(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 June 2010 (03.06.2010)

PCT

(10) International Publication Number
WO 2010/061357 A1

(51) **International Patent Classification**:
A61K 39/00 (2006.01) ***A61P 35/00*** (2006.01)
A61K 31/00 (2006.01)

(21) **International Application Number**:
PCT/IB2009/055389

(22) **International Filing Date**:
27 November 2009 (27.11.2009)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
08291116.5 28 November 2008 (28.11.2008) EP

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2010/061357 A1

(54) **Title**: ANTITUMOR COMBINATIONS CONTAINING ANTIBODIES RECOGNIZING SPECIFICALLY CD38 AND MELPHALAN

(57) **Abstract**: Pharmaceutical composition comprising an antibody specifically recognizing CD38 and melphalan.

Antitumor combinations containing antibodies recognizing specifically CD38 and melphalan.

The present invention relates to combinations of monoclonal antibodies directed against CD38 and melphalan which are therapeutically useful in the treatment of neoplastic diseases.

CD38 is a 45 kD type II transmembrane glycoprotein with a long C-terminal extracellular domain and a short N-terminal cytoplasmic domain. The CD38 protein is a bifunctional ectoenzyme that can catalyze the conversion of NAD^+ into cyclic ADP-ribose (cADPR) and also hydrolyze cADPR into ADP-ribose. CD38 is upregulated and has been implicated in many hematopoietic malignancies.

Monoclonal antibodies 38SB13, 38SB18, 38SB19, 38SB30, 38SB31, and 38SB39, which specifically recognize CD38, are described in PCT application WO2008/047242. Said anti-CD38 antibodies are capable of killing $CD38^+$ cells by three different cytotoxic mechanisms, induction of apoptosis, antibody-dependent cell-mediated cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC). In addition, these antibodies are able to directly induce apoptosis of $CD38^+$ cells, even without the presence of stroma cells or stroma-derived cytokines. Melphalan is an alkylating agent used in chemotherapy. Nevertheless, there is still a need for new and efficacious medicaments for treating cancer.

It has now been found, and for this invention, that the efficacy of the humanized anti-CD38 antibodies may be considerably improved when it is administered in combination with at least one substance which is therapeutically useful in anticancer treatments and has a mechanism identical to or different from the one of the humanized anti-CD38 antibodies and which is limited in the present invention to melphalan.

The term “antibody” is used herein in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies) of any isotype such as IgG, IgM, IgA, IgD and IgE, polyclonal antibodies, multispecific antibodies, chimeric antibodies, and antibody fragments. A typical IgG antibody is comprised of two identical heavy chains and two identical light chains that are joined by disulfide bonds. Each heavy and light chain contains a constant region and a variable region. Each variable region contains three segments called “complementarity-determining regions” (“CDRs”)

or “hypervariable regions”, which are primarily responsible for binding an epitope of an antigen. They are usually referred to as CDR1, CDR2, and CDR3, numbered sequentially from the N-terminus. The more highly conserved portions of the variable regions outside of the CDRs are called the “framework regions”.

As used herein, “V_H” or “VH” refers to the variable region of an immunoglobulin heavy chain of an antibody, including the heavy chain of an Fv, scFv, dsFv, Fab, Fab’ or F(ab’)2 fragment. Reference to “V_L” or “VL” refers to the variable region of the immunoglobulin light chain of an antibody, including the light chain of an Fv, scFv, dsFv, Fab, Fab’ or F(ab’)2 fragment.

The 38SB13 antibody comprises at least one heavy chain having an amino acid sequence consisting of SEQ ID NO: 50 and at least one light chain having an amino acid sequence consisting of SEQ ID NO: 38, said heavy chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 1, 2, and 3, and said light chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 4, 5, and 6.

The 38SB18 antibody comprises at least one heavy chain having an amino acid sequence consisting of SEQ ID NO: 52 and at least one light chain having an amino acid sequence consisting of SEQ ID NO: 40, said heavy chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 7, 8, and 9, and said light chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 10, 11, and 12.

The 38SB19 antibody comprises at least one heavy chain having an amino acid sequence consisting of SEQ ID NO: 54 and at least one light chain having an amino acid sequence consisting of SEQ ID NO: 42, said heavy chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 13, 14, and 15, and said light chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 16, 17, and 18.

