancer, colon carcinoma, Hodgkin's and
 non-Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic
 5 leukemia (ALL), acute myeloblastic leukemia (AML), a solid tissue sarcoma or a
 colon carcinoma. An effector molecule of the immunoconjugate may, in this
 embodiment, exhibit, in its native form, high non-selective cytotoxicity.

The invention is also directed to a method for inhibiting, delaying and/or
 10 preventing the growth of a tumor and/or spread of malignant tumor cells in a patient
 in need thereof, comprising

- (a) administering to the patient one or more cancer drugs and/or radiation in an
 amount effective to reduce tumor load; and
- (b) administering to the patient at least one immunoconjugate in a growth of a tumor
 15 and/or spreading of tumor cells inhibiting, delaying or preventing amount,
 wherein the immunoconjugate selectively inhibits, delays or prevents the growth
 and/or spread of syndecan-1 expressing cells; (a) and (b) may hereby be performed
 consecutively in two consecutive treatment regimes. The drug of (a) and the
 immunoconjugate of (b) may also be administered in a single administration step.

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The present invention is also directed to a method for treating a subject
 having a condition that would benefit from the selective suppression of myeloma cell
 survival, the method comprising:

- (a) providing at least one immunoconjugate that selectively binds to syndecan-1
 25 expressed on myeloma cells; and
- (b) administering the immunoconjugate to the subject to selectively decrease survival
 or growth of said myeloma cells of the subject. The immunoconjugate may comprise
 a B-B4 targeting antibody. The immunoconjugate may, in this embodiment, comprise
 a maytansinoid effector molecule. The selective suppression of myeloma cell survival
 30 may also induce growth arrest or apoptosis in myeloma cells.

Brief Description of the Drawings

FIGS. 1A-1F show the expression of CD138 in multiple myeloma (MM) cells.

FIGS. 2A-2C show the effect of B-B4-DM1 in comparison with that produced by the
 35 naked antibody or by non-conjugated drug on survival of CD138⁺ and CD138⁻
 MM cells.

FIGS. 3A to 3C show the inhibitory effect of B-B4-DM1 on proliferation of CD138⁺ and CD138⁻ cells adherent to bone marrow stromal cells (BMSCs).

FIGS. 4A and 4B show the survival and cell cycle effects of B-B4-DM1 on CD138⁺ MM cells.

5 **FIGS. 5A to 5D** show the activity of B-B4-DM1 in a tumor xenograft model of human CD138⁺ multiple myeloma.

FIGS. 6A to F show the activity of B-B4-DM1 on large tumor xenografts of human CD138⁺ OPM multiple myeloma.

10 **FIG. 7A** shows the expression of GFP in the cells (GFP stands for Green Fluorescent Protein). **FIGS. 7B to 7F** show the activity of B-B4-DM1 on GFP⁺ human MM xenografts.

FIGS. 8A to 8C show that B-B4-DM1 reduces MM tumor burden in SCID-hu hosts implanted with patient MM cells.

15 **FIGS. 9A to 9C** show that B-B4-DM1 increases survival in SCID-hu hosts implanted with the Ocy-My5 MM cell line.

Detailed Description of Various and Preferred Embodiments

The present invention relates to immunoconjugates and the delivery of their effector molecule(s) to target sites and the site specific release of effector(s) molecule in, at or near target cells, tissues and organs. More particularly, the present invention relates to immunoconjugates comprising syndecan-1 targeting agents and potent effector molecules. The effector molecules are covalently linked, chelated or otherwise associated with the targeting agent. The effector molecules may be activated by cleavage/dissociation from the targeting agent portion of the immunoconjugate at the target site.

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The immunoconjugates according to the present invention are administered to a subject in need of therapeutic treatment or to cells isolated from such a subject in need of therapeutic treatment. The effector molecule or molecules may be released from the immunoconjugate by cleavage/dissociation in, at or close to the target cell, tissue or organ.

30

As one example, the immunoconjugate comprises the antibody B-B4 and at least one highly cytotoxic drug or toxin as an effector molecule and is administered to a patient with cancer. In this example, a therapeutically effective amount of the immunoconjugate is administered intravenously to a patient so that it concentrates in

the cancer cells. The effector molecule or molecules are released from the antibody target by natural means.

5 As a second example, the immunoconjugate comprises the antibody B-B4 and at least one highly cytotoxic drug and is administered to a cell population isolated from a patient with cancer. In this example, a cell death or continuous cell cycle arrest inducing amount of the immunoconjugate is administered to the cell population so that it concentrates in the cancerous cells. The effector molecule or molecules are released from the targeting antibody by natural means or external means to induce cell death or continuous cell cycle arrest in the cancer cells.

10 As a third example, the immunoconjugate comprises the antibody B-B4 and at least one highly cytotoxic drug or an immunotoxin as an effector molecule and is administered to a patient with cancer. In this example, a therapeutically effective amount of the immunoconjugate is administered intravenously to a patient so that it concentrates in the cancerous cells. The effector molecule or molecules are released from the antibody target by an external means to induce cell death or
15 continuous cell cycle arrest in the cancer cells.

Targeting agent: A targeting agent according to the present invention is able to associate with a molecule expressed by a target cell and includes peptides and non-peptides. In particular, targeting agents according to the present invention
20 include targeting antibodies and non-immunoglobulin targeting molecules, which may be based on non-immunoglobulin proteins, including, but not limited to, AFFILIN® molecules, ANTICALINS® and AFFIBODIES®. Non-immunoglobulin targeting molecules also include non-peptidic targeting molecules including targeting DNA and RNA oligonucleotides (aptamers).

25 **Targeting antibody:** A targeting antibody according to the present invention is or is based on a natural antibody or is produced synthetically or by genetic engineering and binds to an antigen on a cell or cells (target cell(s)) of interest. A targeting antibody according to the present invention includes a monoclonal antibody, a polyclonal antibody, a multispecific antibody (for example, a bispecific antibody), or
30 an antibody fragment. The targeting antibody may be engineered to, for example, improve its affinity to the target cells (Ross, 2003) or diminish its immunogenicity. The targeting antibody may be attached to a liposomal formulation including effector molecules (Carter, 2003). An antibody fragment comprises a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody.

Examples of antibody fragments according to the present invention include Fab, Fab', F(ab')₂, and Fv fragments, but also diabodies; domain antibodies (dAb) (Ward, 1989; United States Patent 6,005,079); linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. In a single chain
5 variable fragment antibody (scFv) the heavy and light chains (VH and VL) can be linked by a short amino acid linker having, for example, the sequence (glycine₄serine)_n, which has sufficient flexibility to allow the two domains to assemble a functional antigen binding pocket. Addition of various signal sequences may allow for more precise targeting of the targeting antibody. Addition of the light chain
10 constant region (CL) may allow dimerization via disulphide bonds, giving increased stability and avidity. Variable regions for constructing the scFv can, if a mAb against a target of interest is available, be obtained by RT-PCR which clones out the variable regions from mRNA extracted from the parent hybridoma. Alternatively, the scFv can be generated *de novo* by phage display technology (Smith, 2001). A bispecific
15 antibody according to the present invention may, for example, have at least one arm that is reactive against a target tissue and one arm that is reactive against a linker moiety (United States Patent Publication 20020006379). A bispecific antibody according to the present invention may also bind to more than one antigen on a target cell (Carter, 2003). An antibody according to the present invention may be
20 modified by, for example, introducing cystein residues to introduce thiol groups (Olafsen, 2004).

In accordance with the present invention, the targeting antibody may be derived from any source and may be, but is not limited to, a camel antibody, a murine antibody, a chimeric human/mouse antibody or a chimeric human/monkey antibody,
25 in particular, a chimeric human/monkey antibody with the monkey portion stemming from a cynomolgus monkey. Humanized antibodies are antibodies that contain sequences derived from a human-antibody and from a non-human antibody and are also within the scope of the present invention. Suitable methods for humanizing antibodies include CDR-grafting (complementarity determining region grafting) (EP 0
30 239 400; WO 91/09967; United States Patents 5,530,101; and 5,585,089), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan, 199; Studnicka et al., 1994; Roguska et al., 1994), chain shuffling (United States Patent 5,565,332) and Delimmunisation™ (Biovation, LTD). In CDR-grafting, the mouse complementarity-determining regions (CDRs) from, for example, mAb B-B4 are grafted into human
35 variable frameworks, which are then joined to human constant regions, to create a human B-B4 antibody. Several antibodies humanized by CDR-grafting are now in

clinical use, including MYLOTARG (Sievers et al., 2001) and HECEPTIN (Pegram et al, 1998).

The resurfacing technology uses a combination of molecular modeling, statistical analysis and mutagenesis to alter the non-CDR surfaces of antibody variable regions to resemble the surfaces of known antibodies of the target host. Strategies and methods for the resurfacing of antibodies, and other methods for reducing immunogenicity of antibodies within a different host, are disclosed, for example, in United States Patent 5,639,641. Human antibodies can be made by a variety of methods known in the art including phage display methods. See also
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10 United States Patents 4,444,887, 4,716,111, 5,545,806, and 5,814,318; and international patent application publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741.

Fully human antibodies may also been used. Those antibodies can be selected by the phage display approach, where CD138 or an antigenic determinant thereof is used to selectively bind phage expressing, for example, B-B4 variable regions (see, Krebs, 2001). This approach is advantageously coupled with an affinity maturation technique to improve the affinity of the antibody.
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In one embodiment, the targeting antibody is, in its unconjugated form, moderately or poorly internalizable. Moderate internalization constitutes about 30% to about 75% internalization of antibody, poor internalization constitutes about 0.01% to up to about 30% internalization after 3 hours incubation at 37°C. In another preferred embodiment the targeting antibody binds to CD138, for example, antibodies B-B4, BC/B-B4, B-B2, DL-101, 1 D4, MI15, 1.BB.210, 2Q1484, 5F7, 104-9, 281-2 in particular B-B4. Preferably the targeting antibody binds primarily to cell-surface expressed CD138. In another embodiment, the targeting antibody does not substantially bind non-cell-surface expressed CD138. When, in the context of the present invention, the name of a specific antibody is combined with the term "targeting antibody" such as "B-B4 targeting antibody," this means that this targeting antibody has the binding specificity of the antibody B-B4. If a targeting antibody is said to be "derived from" a specified antibody, this means that this targeting antibody has the binding specificity of this antibody, but might take any form consistent with the above description of a targeting antibody. If, in the context of the present invention, for example, a targeting antibody is said to do something "selectively" such as "selectively targeting cell-surface expressed syndcan-1" or, to be "selective" for something, this means that there is a significant selectivity (i.e. a higher affinity towards CD138-positive cells compared with CD138-negative cells) for, in case of the example provided, cell-surface expressed syndecan-1, compared to any other
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antigens and adverse side effects in a given environment are substantially avoided due to this selectivity.

Non-immunoglobulin targeting molecules: Non-immunoglobulin targeting molecules according to the present invention include targeting molecules derived from non-immunoglobulin proteins as well as non-peptidic targeting molecules. Small non-immunoglobulin proteins which are included in this definition are designed to have specific affinities towards, in particular surface expressed CD138. These small non-immunoglobulin proteins include scaffold based engineered molecules such as Affilin® molecules that have a relatively low molecular weight such as between 10 kDa and 20 kDa. Appropriate scaffolds include, for example, gamma crystalline. Those molecules have, in their natural state, no specific binding activity towards the target molecules. By engineering the protein surfaces through locally defined randomization of solvent exposed amino acids, completely new binding sites are created. Former non-binding proteins are thereby transformed into specific binding proteins. Such molecules can be specifically designed to bind a target, such as CD138, and allow for specific delivery of one or more effector molecules (see, scil Proteins GmbH at www.scilproteins.com, 2004). Another kind of non-immunoglobulin targeting molecules are derived from lipocalins, and include, for example ANTICALINS®, which resemble in structure somewhat immunoglobulins. However, lipocalins are composed of a single polypeptide chain with 160 to 180 amino acid residues. The binding pocket of lipocalins can be reshaped to recognize a molecule of interest with high affinity and specificity (see, for example, Beste et al., 1999). Artificial bacterial receptors such as those marketed under the trademark Affibody® (Affibody AB) are also within the scope of the present invention. These artificial bacterial receptor molecules are small, simple proteins and may be composed of a three-helix bundle based on the scaffold of one of the IgG-binding domains of Protein A (*Stylococcus aureus*). These molecules have binding properties similar to many immunoglobulins, but are substantially smaller, having a molecular weight often not exceeding 10kDa and are also comparatively stable. Suitable artificial bacterial receptor molecules are, for example, described in United States Patents 5,831,012; 6,534,628 and 6,740,734. Non-peptidic targeting molecules include, but are limited to, to DNA and RNA oligonucleotides that bind to CD138 (aptamers).

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Effector molecule: An effector molecule according to the present invention is a molecule or a derivative, or an analogue thereof that is attached to a targeting

agent and exerts a desired effect, for example apoptosis, or another type of cell death, or a continuous cell cycle arrest on the target cell or cells. Effector molecules according to the present invention include molecules that can exert desired effects in a target cell and include, but are not limited to, toxins, drugs, in particular low
5 molecular weight cytotoxic drugs, radionuclides, biological response modifiers, pore-forming agents, cytotoxic enzymes, prodrug activating enzymes, antisense oligonucleotides, antibodies or cytokines as well as functional derivatives or analogues/fragments thereof.

In a preferred embodiment, the effector increases internal effector delivery of
10 the immunoconjugate, in particular when the natural form of the antibody on which the targeting antibody of the immunoconjugate is based is poorly internalizable. In another preferred embodiment the effector is, in its native form, non-selective. In certain embodiments the effector has high non-selective toxicity, including systemic toxicity, when in its native form. The "native form" of an effector molecule of the
15 present invention is an effector molecule before being attached to the targeting agent to form an immunoconjugate. In another preferred embodiment, the non-selective toxicity of the effector molecule is substantially eliminated upon conjugation to the targeting agent. In another preferred embodiment, the effector molecule causes, upon reaching the target cell, death or continuous cell cycle arrest in the target cell.
20 A drug-effector molecule according to the present invention includes, but is not limited to, a drug including, for example, small highly cytotoxic drugs that act as inhibitors of tubulin polymerization such as maytansinoids, dolastatins, auristatin and cryptophycin; DNA alkylating agents like CC-1065 analogues or derivatives (United States Patents 5,475,092; 5,585,499; 6,716,821) and duocarmycin; enediyne
25 antibiotics such as calicheamicin and esperamicin; and potent taxoid (taxane) drugs (Payne, 2003). Maytansinoids and calicheamicins are particularly preferred. An effector maytansinoid includes maytansinoids of any origin, including, but not limited to synthetic maytansinol and maytansinol analogue and derivative. Doxorubicin, daunomycin, methotrexate, vinblastine, neocarzinostatin, macromycin, trenimon and
30 α -amanitin are some other effector molecules within the scope of the present invention. Also within the scope of the present invention are antisense DNA molecules as effector molecules. When the name of, for example, a specific drug or class of drugs is combined herein with the term "effector" or "effector molecule," reference is made to an effector of an immunoconjugate according to the present
35 invention that is based on the specified drug or class of drugs.

Maytansine is a natural product originally derived from the Ethiopian shrub *Maytenus serrata* (Remillard, 1975; United States Patent 3,896,111). This drug

inhibits tubulin polymerization, resulting in mitotic block and cell death (Remillard, 1975; Bhattacharyya, 1977; Kupchan, 1978). The cytotoxicity of maytansine is 200-1000-fold higher than that of anti-cancer drugs in clinical use that affect tubulin polymerization, such as *Vinca* alkaloids or taxol. However, clinical trials of
5 maytansine indicated that it lacked a therapeutic window due to its high systemic toxicity. Maytansine and maytansinoids are highly cytotoxic but their clinical use in cancer therapy has been greatly limited by their severe systemic side-effects primarily attributed to their poor selectivity for tumors. Clinical trials with maytansine showed serious adverse effects on the central nervous system and gastrointestinal
10 system.

Maytansinoids have also been isolated from other plants including seed tissue of *Trewia nudiflora* (United States Patent 4,418,064)

Certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (United States Patent 4,151,042).

15 The present invention is directed to maytansinoids of any origin, including synthetic maytansinol and maytansinol analogues which are disclosed, for example, in United States Patents 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,362,663; 4,364,866; 4,371,533;
20 4,424,219 and 4,151,042.

In a preferred embodiment, the maytansinoid is a thiol-containing maytansinoid and is more preferably produced according to the processes disclosed in United States Patent 6,333,410 to Chari et al or in Chari et al.(Chari, 1992).

25 DM-1 (N²-deacetyl-N²-(3-mercapto-1-oxopropyl)-maytansine) is a preferred effector molecule in the context of the present invention. DM1 is 3- to 10-fold more cytotoxic than maytansine, and has been converted into a pro-drug by linking it *via* disulfide bond(s) to a monoclonal antibody directed towards a tumor-associated antigen. Certain of these conjugates (sometimes called "tumor activated prodrugs" (TAPs)) are not cytotoxic in the blood compartment, since they are activated upon
30 associating with a target cells and internalized, thereby releasing the drug (Blättler, 2001). Several antibody-DM1 conjugates have been developed (Payne, 2003), and been evaluated in clinical trials. For example, huC242-DM1 treatment in colorectal cancer patients was well tolerated, did not induce any detectable immune response, and had a long circulation time (Tolcher, 2003).

35 Other particularly preferred maytansinoids comprise a side chain that contains a sterically hindered thiol bond such as, but not limited to, maytansinoids N²-deacetyl- N²-(4-mercapto-1-oxopentyl)-maytansine, also referred to as "DM3," and

N²-deacetyl- N²-(4-methyl-4-mercapto-1-oxopentyl)-maytansine, also referred to as "DM4."

DNA alkylating agents are also particularly preferred as effector molecules and include, but are not limited to, CC-1065 analogues or derivatives. CC-1065 is a
5 potent antitumor-antibiotic isolated from cultures of *Streptomyces zelensis* and has been shown to be exceptionally cytotoxic *in vitro* (United States Patent 4,169,888). Within the scope of the present invention are, for examples the CC-1065 analogues or derivatives described in United States Patents 5,475,092, 5,585,499 and 5,739,350. As the person skilled in the art will readily appreciate, modified CC-1065
10 analogues or derivatives as described in United States Patent 5,846,545 and prodrugs of CC-1065 analogues or derivatives as described, for example, in United States Patent 6,756,397 are also within the scope of the present invention. In certain embodiments of the invention, CC-1065 analogues or derivatives may, for example, be synthesized as described in United States Patent 6,534,660.

15 Another group of compounds that make preferred effector molecules are taxanes, especially highly potent ones and those that contain thiol or disulfide groups. Taxanes are mitotic spindle poisons that inhibit the depolymerization of tubulin, resulting in an increase in the rate of microtubule assembly and cell death. Taxanes that are within the scope of the present invention are, for example,
20 disclosed in United States Patents 6,436,931; 6,340,701; 6,706,708 and United States Patent Publications 20040087649; 20040024049 and 20030004210. Other taxanes are disclosed, for example, in United States Patent 6,002,023, United States Patent 5,998,656, United States Patent 5,892,063, United States Patent 5,763,477, United States Patent 5,705,508, United States Patent 5,703,247 and United States
25 Patent 5,367,086. As the person skilled in the art will appreciate, PEGylated taxanes such as the ones described in United States Patent 6,596,757 are also within the scope of the present invention.

Calicheamicin effector molecules according to the present invention include gamma 1I, N-acetyl calicheamicin and other derivatives of calicheamicin.
30 Calicheamicin binds in a sequence-specific manner to the minor groove of DNA, undergoes rearrangement and exposes free radicals, leading to breakage of double-stranded DNA, resulting in cell apoptosis and death. One example of a calicheamicin effector molecule that can be used in the context of the present invention is described in United States Patent 5,053,394.

35

Immunoconjugate: An immunoconjugate according to the present invention comprises at least one targeting agent, in particular targeting antibody, and one

effector molecule. The immunoconjugate might comprise further molecules for example for stabilization. For immunoconjugates, the term "conjugate" is generally used to define the operative association of the targeting agent with one or more effector molecules and is not intended to refer solely to any type of operative association, and is particularly not limited to chemical "conjugation". So long as the targeting agent is able to bind to the target site and the attached effector functions sufficiently as intended, particularly when delivered to the target site, any mode of attachment will be suitable. The conjugation methods according to the present invention include, but are not limited to, direct attachment of the effector molecule to the targeting antibody, with or without prior modification of the effector molecule and/or the targeting antibody or attachment via linkers. Linkers can be categorized functionally into, for example, acid labile, photosensitive, enzyme cleavable linkers etc. Other suitable linkers may include disulfide bonds and non-cleavable bonds, such as, but not limited to Sulfosuccinimidyl maleimidomethyl cyclohexane carboxylate (SMCC), which is a heterobifunctional linker capable of linking compounds with SH-containing compounds. Bifunctional and heterobifunctional linker molecules, such as carbohydrate-directed heterobifunctional linker molecules, such as S-(2-thiopyridyl)-L-cysteine hydrazide (TPCH), are also within the scope of the present invention (Vogel, 2004). The effector molecule, such as a maytansinoid, may be conjugated to the targeting antibody via a two reaction step process, including as a first step modification of the targeting antibody with a cross-linking reagent such as N-succinimidyl pyridyldithiopropionate (SPDP) to introduce dithiopyridyl groups into the targeting antibody. In a second step, a reactive maytansinoid having a thiol group, such as DM1, may be added to the modified antibody, resulting in the displacement of the thiopyridyl groups in the modified antibody, and the production of disulfide-linked cytotoxic maytansinoid/antibody conjugate (United States Patent 5,208,020). However, one-step conjugation processes such as the one disclosed in United States Patent Publication 20030055226 to Chari et al are also within the scope of the present invention. In one embodiment of the present invention multiple effector molecules of the same or different kind are attached to a targeting antibody.