The 38SB30 antibody comprises at least one heavy chain having an amino acid sequence consisting of SEQ ID NO: 56 and at least one light chain having an amino acid sequence consisting of SEQ ID NO: 44, said heavy chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 19, 20, and

21, and said light chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 22, 23, and 24.

The 38SB31 antibody comprises at least one heavy chain having an amino acid sequence consisting of SEQ ID NO: 58 and at least one light chain having an amino acid sequence consisting of SEQ ID NO: 46, said heavy chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 25, 26, and 27, and said light chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 28, 29, and 30.

The 38SB39 antibody comprises at least one heavy chain having an amino acid sequence consisting of SEQ ID NO: 60 and at least one light chain having an amino acid sequence consisting of SEQ ID NO: 48, said heavy chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 31, 32, and 33, and said light chain comprising three sequential CDRs having amino acid sequences consisting of SEQ ID NOS: 34, 35, and 36.

The hybridoma cell lines producing the 38SB13, 38SB18, 38SB19, 38SB30, 38SB31, and 38SB39 murine anti-CD38 antibodies have been deposited at the American Type Culture Collection (10801 University Bld, Manassas, VA, 20110-2209, USA), on June 21, 2006, under the deposit numbers PTA-7667, PTA-7669, PTA-7670, PTA-7666, PTA-7668, and PTA-7671, respectively (as described in WO2008/047242).

The term “humanized antibody”, as used herein, refers to a chimeric antibody which contain minimal sequence derived from non-human immunoglobulin. The goal of humanization is a reduction in the immunogenicity of a xenogenic antibody, such as a murine antibody, for introduction into a human, while maintaining the full antigen binding affinity and specificity of the antibody. Humanized antibodies, or antibodies adapted for non-rejection by other mammals, may be produced using several technologies such as resurfacing and CDR grafting. As used herein, the resurfacing technology uses a combination of molecular modelling, 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. The CDR grafting technology involves substituting the complementarity determining regions of, for example, a mouse antibody, into a human framework domain, e.g., see W0 92/22653. Humanized chimeric antibodies preferably have constant regions and variable regions other than

the complementarity determining regions (CDRs) derived substantially or exclusively from the corresponding human antibody regions and CDRs derived substantially or exclusively from a mammal other than a human.

Strategies and methods for the resurfacing of antibodies, and other methods for reducing immunogenicity of antibodies within a different host, are disclosed in US Patent 5,639,641, which is hereby incorporated in its entirety by reference. Antibodies can be humanized using a variety of other techniques including CDR-grafting (EP 0 239 400; WO 91/09967; U.S. Pat. Nos. 5,530,101; and 5,585,089), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan E. A., 1991, *Molecular Immunology* **28(4/5)**: 489-498; Studnicka G. M. *et al.*, 1994, *Protein Engineering*, **7(6)**: 805-814; Roguska M.A. *et al.*, 1994, *PNAS*, **91**: 969-973), chain shuffling (U.S. Pat. No. 5,565,332), and identification of flexible residues (PCT/US2008/074381). Human antibodies can be made by a variety of methods known in the art including phage display methods. See also U.S. Pat. Nos. 4,444,887, 4,716,111, 5,545,806, and 5,814,318; and international patent application publication numbers WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741 (said references incorporated by reference in their entireties).

The anti-CD38 antibodies of the pharmaceutical combination of the present invention are humanized antibodies which recognize CD38 and kill $CD38^+$ cells by apoptosis, ADCC, and CDC. In a further embodiment, the humanized antibodies of the invention are capable of killing said $CD38^+$ cells by apoptosis even in the absence of stroma cells or stroma-derived cytokines.

A preferred embodiment of such a humanized antibody is a humanized 38SB13, 38SB18, 38SB19, 38SB30, 38SB31, or 38SB39 antibody, or an epitope-binding fragment thereof.