CC-1065 analogues or derivatives may be conjugated to the targeting agent via for example PEG linking groups as described in United States Patent 6,716,821.

Calicheamicins may be conjugated to the targeting antibodies via linkers (United States Patent 5,877,296 and United States Patent 5,773,001) or according to the conjugation methods disclosed in United States Patent 5,712,374 and United

States Patent 5,714,586. Another preferred method for preparing calicheamicin conjugates is disclosed in Unites States Patent Publication 20040082764.

The immunoconjugates of the present invention also include recombinant fusion proteins.

5 The present invention takes advantage of the property of antibodies, in particular monoclonal antibodies, to bind to specific antigen targets, in particular, the property of certain antibodies to bind to CD138.

CD138 or sydecan-1 (also described as SYND1; SYNDECAN; SDC; SCD1; CD138 ANTIGEN, SwissProt accession number: P18827 human) is a membrane
10 glycoprotein that was originally described to be present on cells of epithelial origin, and subsequently found on hematopoietic cells (Sanderson, 1989). In malignant hematopoiesis, CD138 is highly expressed on the majority of MM cells, ovarian carcinoma, kidney carcinoma, gall bladder carcinoma, breast carcinoma, prostate cancer, lung cancer, colon carcinoma cells and cells of Hodgkin's and non-Hodgkin's
15 lymphomas, chronic lymphocytic leukemia (CLL) (Horvathova, 1995), acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML) (Seftalioglu, 2003 (a); Seftalioglu, 2003 (b)), solid tissue sarcomas, colon carcinomas as well as other hematologic malignancies and solid tumors that express syndecan-1 (Carbone et al., 1999; Sebestyen et al., 1999; Han et al., 2004; Charnaux et al., 2004; O'Connell et
20 al., 2004; Orosz and Kopper, 2001).

Other cancers that have been shown to be positive for CD138 expression are many ovarian adenocarcinomas, transitional cell bladder carcinomas, kidney clear cell carcinomas, squamous cell lung carcinomas; breast carcinomas and uterine cancers (see, for example, Davies et al., 2004; Barbareschi et al., 2003; Mennerich
25 et al., 2004; Anttonen et al., 2001; Wijdenes, 2002).

In the normal human hematopoietic compartment, CD138 expression is restricted to plasma cells (Wijdenes, 1996; Chilosi, 1999) and is not expressed on peripheral blood lymphocytes, monocytes, granulocytes, and red blood cells. In particular, CD34⁺ stem and progenitor cells do not express CD138 and anti-CD138
30 mAbs do not affect the number of colony forming units in hematopoietic stem cell cultures (Wijdenes, 1996). In non-hematopoietic compartments, CD138 is mainly expressed on simple and stratified epithelia within the lung, liver, skin, kidney and gut. Only a weak staining was seen on endothelial cells (Bernfield, 1992; Vooijs,

1996). It has been reported that CD138 exists in polymorphic forms in human lymphoma cells (Gattei, 1999).

Monoclonal antibodies antibodies B-B4, BC/B-B4, B-B2, DL-101, 1 D4, MI15, 5 1.BB.210, 2Q1484, 5F7, 104-9, 281-2 in particular B-B4 have been reported to be specific to CD138. Of those B-B4, 1D4 and MI15 recognized both the intact molecule and the core protein of CD138 and were shown to recognize either the same or closely related epitopes (Gattei, 1999). B-B4 has the advantage of not recognizing soluble CD138, but only CD138 in membrane bound form (Wijdenes, 10 2002).

B-B4, a murine IgG1 mAb, binds to a linear epitope between residues 90-95 of the core protein on human syndecan-1 (CD138) (Wijdenes, 1996; Dore, 1998). Consistent with the expression pattern of CD138, B-B4 was shown to strongly react 15 with plasma cell line RPMI8226, but not to react with endothelial cells. Also consistent with the expression pattern of CD138, B-B4 also reacted with epithelial cells lines A431 (keratinocyte derived) and HepG2 (hepatocyte derived). An immunotoxin B-B4-saporin was also highly toxic towards the plasma cell line RPMI8226, in fact considerably more toxic than free saporin. However, from the two 20 epithelial cell lines tested, B-B4-saporin showed only toxicity towards cell line A431, although in a clonogenic assay B-B4 saporin showed no inhibitory effect on the outgrowth of A431 cells (Vooijs, 1996). Other researchers reported lack of specificity of MM-associated antigens against tumors (Couturier, 1999).

25 The reactivity of B-B4 with tissue of various organs is shown in Table 1, the reactivity of B-B4 with cell lines of different origins is shown in Table 2. The reactivity was determined by immunohistochemistry (Table 1) and cytofluorography (Table 2). The number of (+) signs indicate the intensity of the reaction.

30

Table 1

Reactivity of B-B4 with tissues of various organs (immunohistochemistry)

Organ	Tissue	B-B4
Blood	Normal plasma cells	+++
Blood	MM patient cells	+++
Kidney	Tubular epithelium	-
Kidney	Glomerular	-
Kidney	Urothelium	++
Kidney	Smooth muscle of hilus	-
Liver	Sinusoid endothelium	-

Liver	Biliary epithelium	-
Liver	Hepatocytes	++
Lung	Alveolar epithelium	++
Lung	Bronchial epithelium	+
Lung	Blood vessel	-
Lung	Bronchial gland	++
Duodenum	Crypts epithelium	++
Duodenum	Glands	++
Duodenum	Chorion lymphocytes	++
Duodenum	Smooth muscle	-
Duodenum	Blood vessels	-
Heart	Myocytes	++ (cytoplasmic)
Spleen	Red pulp	+
Various organs	Muscle	-
Various organs	Connective tissue	-
Various organs	Nervous tissue	-
Various organs	Epithelium	+++
Various organs	Endothelium	+
Cell lines	MM cell lines	+++

Table 2
Reactivity of B-B4 with cell lines of different origins (Cytofluorography)

5

Cell line	Cell type	B-B4
RPMI 8226	Multiple myeloma	+++
U266	Multiple myeloma	+++
UM-1	Multiple myeloma	+++
XG-1	Multiple myeloma	+++
Daudi	EBV-infected LCL	-
Ramos	EBV-infected LCL	-
Jijoye	EBV-infected LCL	-
BJAB	Burkitt lymphoma	-
Raji	Burkitt lymphoma	-
BTL-1	LCL	-
BTL-6	LCL	-
KM-3	Pre-B	+
REH	Pre-B	-
NALM-6	Pre-B	+
ROS	Pre-B	-
697	Pre-B	-
CEM	T-cell	-
Jurkat	T-cell	-
HL-60	Myeloid	-
U937	Myeloid	+
HEL	Myeloid	-
KG1A	Myeloid	-
K562	Erythroid	++
A341	Epithelial	+++
HepG	Hepatocytic	++
HUVEC	Endothelial	+
Peripheral blood	Monocyte	-
Peripheral blood	B-cell (CD19+)	-
Peripheral blood	T-cell (CD3+)	-
Peripheral blood	Granulocytes	-
Bone marrow	(CD34+, CD33+, CD19+,	-

Cell line	Cell type	B-B4
	CD20+, CD10+, CD3+, CD19+, CD14+, CD38+) cells	
Bone marrow	Plasma cells	++
Bone marrow	Myeloma cells/CD38 high	+++
Tonsil	(CD19+, CD38+) cells	-
Patient sample ALL	B-cell	-
Patient sample CLL	B-cell	-
Patient sample Hodgkin	Reed-Sternberg cell	++

The activity of immunoconjugates on a cellular level has been described, for example, for huC242-DM1 (Immunogen, Inc.), an immunoconjugate comprising the antibody huC242 and the maytansinoid DM1, an inhibitor of tubulin polymerization described above. The activity of this immunoconjugate at the cellular level was described to include the following steps: (1) binding of the immunoconjugate to the antigen expressed on a cancer cell, (2) the internalization of the conjugate-antigen complex by the cancer cell, and (3) release of DM1, thereby allowing DM1 to reach its intracellular target tubulin and to inhibit tubulin polymerization (Xie, 2003). This multi-step attachment, internalization and release model forms the rationale behind the development of tumor activated prodrugs (TAPs) (Immunogen, 2003). Similar uptake mechanisms have been described for immunoconjugates based on anti-PSCA antibodies, which were reported to be internalized via caveolae (Ross, 2002).

The present invention is useful in the treatment of, but is not limited to, cancers, in particular, multiple myeloma, ovarian carcinoma, kidney carcinoma, gall bladder carcinoma, breast carcinoma, prostate cancer, lung cancer, colon carcinoma, Hodgkin's and non-Hodgkin's lymphomas, chronic lymphocytic leukemia (CLL) (Horvathova, 1995), acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML) (Seftalioglu, 2003 (a); Seftalioglu, 2003 (b)), solid tissue sarcomas, colon carcinomas as well as other hematologic malignancies and solid tumors that express syndecan-1 (Carbone et al., 1999; Sebestyen et al., 1999; Han et al., 2004; Charneau et al., 2004; O'Connell et al., 2004; Orosz and Kopper, 2001).

The immunoconjugates according to the present invention can be administered by any route, including intravenously, parenterally, orally, intramuscularly, intrathecally or as an aerosol. The mode of delivery will depend on the desired effect. A skilled artisan will readily know the best route of administration for a particular treatment in accordance with the present invention. The appropriate dosage will depend on the route of administration and the treatment indicated, and can readily be determined by a skilled artisan in view of current treatment protocols.

Pharmaceutical compositions containing an immunoconjugate of the present invention as the active ingredient can be prepared according to conventional pharmaceutical compounding techniques. See, for example, Remington's Pharmaceutical Sciences, 17th Ed. (1985, Mack Publishing Co., Easton, Pa.).

- 5 Typically, an antagonistic amount of active ingredient will be admixed with a pharmaceutically acceptable carrier. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, for example, intravenous, oral, parenteral, intrathecal, transdermal, or by aerosol.

For oral administration, the immunoconjugate can be formulated into solid or
10 liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example,
15 suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are
20 obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent must be stable to passage through the gastrointestinal tract. If necessary, suitable agents for stable passage can be used, and may include phospholipids or lecithin derivatives described in the literature, as well as liposomes, microparticles (including microspheres and macrospheres).

25 For parenteral administration, the immunoconjugate may be dissolved in a pharmaceutical carrier and administered as either a solution or a suspension. Illustrative of suitable carriers are water, saline, phosphate buffer solution (PBS), dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example,
30 preservatives, suspending agents, solubilizing agents, buffers and the like. When the immunoconjugate are being administered intracerebroventricularly or intrathecally, they may also be dissolved in cerebrospinal fluid.

In accordance with the present invention, MM is treated as follows, with use of the B-B4-DM1 conjugate as an example. This example is not intended to limit the
35 present invention in any manner, and a skilled artisan could readily determine other immunoconjugates of the present invention and other treatment regimes which could be utilized for the treatment of diseases such as MM. Due to the selective

expression of CD138 on patient MM cells on via the blood stream accessible cells, the specificity of B-B4 and the stability of the B-B4-DM1 conjugate in the bloodstream, the immunoconjugate removes the systemic toxicity of DM1 and provides an opportunity to target the delivery of the DM1-effector molecule(s). The immunoconjugates of this invention provide a means for the effective administration of the effector molecules to cell sites where the effector molecules can be released from the immunoconjugates. This targeted delivery and release provides a significant advance in the treatment of multiple myeloma, for which current chemotherapy methods sometimes provide incomplete remission.

10 In accordance with the present invention, in particular solid tumors may also be treated as follows with use of B-B4-DM1, as an example. This example is not intended to limit the present invention in any manner, and a skilled artisan could readily determine other immunoconjugates of the present invention and other treatment regimes which could be utilized for the treatment of solid tumors. The tumor is first treated to reduce the size of the tumor, for example chemotherapeutically or radioactively. Subsequent administration of the immunoconjugates of this invention provides a means for eliminating residual cancer cells. The administration of the immunoconjugate allows specific targeting of these residual cells and release of the effector molecules at the target site. This targeted delivery and release provides a significant advance in the treatment of residual cancer cells of solid tumors, for which current chemotherapy methods sometimes provide incomplete remission.

15 The present invention is further described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below were utilized.

MATERIALS AND METHODS

Preparation of mAb-DM1 conjugate

30 The thiol-containing maytansinoid DM1 was synthesized from the microbial fermentation product ansamitocin P-3, as previously described by Chari (Chari et al, 1992). Characterization of murine B-B4 (Wijdenes, 1996) and preparation of humanized C242 (huC242) (Roguska, 1994) have been previously described. Antibody-drug conjugates were prepared as described by Liu et al (Liu, 1996). An average of 3.5 DM1 molecules was linked per antibody molecule.

Cell lines and patient cells

CD138⁺ dexamethasone (Dex)-sensitive MM.1S and Dex-resistant MM.1R, Ocy-My5, OPM1 and OPM2 human MM cell lines and CD138⁻ Waldenstrom Macroglobulinemia (WM) WSU-WM and the lymphoma (LB) SUDHL4 cell lines were used. Cell lines were cultured in RPMI-1640 medium (GIBCO) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT), L-glutamine, penicillin, and streptomycin (GIBCO) (denoted below as RPMI complete medium). Plasma cells (PC) and bone marrow (BM) cells were isolated using Ficoll-Hypaque density gradient sedimentation from BM aspirates, obtained from MM patients following informed consent. BM cells were separated. BMSCs were obtained by long-term cultures of BM cells (4-8 weeks) in RPMI 1640 medium supplemented with 20% FBS.

Gene Expression Analysis and Data analysis: Expression of CD138 in MM patients

Expression of CD138 on normal plasma cells and patient MM cells was evaluated. BM aspirate samples from normal donors and patients with MM were treated with 0.86% ammonium chloride to lyse red blood cells. PC were then isolated by positive immunomagnetic bead selection using anti-CD138 antibodies and Magnet Assisted Cell Sorting ("MACS," Miltenyi Biotech). Purity of plasma cells (>95%) was assessed by flow cytometric (Becton-Dickinson "FACSsort") monitoring for CD38⁺/CD45^b phenotype as well as forward and side scatter and morphological characteristics.

Total RNA was isolated from 5×10^6 cells utilizing an "RNeasy® kit" (Qiagen Inc., Valencia, CA). Total RNA (10–15 µg) was reverse-transcribed to get cDNA using the "Superscript® II RT kit" (Invitrogen Life Technologies, Carlsbad, CA). cDNA was used in an *in vitro* transcription reaction to synthesize biotin-labeled cRNA utilizing "ENZO® RNA labeling kit" (Enzo Diagnostics, Inc., Farmingdale, NY). Labeled cRNA was purified with the "RNeasy® Mini-kit" (Qiagen Inc., Valencia, CA) and quantitated. Purified cRNA (15 µg) was hybridized to Human Genome U133 (HG-U133) GeneChip® arrays (Affymetrix, Inc.) representing approximately 33,000 human genes, and GeneChip® arrays were scanned on a GeneArray® Scanner (Affymetrix, Inc., Santa Clara, CA). Normalization of arrays and calculation of expression values was performed using the DNA-Chip Analyzer ("dChip") program. Arrays were normalized based on relative signal produced for an invariant subset of genes. This model-based method was used for probe selection and computing expression values. By pooling hybridization information across multiple arrays, it

was possible to assess standard errors for the expression level indexes. This approach also allowed automatic probe selection in the analysis stage to reduce errors due to cross-hybridizing probes and image contamination.

5 **Antibody Internalization**

Internalization of B-B4 antibody was examined with a cultured CD138⁺ cell line by flow cytometry and under a fluorescent microscope. The antibody was modified by Alexa 488 dye (Molecular Probes), and the fluorescence of the non-internalized antibody bound to cells was quenched by exposure to an “anti-Alexa
10 antibody” (Molecular Probes). Thus, semi-quantitatively discrimination between surface-bound and internalized antibody was possible. B-B4 was poorly internalized.

Colorimetric survival assay

Survival of CD138⁺ and CD138⁻ cells upon administration of B-B4-DM1, B-B4
15 and DM1 was examined using a tetrazolium colorimetric assay (CellTiter 96[®] Non-Radioactive Cell Proliferation Assay; Promega, WI), as previously described (Mossmann, 1983). Cells (1×10^4) were plated in 24-well plates in 1 ml RPMI complete medium and then treated as indicated. At the end of each treatment, cells were incubated with 150 μ l of Dye Solution and then incubated for 4 h at 37°C. A
20 solubilization/stop solution was then added to each well under vigorous pipetting to dissolve the formazan crystals. Absorbance was measured at 570 nm, and cell viability was estimated as percentage of untreated controls. All experiments were repeated 3 times, and each experimental condition was repeated in triplicate wells in each experiment. Data reported are average values \pm SD of 3 representative
25 experiments.

Cell proliferation assay

The effect of B-B4-DM1 on cell proliferation was measured by the extent of [³H]-thymidine (NEN Life Science Products, Boston, MA) incorporation. Cells (2×10^4 cells/well) were incubated in 96-well culture plates in the presence of 70%-80%
30 confluent BMSCs at 37°C with or without a test-agent (in triplicate wells). [³H]-thymidine (0.5 μ Ci) was then added to each well for the last 8 h. Cells were harvested onto glass filters with an automatic cell harvester (Cambridge Technology, Cambridge, MA) and counted using a Micro-Beta[®] Trilux counter (Wallac, Gaithersburgh, MD).

35

Detection of apoptosis

Dual staining with FITC-labeled Annexin V and propidium iodide (PI) was carried out to detect induction of apoptotic cell death by B-B4-DM1. After treatment of 1×10^6 tumor cells for 48 h, cells were washed with PBS and re-suspended in 100 μ l of HEPES buffer containing Annexin V-FITC and propidium iodide (PI) (Annexin V-FLUOS staining kit; Roche Diagnostic, Indianapolis, IN). Following 15 min incubation at room temperature, cells were analyzed using a Coulter Epics XL flow cytometer for the presence of an Annexin V-FITC-positive/PI-negative apoptotic cell population.

10 Cell cycle analysis

1×10^6 MM cells were incubated with or without a test-agent for 48 h, washed with PBS, permeabilized by a 30 min exposure to 70% ethanol at 4°C, incubated with PI (50- μ g/mL) in 0.5 ml PBS containing 20 U/mL Rnase A (Roche) for 30 min at room temperature, and analyzed for DNA content by cell-associated fluorescence using a flow cytometer and CellQuest™ software.

In vivo activity

Human MM xenograft murine model

In this model, CB-17 SCID mice were subcutaneously (s.c.) inoculated in the interscapular area with 5×10^6 OPM1 or OPM2 cells in 100 μ l of RPMI-1640 medium. Treatment was initiated after the detection of palpable tumors. Tumor growth was measured weekly in two dimensions using a caliper, and volume was expressed in mm^3 using the formula: $V = 0.5a \times b^2$, where a and b are the long and short diameter of the tumor, respectively. Tumor size was evaluated from the first day of treatment until day of first sacrifice. The survival time is defined as the time interval between start of the experiment and either death or day of sacrifice. Mice were treated intravenously (i.v.) with vehicle alone (PBS), unconjugated B-B4 (13.3 μ g/ml), B-B4-DM1 (conjugate containing 75 or 150 μ g DM1/Kg per day), or control huC242-DM1 (150 μ g DM1/Kg per day), for a total of 3 days. In addition, two mice bearing very large tumors (average size of $1309 \pm 60 \text{ mm}^3$) were treated with B-B4-DM1 (150 μ g DM1/kg per day) for a total of 3 days and observed for changes in the tumor size.

Autofluorescent GFP⁺ human MM xenograft model

Procedures for stably transfection of green fluorescent protein (GFP) in tumor cells and use have been previously described (Yang, 1999; Yang 2000). Five mice

were injected s.c. with GFP⁺ OPM1 cells as described above. Mice were monitored by whole-body fluorescence imaging using "Illumatool Bright Light System LT-9900" (Lighttools Research, Encinitas, CA). After accurate cutaneous shave of tumor area, fluorescence imaging results were digitally captured by a Sony® DSC-P5™ digital camera (Sony, New York, NY) and analyzed with Adobe PhotoShop® 4.0.