The CDRs of the 38SB13, 38SB18, 38SB19, 38SB30, 38SB31, and 38SB39 antibodies are identified by modelling and their molecular structures have been predicted. Thus, in one embodiment, this invention provides humanized antibodies or epitope-binding fragment thereof comprising one or more CDRs having an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36. In a preferred embodiment, a humanized version of 38SB13 is provided, which

comprises at least one heavy chain and at least one light chain, wherein said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 1, 2, and 3, and wherein said light chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 4, 5, and 6. In another preferred embodiment, a humanized version of 38SB18 is provided, which comprises at least one heavy chain and at least one light chain, wherein said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 7, 8, and 9, and wherein said light chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 10, 11, and 12. In another preferred embodiment, a humanized version of 38SB19 is provided, which comprises at least one heavy chain and at least one light chain, wherein said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 13, 14, and 15, and wherein said light chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 16, 17, and 18. In another preferred embodiment, a humanized version of 38SB30 is provided, which comprises at least one heavy chain and at least one light chain, wherein said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 19, 20, and 21, and wherein said light chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 22, 23, and 24. In another preferred embodiment, a humanized version of 38SB31 is provided, which comprises at least one heavy chain and at least one light chain, wherein said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 25, 26, and 27, and wherein said light chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 28, 29, and 30. In another preferred embodiment, a humanized version of 38SB39 is provided, which comprises at least one heavy chain and at least one light chain, wherein said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 31, 32, and 33, and wherein said light chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 34, 35, and 36.

In one embodiment, this invention provides humanized antibodies or fragments thereof which comprise a V_H having an amino acid sequence selected from the group of SEQ ID NOS: 66 and 72. In a preferred embodiment, a humanized 38SB19 antibody is provided which comprises a V_H having an amino acid sequence represented by SEQ ID NO: 66. In another preferred embodiment, a humanized 38SB31 antibody is provided which comprises a V_H having an amino acid sequence represented by SEQ ID NO: 72.

In another embodiment, this invention provides humanized antibodies or fragments thereof which comprise a V_L having an amino acid sequence selected from the group of SEQ ID NOS: 62, 64, 68, and 70. In a preferred embodiment, a humanized 38SB19 antibody is provided which comprises a V_L having an amino acid sequence chosen from the group of SEQ ID NOS: 62 and 64. In another preferred embodiment, a humanized 38SB31 antibody is provided which comprises a V_L having an amino acid sequence chosen from the group of SEQ ID NOS: 68 and 70.

Each of the humanized versions of the 38SB13, 38SB18, 38SB19, 38SB30, 38SB31, and 38SB39 antibodies has been shown to be particularly advantageous as an anticancer agent. The preparation, physical properties and beneficial pharmacological properties thereof are described in WO 2008/047242, which is incorporated by reference herein in its entirety. Generally, the doses used for treating human beings, which depend on factors distinctive to the subject to be treated, are between 1 and 150 mg/kg administered orally or between 1 and 150 mg/kg administered intravenously.

Melphalan (brand name, Alkeran™), is a chemotherapy drug belonging to the class of nitrogen mustard alkylating agents. Otherwise known as L-Phenylalanine Mustard, or L-PAM, melphalan is a phenylalanine derivative of mechlorethamine and is a bifunctional alkylating agent. Formation of carbonium intermediates from each of the two bis-2-chloroethyl groups enables alkylation through covalent binding with the 7-nitrogen of guanine on DNA, cross-linking two DNA strands and thereby preventing cell replication. Melphalan is used primarily to treat multiple myeloma and ovarian cancer, and occasionally malignant melanoma. It is usually administered orally or intravenously.

One aspect of the invention is a pharmaceutical composition comprising an anti-CD38 antibody in combination with at least melphalan. Since the activity of the products depends on the doses used, it is thus possible to use lower doses and to increase the

activity while decreasing the toxicity phenomena. The improved efficacy of a combination according to the invention may be demonstrated by determination of the therapeutic synergy. A combination manifests therapeutic synergy if it is therapeutically superior to the best agent of the study used alone at its maximum tolerated dose or at its highest dose tested when toxicity cannot be reached in the animal species.