SCID-hu mouse model

Human fetal bones were obtained from products of conceptions of second trimester abortions in compliance with state and federal regulations (Advanced Bioscience Resources, ABR; Alameda, CA). The implantation of human fetal long bone grafts into SCID mice to produce SCID-hu mice has been previously described (McCune et al, 1988; Namikawa et al, 1988; Kyoizumi et al, 1993; Akkina et al, 1994; Chen et al, 1994; Sandhu et al, 1996; Urashima, 1997). In brief, the femurs or tibias of 19 to 23 gestational week fetuses were cut into fragments and implanted s.c. into SCID mice. After approximately 8 weeks, 2 to 5 x 10⁶ BM cells from a MM patient or Ocy-My5 MM cells were injected in 50 µl PBS directly into human bone of SCID-hu hosts. Production and level of human paraprotein in mouse serum was an indicator of myeloma engraftment and growth. At least 2 consecutive measurements, of increasing levels of circulating human immunoglobulin (hulg), signified human MM cell growth.

Measurement of serum paraprotein concentration

Blood (50-100 µl) was withdrawn from the tail vein for measurement of human paraprotein in murine serum using ELISA (Bethyl, Montgomery, TX). Goat anti-human λ and κ antisera were used for capture and goat anti-human λ or κ HRP conjugates were used for detection.

Histopathological analysis

Excised bone grafts were fixed in 10% buffered formalin; skeletal tissues were decalcified with 14% EDTA and embedded in paraffin by previously described standard techniques (Sasaki, 1995). Sections were then stained with H & E (Hematoxylin and eosin) for histopathological examination. Immunoperoxidase studies were performed on paraffin sections using an indirect technique as described (Urashima, 1997). Rabbit anti-human λ and κ antisera were used for detection of MM cells in fetal bone.

Statistical analysis

Statistical significance of differences was determined using Student's t-test. Differences were considered significant when $p < 0.05$.

5 Results and Discussion

CD138 is expressed on patient MM cells and is the most important target Ag for identification and selection of these cells. However previous reports show heterogenous CD138 expression on MM cells (Wijdenes, 1996; Dhodapkar, 1998; Witzig, 1996; Schneider, 1997; Rawstron, 1997). The expression of CD138 on patient MM cells was measured by gene profiling and flow cytometry. FIGS. 1A to 1C show the CD138 gene expression profiles of normal plasma cells ($n=3$) and patient MM cells ($n=15$) measured utilizing HG-U133 GeneChip® array (Affymetrix) data. FIG. 1A shows the individual fold increase in intensity of CD138 gene expression compared to normal PC; FIG. 1B shows the mean of intensity of CD138 gene expression in normal PC ($n=3$) and patient MM cells ($n=15$) and FIG. 1C the mean fold increase intensity of CD138 gene expression in MM cells ($n=15$) compared to normal PC ($n=3$). As can be seen, CD138 was expressed in all 15 MM specimens (100 %) examined at a 95 ± 8 -fold mean increase in intensity relative to normal plasma cells.

Furthermore, flow cytometry was used to assess cell surface expression of CD138 on MM cells from 25 patients. Expression of CD138 on the $CD38^{bright}CD45^{lo}$ cell population was assessed both by percentage of positive cells, and by mean fluorescence intensity (MFI). Figure 1D shows the percentage of patients expressing $CD138^{+}$ MM cells, as determined by flow cytometry on fresh BM aspirate samples. Fig. 1E shows the percentage of $CD138^{+}$ MM cells in $CD138^{+}$ patients on fresh BM aspirates and FIG. 1F shows MFI of $CD138^{+}$ or $CD138^{-}$ MM cells within $CD38^{bright}CD45^{lo}$ population. As can be seen from FIG. 1D 18 of 25 patients (72 %) expressed CD138, with a mean of $68 \pm 31\%$ $CD138^{+}$ cells (FIG. 1E) and MFI of 1234 ± 539 (range: 166-2208) (FIG. 1F). Taken together, these results indicate that CD138 is highly expressed in patient MM cells.

These results were consistent with previous reports showing CD138 expression on 60% and 100% of cases (Horvathova, 1995; Wijdenes, 1996). Possible explanations for the variability in CD138 detection by flow cytometry are the rapid shedding of protein during flow cytometric manipulation of specimens, the high turnover rate of the molecule on the cell membrane, lack of cell surface antigen (Ag) in pre-apoptotic plasma cells or Ag expression dependent on stage of the cell cycle (Clement, 1995). By immunohistochemistry, CD138 has been reported to be highly sensitive and specific marker of MM cells in 100% of BM biopsies (Chilosi, 1999). These data support the potential value of CD138 as a target for immunotherapeutic approaches in MM.

The effects of B-B4-DM1 on survival of CD138⁺ (MM-1S, MM-1R, Ocy-My5) and CD138⁻ cells (SUDHL-4 and WSU-WM) were determined using an MTT assay. MM cell lines were exposed to unconjugated B-B4 mAb (FIG. 2A), immunoconjugate B-B4-DM1 (FIG. 2B), or free DM1 drug at equimolar concentrations (FIG. 2C). Cell survival was measured using an MTT assay. Data (mean \pm SD of triplicate experiments) are shown in FIGS. 2A to 2C as percentage of untreated controls. CD138⁺ MM cell lines MM-1S, MM-1R and Ocy-My5 were evaluated as well as CD138⁻ cell lines including the lymphoma cell line SUDHL4 and the Waldstrom's Macroglobulinemia cell line WSU-WM.

As can be seen from FIG. 2B, treatment with B-B4-DM1 (1-50 nM) induced growth inhibition in CD138⁺ tumor cells in a time- and dose-dependent manner. This effect was clearly detected after 72 h in all CD138⁺ cells. B-B4-DM1 treatment of CD138⁺ OPM1 and OPM2 MM cells further confirmed these observations (data not shown). In contrast, B-B4-DM1 (1-50 nM) was not toxic to CD138⁻ cells, even after treatment for 96 h. To confirm that inhibitory activity of the immunoconjugate is specifically related to mAb-delivered cytotoxicity, the effect of equimolar concentrations of B-B4 antibody or unconjugated drug DM1 were tested. Even the highest concentrations of B-B4 did not affect the growth of cells at 96 h, (FIG. 2A), whereas free DM1 was equally and highly cytotoxic in both CD138⁺ and CD138⁻ cell lines (FIG. 2C). These data indicate that activity of the immunoconjugate is not related to the differential sensitivity of cells to the drug nor the intrinsic properties of the antibody.

Since adhesion of MM cells to BMSC (bone marrow stromal cells) protects MM cells against drug-induced apoptosis, the effect of B-B4-DM1 on proliferation of CD138⁺ (Ocy-My5) MM and CD138⁻ (SUDHL-4) LB cells adherent to BMSC was evaluated.

Ocy-My5 (FIG. 3A) or SUDHL-4 (FIG. 3B) cells (2×10^4) were seeded on 70%-80% confluent BMSC for 24 h. Cell proliferation was measured by [3 H]thymidine incorporation following 72 h treatment with B-B4-DM1 (10 nM). Values represent the mean [3 H]-TdR incorporation (cpm) of triplicate cultures. As seen in
5 FIGS. 3A and 3B, B-B4-DM1 (10 nM) significantly inhibited the proliferation of CD138⁺ Ocy-My5 cells, but had no significant effect on CD138⁻ SUDHL-4 cells. Unconjugated B-B4 did not exert any significant effect, whereas free DM1 (10 nM) was cytotoxic to both cell lines.

CD56, another CD associated with MM, and CD138 expression was
10 evaluated by flow cytometry. Previous experiments established that B-B4-DM1, even at a concentrations as high as 240 μ M, did not affect binding of FITC-labeled anti-CD138 antibody to CD138-expressing cells. FIG. 3C shows the cytotoxic activity of B-B4-DM1 (10 nM) on CD138⁺/CD56⁺ patient MM cells cultured with BMSCs using flow cytometry. Following 72 h of treatment with the immunoconjugate, >90%
15 reduction in the MM cells was observed. Taken together, these results indicate that B-B4-DM1 overcomes cell adhesion mediated drug resistance (CAM-DR).

To determine whether apoptotic cell death occurs in cells exposed to the immunoconjugate, CD138⁺ Ocy-My5 MM cells were incubated with B-B4-DM1 (10 nM) for 72 h. Apoptotic cell death was then measured by staining with annexin V and
20 PI and flow cytometric analysis.

Figure 4A shows the induction of apoptotic cell death in CD138⁺ Ocy-My5 MM cells after 48h exposure to B-B4-DM1 (10 nM). Percentages of stained cells are reported in each quadrant. FIG. 4A shows a significant increase in both annexin V⁺/PI⁻ and annexin V⁺/PI⁺ fractions in CD138⁺ cells exposed to B-B4-DM1 and free
25 DM1, whereas no significant differences were detected in cells treated with unconjugated mAb alone. Figure 4B shows the effects of B-B4-DM1 treatment on the cell cycle. Ocy-My5 MM cells were exposed to B-B4 mAb (13.3 μ g/ml) or B-B4-DM1 (10 nM) for 48 h, labeled with PI, and analyzed using flow cytometry. Percentages of cells in the S-phase (S) and G2/M phase (G2) are indicated. As
30 shown in FIG. 4B, B-B4 mAb alone had no significant effect on the proportion of cells in G2/M phase compared to untreated cells (15% vs 16%), whereas exposure of MM cells to B-B4-DM1 induced a majority (88%) of cells into the G2/M phase.

A human MM s.c. xenograft model in SCID mice was used to study the *in vivo* activity of B-B4-DM1 against CD138⁺ OPM1 cells. In this model, the therapeutic
35 efficacy of B-B4-DM1 was measured in mice bearing large palpable tumors (average size 453 ± 74 mm³). Animals were treated daily i.v. for 3 consecutive days with vehicle alone (PBS 9phosphate buffered saline); n = 5), unconjugated B-B4 (13.3

µg/ml;n=5), B-B4-DM1 (150 µg DM1/kg; n =5), or control huC242-DM1 (150 µg DM1/kg; n =5) which does not bind OPM1 cells. Tumor size and overall survival were monitored serially in this cohorts.

5 FIG. 5 shows the results obtained after CB-17 SCID mice were inoculated s.c. in the interscapular area with 5×10^6 OPM1 (A and B) or OPM2 (C and D) MM cells. Mice were treated *i.v.* with B-B4-DM1 or control mAbs for 3 consecutive days. Tumor volume was assessed in two dimensions using an caliper eletronic, and the volume was expressed in mm^3 using the formula: $V= 0.5a \times b^2$, where a and b are the long and short diameter of the tumor, respectively. Tumor volume and survival were
10 calculated as described previously.

As shown in FIGS. 5A and 5B, vehicle alone, unconjugated B-B4 and huC242-DM1, had no significant effect on tumor growth (panel A) or survival (panel B). Importantly, treatment with 150 µg/kg of B-B4-DM1 induced tumor regression and a significant increase in survival ($p<0.001$). We also studied the effect induced
15 by B-B4-DM1 (75 or 150 µg DM1/kg; n =10) against OPM2 MM cells. As shown in FIG. 5C and D, treatment with 75 µg/kg of B-B4-DM1 induced a significant delay in tumor growth, and 150 µg/kg of B-B4-DM1 completely inhibited tumor growth. A significant increase in survival was also observed in mice treated at both dose levels ($p<0.05$) relative to animals treated with vehicle or huC242-DM1 alone. To confirm
20 the activity of B-B4-DM1 (150 µg DM1/kg), animals bearing a significant burden of disease (average tumor size was $1309 \pm 60 \text{ mm}^3$) were treated. FIGS. 6A to 6F also shows the results obtained when CB-17 SCID mice were inoculated s.c. in the interscapular area with 5×10^6 OPM1 MM cells. Again, mice were treated *i.v.* with B-B4-DM1 (150 µg DM1/kg) for a total of 3 consecutive days. Tumor volume was
25 measured in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V= 0.5a \times b^2$, where a and b are the long and short diameter of the tumor, respectively.

As shown in FIGS 6A to 6F, significant tumor regression was induced by B-B4-DM1 treatment. Taken together, these results indicate that B-B4-DM1 is highly
30 active in controlling tumor growth in a murine xenograft model of human MM.

Since expression of reporter genes encoding fluorescent proteins are sensitive method for *in vivo* detection of localized tumor growth as well as distant metastasis, the OPM1 MM cells were transfected with green fluorescent protein (GFP) and B-B4-DM1 activity was further characterized. In particular, mice were
35 injected s.c. with GFP⁺ OPM1 cells, followed by serial whole-body fluorescence imaging to assess development of GFP⁺ tumors. Mice were then treated with B-B4-DM1 (150 µg DM1/kg; n=5). FIG. 7A shows a flow cytometry analysis of GFP⁺

OPM1 cells, indicating a ~2-log difference in MFI of transfected cells. FIG. 7B shows results from five animals being injected with 5×10^6 GFP⁺ cells, monitored with whole-body fluorescence imaging for tumor development, and then treated with B-B4-DM1 (150 µg DM1/kg). Tumor sizes were determined directly by imaging the GFP-expressing tumor. FIGS. 7 C and D are representative whole-body fluorescence imaging from a mouse treated with B-B4-DM1. FIGS. 7E and 7F are negative images of the representative mouse. As seen in FIG. 7B and 7C to 7F, B-B4-DM1 induced significant regressions of GFP⁺ tumors, confirming high activity of the immunoconjugate against CD138⁺ MM cells.

10 Since the SCID-hu model of MM accurately reproduces the pathological behaviours of the disease, the efficacy of B-B4-DM1 treatment was tested in (i) SCID-hu mice injected with patient MM cells and (ii) SCID-hu mice injected with Ocy-My5 MM cell line (Urashima, 1997). The activity of the immunoconjugate on disease confined to the human fetal bone chip implanted s.c. in mice was studied. Four mice with patient MM cells growing in human bone environment increasing serum hulg levels, were treated with either B-B4-DM1 (150 µg DM1/kg) or the control huC242-DM1 (150 µg DM1/kg).

In FIGS. 8A and 8B the results of monitoring mice for changes in levels of human κ chain as an indicator of disease burden are shown. The Figure shows a significant reduction of κ levels after treatment with B-B4-DM1. FIG. 8C shows final human κ chain levels (mean ± SD) (n=4) after treatment. As seen in FIGS. 8A to 8C, treatment with B-B4-DM1 induced a significant reduction of human paraprotein, whereas human paraprotein continued to rise in mice treated with control antibody.

The activity of B-B4-DM1 after injection of Ocy-My5 cells in human fetal bone, tumor cell growth in bone (FIG. 9A and 9B) and subsequent spread to surrounding tissues was also studied (Urashima, 1997) were treated with either B-B4-DM1 (150 µg DM1/kg) or the control huC242-DM1 (150 µg DM1/kg). FIG. 9A and 9B show representative human bone sections after implantation of Ocy-My5 cells and before treatment. Sections are respectively stained by H & E and with anti-λ mAb. Finally, the activity of B-B4-DM1 on survival of tumor bearing mice was studied. Figure 9C shows the survival of mice measured from the first day of treatment to the day of death or sacrifice. Figure shows a significant prolongation of survival after treatment with B-B4-DM1 As seen in FIG. 9C, treatment with B-B4-DM1 (150 µg DM1/kg) induced a significant prolongation in survival compared with control huC242-DM1 (150 µg DM1/kg) therapy. Taken together, these results confirm *in vivo* B-B4-DM1 activity in preclinical models which mimic many features of human MM.

It will be appreciated that the methods and compositions of the instant invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the invention. Thus, the described
5 embodiments are illustrative and should not be construed as restrictive.

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CLAIMS:

1. An immunoconjugate comprising:

at least one targeting antibody selectively targeting cell-surface expressed syndecan-1,

at least one effector molecule, wherein said effector molecule is a low molecular weight cytotoxic drug having a molecular weight of less than 2kD, wherein said targeting antibody is functionally attached to said effector molecule to form said immunoconjugate, and wherein, in the immunoconjugate systemic cytotoxicity of said effector is substantially eliminated.

2. The immunoconjugate of claim 1, wherein the targeting antibody binds to a linear epitope between residues 90-95 of the core protein on human CD138.

3. The immunoconjugate of claim 1, wherein said effector molecule has, a potency of about 10^{-11} - 10^{-8} M before being attached to the targeting antibody to form the immunoconjugate.

4. The immunoconjugate of claim 1, wherein said effector molecule is a maytansinoid, a CC1065 analogue, a calicheamicin or a taxane.

5. The immunoconjugate of claim 4, wherein said effector molecule is a maytansinoid.

6. The immunoconjugate of claim 5, wherein said maytansinoid is DM1.

7. The immunoconjugate of claim 5, wherein said maytansinoid is DM3.

8. The immunoconjugate of claim 5, wherein said maytansinoid is DM4.

9. The immunoconjugate of claim 1, wherein said effector molecule has a molecular weight of between 600 and 800 Da.

10. The immunoconjugate of claim 1, wherein said targeting antibody selectively targeting cell-surface expressed syndecan-1 is an antibody fragment comprising an antigen binding region of the antibody.

11. The immunoconjugate of claim 1, wherein said targeting antibody selectively targeting cell-surface expressed syndecan-1 is derived from an antibody that internalizes poorly.

12. The immunoconjugate of claim 1, wherein said targeting antibody selectively targeting cell-surface expressed syndecan-1 is derived from the antibody B-B4.

13. A pharmaceutical composition comprising the immunoconjugate according to claim 1 and one or more pharmaceutically acceptable excipients.

14. A kit comprising, in separate containers, pharmaceutical compositions, wherein one container comprises the pharmaceutical composition of claim 13, and wherein, a separate container comprises a second pharmaceutical composition comprising a chemotherapeutic agent.

15. The use of the immunoconjugate of claim 1 to inhibit, delay or prevent the growth of tumor cells in a cell culture containing both syndecan-1 expressing tumor cells and non-tumor cells in an amount effective to inhibit, delay or prevent the growth of syndecan-1 expressing tumor cells.

16. The use of claim 15, wherein said effective amount induces cell death or continuous cell cycle arrest in said syndecan-1 expressing tumor cells.

17. The use of claim 16, wherein the cells in said cell culture are from a patient suffering from an hematologic malignancy or a solid tumor comprising syndecan-1 expressing cells.

18. The use of claim 17, wherein said patient is suffering from one of the following: multiple myeloma, ovarian carcinoma, kidney carcinoma, gall bladder carcinoma, breast carcinoma, prostate cancer, lung cancer, colon carcinoma, Hodgkin's and non-Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML), a solid tissue sarcoma or a colon carcinoma.

19. The use of claim 18, wherein said patient is a multiple myeloma patient.

20. The use of at least one immunoconjugate as in claim 1 to inhibit, delay, or prevent the growth of a tumor comprising syndecan-1 expressing tumor cells or the spread of syndecan-1 expressing tumor cells in a patient.

21. The use of claim 20, wherein said patient suffers from an hematologic malignancy or a solid tumor comprising syndecan-1 expressing cells.

22. The use of claim 21, wherein said patient suffers from one of the following: multiple myeloma, ovarian carcinoma, kidney carcinoma, gall bladder carcinoma, breast carcinoma, prostate cancer, lung cancer, colon carcinoma, Hodgkin's and non-Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myeloblastic leukemia (AML), solid tissue sarcoma or colon carcinoma.

23. The use of claim 22, wherein the disease is multiple myeloma.

24. The use of claim 22, wherein said effector molecule exhibits systemic cytotoxicity before being attached to the targeting antibody to form the immunoconjugate.

25. The use of the immunoconjugate as defined in claim 1 to inhibit, delay or prevent the growth of a tumor or the spread of malignant tumor cells in a patient treated with one or more cancer drugs or radiation.

26. The use of claim 25, wherein the immunoconjugate comprises a B-B4 targeting antibody.

27. The use of claim 25, wherein the immunoconjugate comprises a maytansinoid effector molecule.

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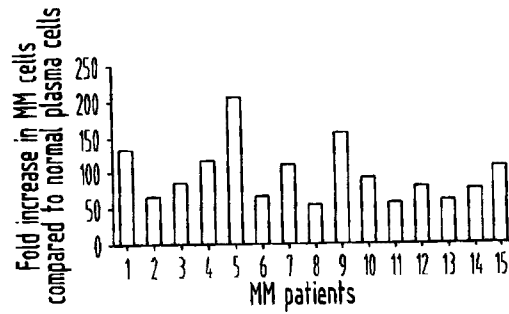