This efficacy may be quantified, for example, by the $\log_{10}$ cells kill, which is determined according to the following formula:

$$\log_{10} \text{ cell kill} = T\text{-}C \text{ (days)}/3.32\times T_d$$

in which T - C represents the tumor growth delay, which is the median time in days for the tumors of the treated group (T) and the tumors of the control group (C) to reach a predetermined value (1 g for example), and T_d represents the time in days needed for the volume of the tumor to double in the control animals [T.H. Corbett et al., *Cancer*, **40**: 2660-2680 (1977); F.M. Schabel et al., *Cancer Drug Development*, Part B, Methods in Cancer Research, **17**: 3-51, New York, Academic Press Inc. (1979)]. A product is considered to be active if $\log_{10}$ cell kill is greater than or equal to 0.7. A product is considered to be very active if $\log_{10}$ cell kill is greater than 2.8.

The combination will manifest therapeutic synergy when the $\log_{10}$ cell kill is greater than the value of the $\log_{10}$ cell kill of the best constituent when it is administered alone and used at its maximum tolerated dose or at its highest dose tested.

The efficacy of the combinations on solid tumors may be determined experimentally in the following manner:

The animals subjected to the experiment, generally mice, are subcutaneously grafted bilaterally with 30 to 60 mg of a tumor fragment on day 0. The animals bearing tumors are randomized based on their tumor size before being subjected to the various treatments and controls. Chemotherapy begins when tumors have reached a predetermined size after grafting, depending on the type of tumor, and the animals are observed every day. The different animal groups are weighed daily during treatment until the maximum weight loss is reached and subsequent full weight recovery has occurred. The groups are then weighed once or twice a week until the end of the trial.

The tumors are measured 1 to 5 times a week, depending on the tumor doubling time, until the tumor reaches approximately 2 g, or until the animal dies (if this occurs before the tumor reaches 2 g). The animals are necropsied immediately after euthanasia or death.

The antitumor activity is determined in accordance with the different parameters recorded.

Results obtained with combinations of hu38SB19 and melphalan used at their optimal doses are indicated hereunder as examples.

The present invention also relates, therefore, to pharmaceutical compositions containing the combinations according to the invention.

The constituents of which the combination are composed may be administered simultaneously, semi-simultaneously, separately, or spaced out over a period of time so as to obtain the maximum efficacy of the combination; it being possible for each administration to vary in its duration from a rapid administration to a continuous perfusion.

As a result, for the purposes of the present invention, the combinations are not exclusively limited to those which are obtained by physical association of the constituents, but also to those which permit a separate administration, which can be simultaneous or spaced out over a period of time.

The compositions according to the invention are preferably compositions which can be administered parentally. However, these compositions may be administered orally, subcutaneously or intraperitoneally in the case of localized regional therapies.

The compositions for parental administration are generally pharmaceutically acceptable, sterile solutions or suspensions which may optionally be prepared as required at the time of use. For the preparation of non-aqueous solutions or suspensions, natural vegetable oils such as olive oil, sesame oil or liquid petroleum or injectable organic esters such as ethyl oleate may be used. The sterile aqueous solutions can consist of a solution of the product in water. The aqueous solutions are suitable for intravenous administration provided the pH is appropriately adjusted and the solution is made

isotonic, for example with a sufficient amount of sodium chloride or glucose. The sterilization may be carried out by heating or by any other means which does not adversely affect the composition. The combinations may also take the form of liposomes or the form of an association with carriers as cyclodextrins or polyethylene glycols.

The compositions for oral, subcutaneous or intraperitoneal administration are preferably aqueous suspensions or solutions.

In the combinations according to the invention, the application of the constituents of which may be simultaneous, separate or spaced out over a period of time, it is especially advantageous for the amount of humanized anti-CD38 antibody to represent from 10 to 90 % by weight of the combination, it being possible for this content to vary in accordance with the nature of the associated substance, the efficacy sought and the nature of the cancer to be treated.

The combinations according to the invention are especially useful in the treatment of several types of cancers including (but not limited to) the following: carcinomas and adenocarcinomas, including that of the bladder, breast, colon, head-and-neck, prostate, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin, and including squamous cell carcinoma ; hematopoietic tumors of lymphoid lineage, including multiple myeloma, leukemia, acute and chronic lymphocytic (or lymphoid) leukemia, acute and chronic lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, non-Hodgkin lymphoma (e.g. Burkitt's lymphoma) ; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous (myeloid or myelocytic) leukemias, and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma, osteosarcoma and rhabdomyosarcoma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; and other tumors, including melanoma, teratocarcinoma, xeroderma pigmentosum, keratoacanthoma, and seminoma, and other cancers yet to be determined in which CD38 is expressed. They are mainly useful for treating leukemia, lymphoma and cancers resistant to the commonly used anticancer agents as the anti-CD38 antibodies of the invention have a unique mechanism of action.