FIG.1A

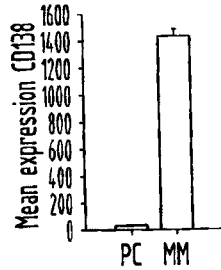


FIG.1B



FIG.1C

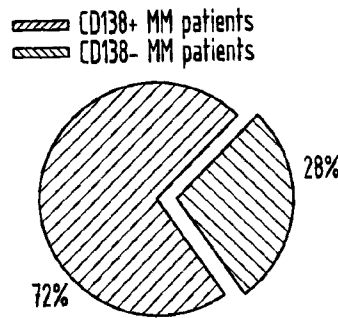


FIG.1D

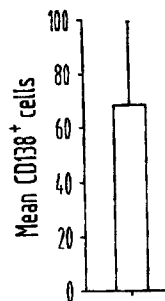


FIG.1E

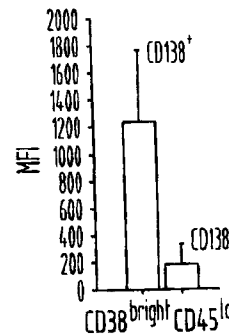


FIG.1F

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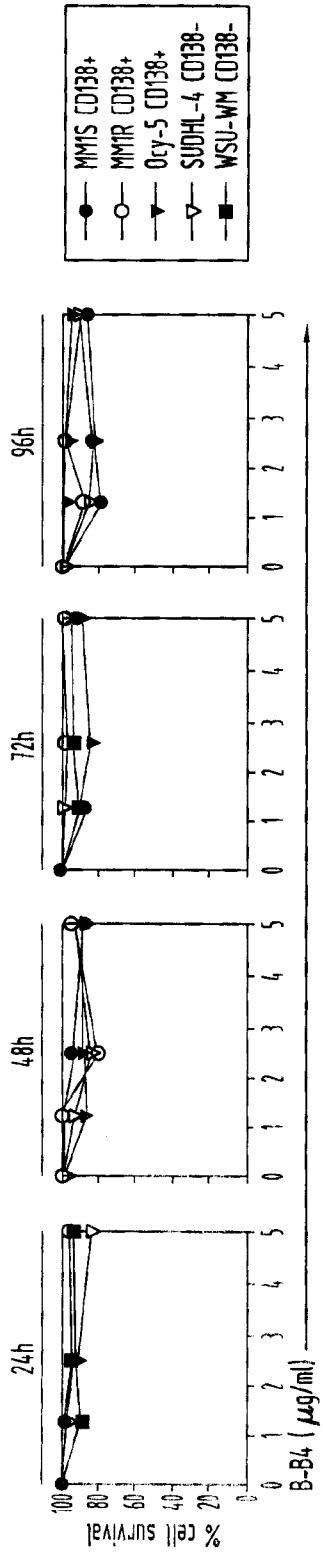


FIG. 2A

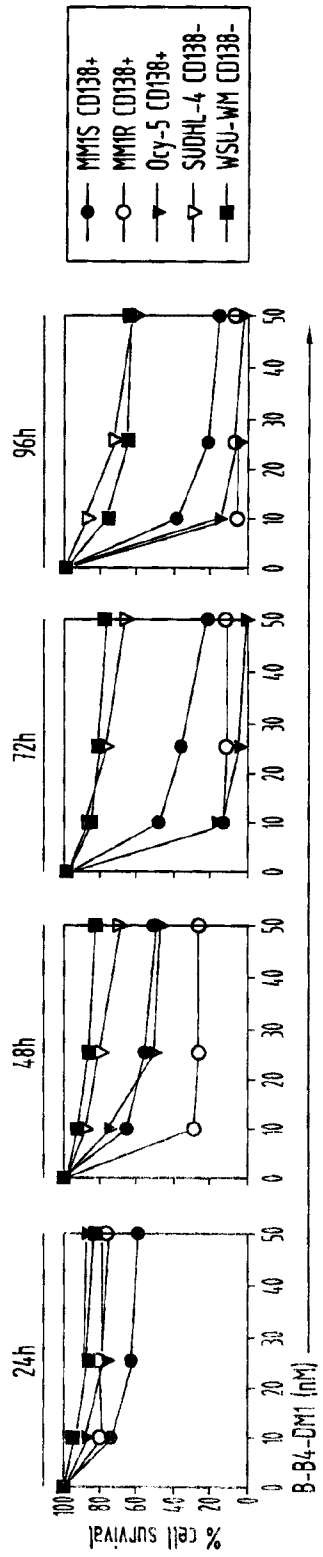


FIG. 2B

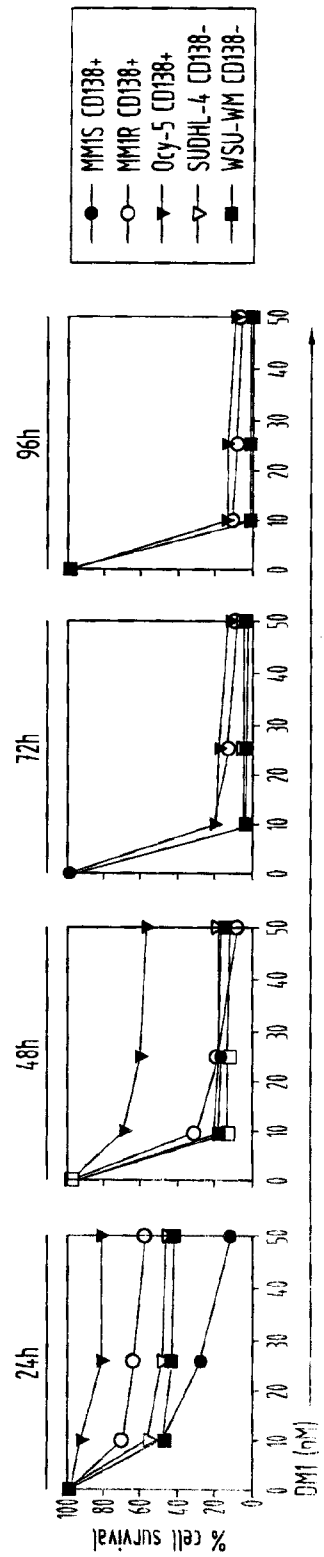


FIG. 2C

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FIG.3A

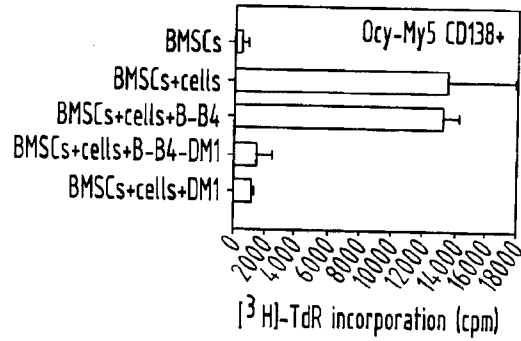


FIG.3B

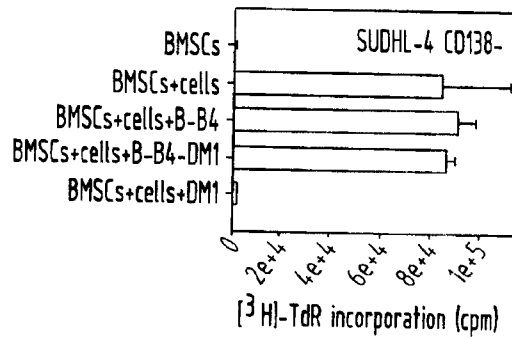
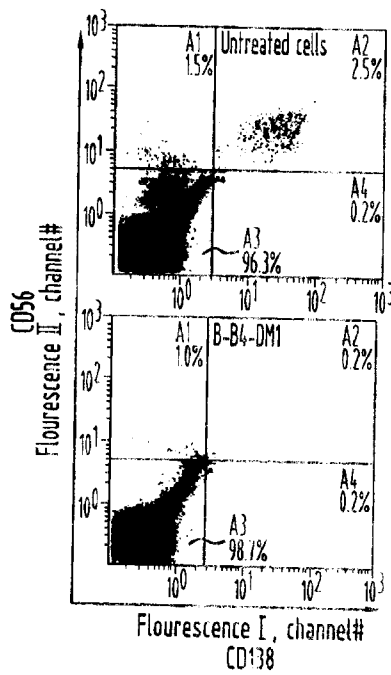


FIG.3C



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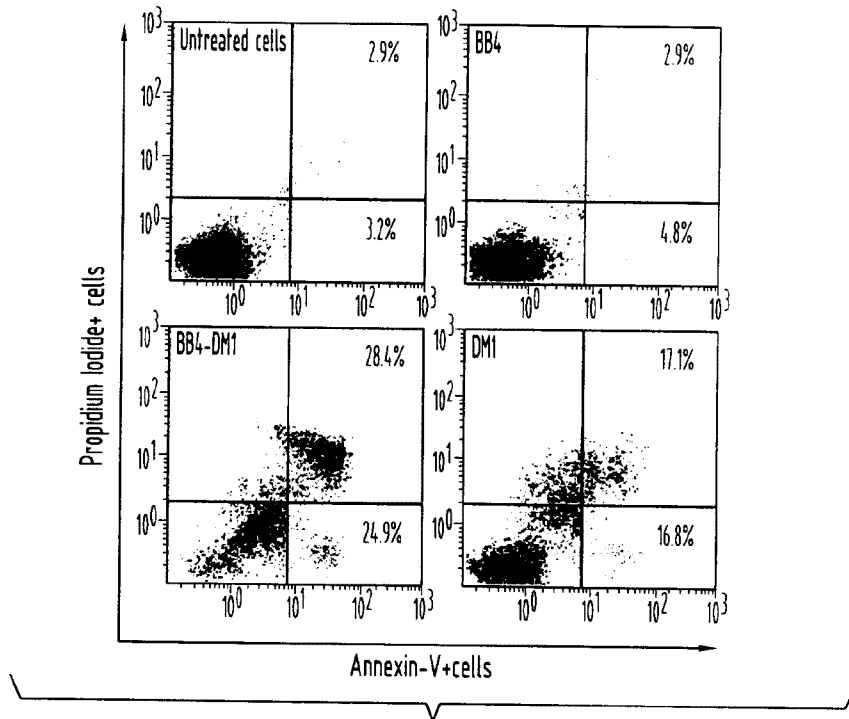


FIG.4A

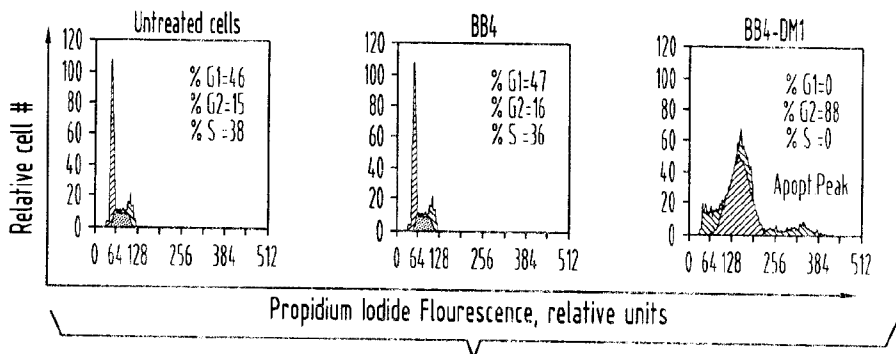


FIG.4B

FIG.5A

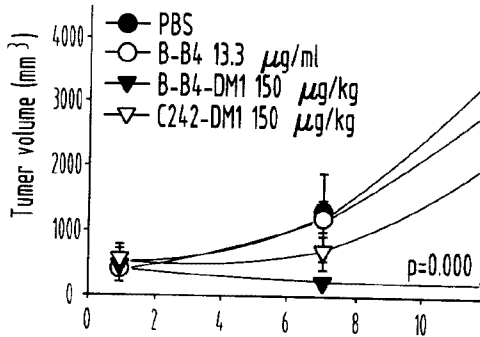


FIG.5C

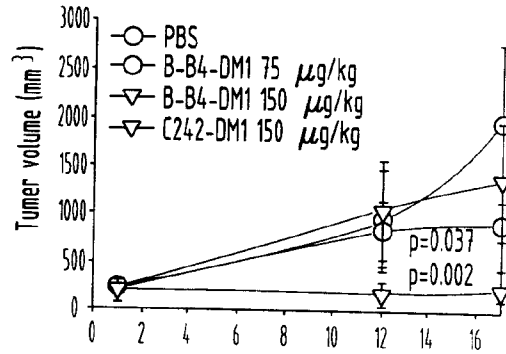


FIG.5B

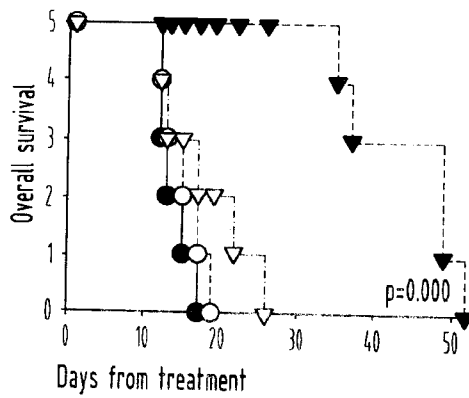
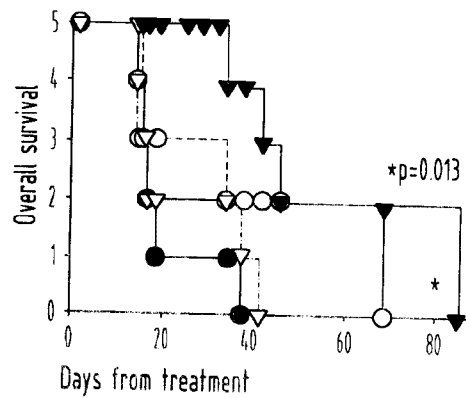


FIG.5D



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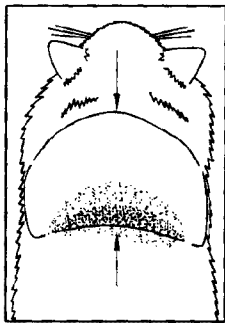


FIG. 6A

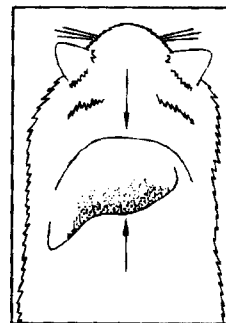


FIG. 6B

FIG. 6C

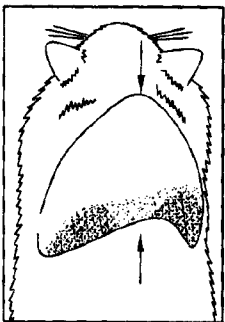
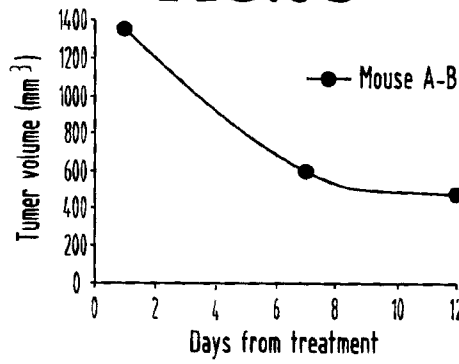


FIG. 6D

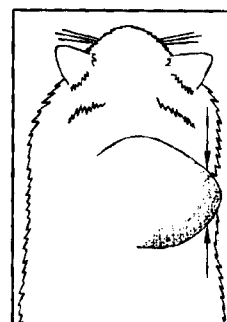


FIG. 6E

FIG. 6F

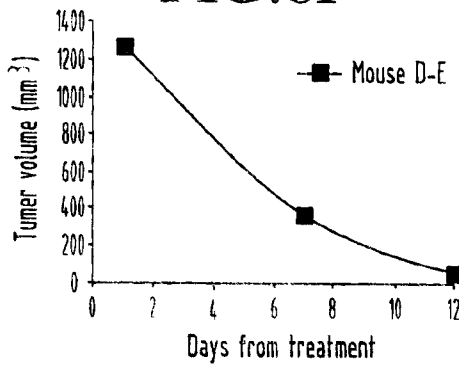


FIG.7A

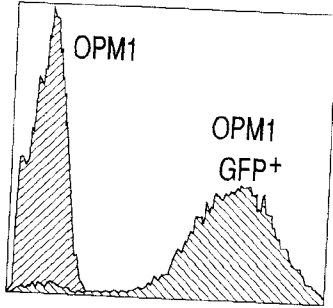


FIG.7B

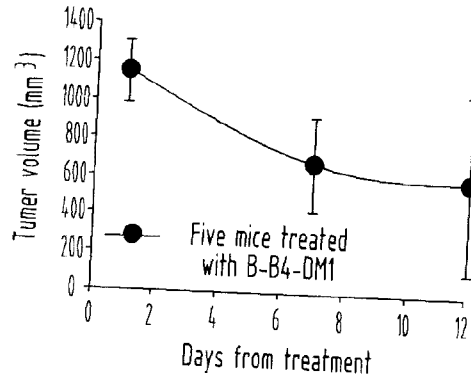


FIG.7C

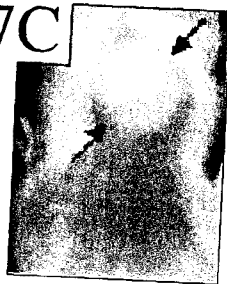


FIG.7D



FIG.7E



FIG.7F



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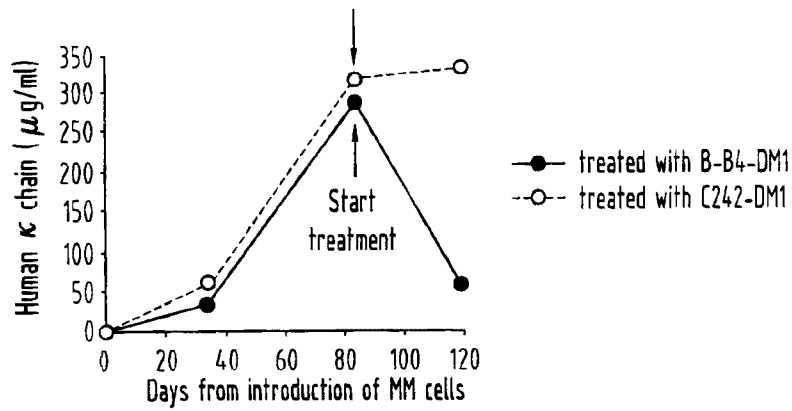


FIG.8A

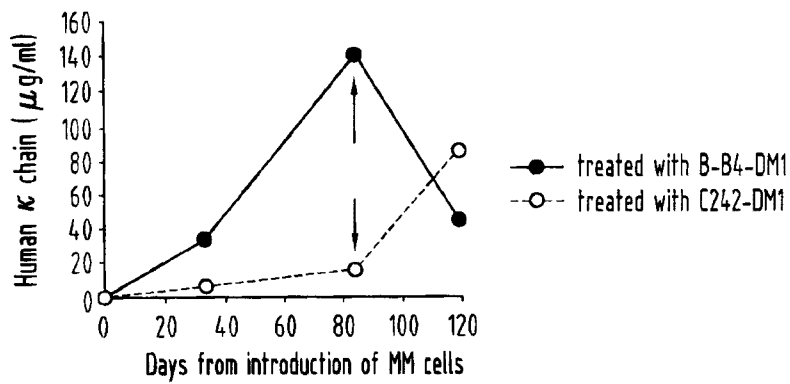


FIG.8B

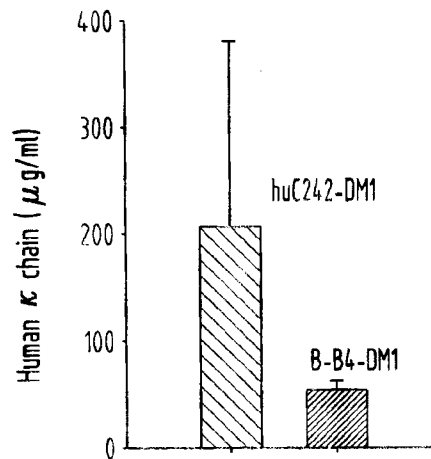


FIG.8C

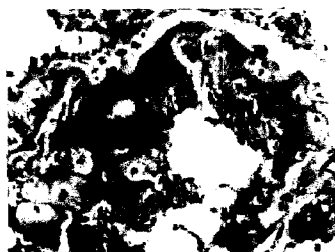


FIG.9A



FIG.9B

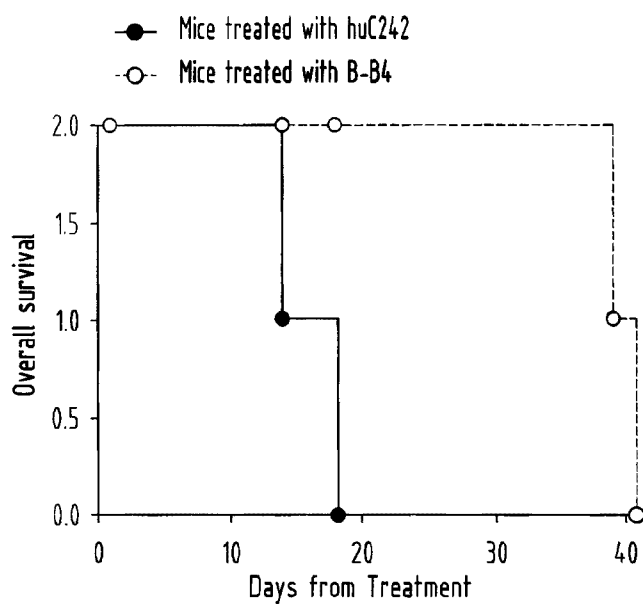
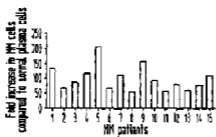
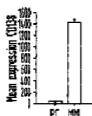


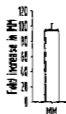
FIG.9C



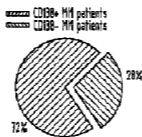
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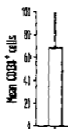
B



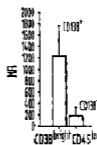
C



D



E



F