Thus, the invention also encompasses the use of the above combinations for the manufacture of a medicament for the treatment of cancer.

Example:

In this example, the effectiveness of an anti-CD38 antibody/melphalan combination of the invention for tumor growth inhibition was demonstrated in vivo.

The first selected tumor model was a transplantable human multiple myeloma cell line, RPMI-8226, implanted in SCID mice.

Hu38SB19 was formulated in phosphate buffer saline without Ca^{2+} and Mg^{2+}, pH7.4. Hu38SB19 was administered intravenously on days 16, 19, 22, 25 after tumor implantation.

Melphalan was formulated in 5 % ethanol, 5 % polysorbate 80, 90 % sodium chloride 0.9 % in water. Melphalan was administered intravenously simultaneously to hu38SB19 on days 16, 19, 22, 25 after tumor implantation (except for the highest dose of the combination for which treatment was stopped on day 19 when toxicity was reached).

The results of the experiment are reported in Table 1.

Tumor doubling time = 3.2 days.

The following end points have been used:

- Toxicity was declared at dosages inducing ≥ 20 % body weight loss or ≥ 10 % drug death,
- Antitumor efficacy was determined by calculating log10 cell kill = (T-C) / [3.32 x (tumor doubling time in days)]
  (T meaning the median time of the treated mice to reach 1000 mg and C the median time (25.3 days) of the control mice to reach the same size; tumor-free survivors are excluded from these calculations and are tabulated separately). No antitumor activity was declared for log cell kill < 0.7, and the treatment was declared highly active for log cell kill ≥ 2.8
- Tumor Free Survivors (TFS): correspond to complete regression below the limit of palpation (63 mg) for the entire duration of the study (>100 days post last treatment).

- Therapeutic Synergism: a combination has therapeutic synergism if it is more active than the best single agent of the study (by at least 1 log cell kill).

Toxicity for melphalan alone was observed at a dose of 16.1 mg/kg/injection, with 3 drug-related deaths out of 5 mice, i.e. above the 10 % threshold. The highest nontoxic dose (HNTD) for melphalan was 10 mg/kg/inj (total injected dose = 40 mg/kg). The 10 mg/kg/inj dose was found to be active with a log cell kill of 1.9.

Regarding hu38SB19, the product was well tolerated at a dose of 40 mg/kg/inj. No toxicity was observed, which can be explained by the lack of cross-reactivity of the antibody with murine CD38. The log cell kill was 0.5, indicating that hu38DB19 was not active under these conditions.

The combination of melphalan at 16.1 mg/kg/inj and hu38SB19 at 40 mg/kg/inj was toxic, with 5 out of 5 drug-related deaths, i.e. very similar to what was observed with melphalan alone at the same dose. The dose of 10 mg/kg/inj of melphalan with 40 mg/kg/inj of hu38SB19 was considered to be the HNTD. At this dose, the log cell kill was 2.2, indicating that the combination was as active as the best agent, i.e. melphalan.

Another experiment was performed with LP1, a human multiple myeloma model highly sensitive to melphalan in comparison to RPMI-8226. The model was implanted on SCID mice.

Hu38SB19 was formulated in glucose 5 % in water. Hu38SB19 was administered intravenously on days 12, 15, 18, 21 after tumor implantation.

Melphalan was formulated in 5 % ethanol, 5 % polysorbate 80, 90 % sodium chloride 0.9 % in water. Melphalan was administered intravenously simultaneously to hu38SB19 on days 12, 15, 18, 21 after tumor implantation.

The results of the experiment are reported in Table 2.

Tumor doubling time = 1.5 days.

The following end points have been used:

- Toxicity was declared at dosages inducing ≥ 20 % body weight loss or ≥ 10 % drug death,
- Antitumor efficacy was determined by calculation of the log10 cell kill = (T-C) / [3.32 x (tumor doubling time in days)]

(T meaning the median time of the treated mice to reach 1000 mg and C the median time (16.8 days) of the control mice to reach the same size; tumor-free survivors are excluded from these calculations and are tabulated separately). No antitumor activity was declared for log cell kill < 0.7, and the treatment was declared highly active for log cell kill ≥ 2.8

- Therapeutic Synergism: A combination has therapeutic synergism if it is more active than the best single agent of the study (by at least 1 log cell kill).

Toxicity for melphalan alone was observed at a dose of 16.1 mg/kg/injection, with 33.6 % body weight loss at nadir on day 22, i.e. above the 20 % threshold and 4/5 drug-related deaths. The HNTD for melphalan was 10 mg/kg/inj (total injected dose = 40 mg/kg). The 10 mg/kg/inj dose was found to be highly active with a 9.3 log cell kill.

Regarding hu38SB19, the product was well tolerated at a dose of 40 mg/kg/inj. No toxicity was observed, which can be explained by the lack of cross-reactivity of the antibody with murine CD38. The log cell kill was 0.2, indicating that hu38DB19 was not active under these conditions.

The combination of melphalan at 16.1 mg/kg/inj and hu38SB19 at 40 mg/kg/inj was toxic, with 34.2 % body weight loss at nadir on day 22 and 5/5 drug-related deaths, i.e. very similar to what was observed with melphalan alone at the same dose. The dose of 10 mg/kg/inj of melphalan with 40 mg/kg/inj of hu38SB19 was considered to be the highest nontoxic dose. Remarkably, this dose displayed a high antitumor efficacy of 18.9 log cell kill (and 1/5 TFS on day 148) and demonstrated therapeutic synergism in comparison to the HNTD of the melphalan alone (9.3 log cell kill). Therapeutic synergism was maintained at lower dose levels of the combination in comparison to equitoxic doses of melphalan alone.

Table I: Combination of hu38SB19 and melphalan against advanced human multiple myeloma RPMI-8226 implanted in SCID female mice.

agent, route and dose in mg/kg/inj (total dose)		Schedule in days	Drug death	% BWC at nadir (day)	T-C in days (1000 mg)	log_{10} cell kill	Comments
hu38SB19 IV	melphalan IV						
40.0 (160.0)	-	16,19,22,25	0/5	+5.3 (26)	5.2	0.5	HDT - inactive
-	16.1 (32.2)	16,19	3/5	-33.4 (26)	-	-	Toxic
-	10.0 (40.0)	16,19,22,25	0/5	-12.2 (26)	20.0	1.9	HNTD, active
-	6.2 (24.8)		0/5	-3.5 (32)	9.5	0.9	Active
-	3.8 (15.2)		0/5	-1.2 (17)	8.5	0.8	Moderately active
40.0 (80.0)	16.1 (32.2)	16,19	5/5	-30.6 (22)	-	-	Toxic
40.0 (160.0)	10.0 (40.0)	16,19,22,25	0/5	-10.0 (26)	23.6	2.2	HNTD, active
40.0 (160.0)	6.2 (24.8)		0/5	-4.0 (28)	13.6	1.3	Active
40.0 (160.0)	3.8 (15.2)		0/5	-1.9 (17)	7.5	0.7	Marginally active

Tumor doubling time = 3.2 days. Median tumor size at start of therapy = 131-148 mg. Time for median tumor to reach 1000 mg = 25.3 days. Formulation: hu38SB19 = phosphate buffer saline without Ca^{2+} and Mg^{2+}, pH 7.4; melphalan = 5 % ethanol, 5 % polysorbate 80, 90 % sodium chloride 0.9 % in water. BWC = body weight change, T-C = tumor growth delay, HNTD = highest nontoxic dose, HDT = highest dose tested, IV = intravenous.

Table II: Combination of hu38SB19 and melphalan against advanced human multiple myeloma LP1 implanted in SCID female mice.

agent, route Dose in mg/kg/inj (total dose)		Schedule in days	% BWC at nadir (day)	T-C in days (1000 mg)	log_{10} cell kill gross	Comments
hu38SB19 IV	melphalan IV					
40.0 (160.0)	-	12, 15, 18, 21	+15.9 (22)	1.1	0.2	HDT-inactive
-	16.1 (48.3)[a]	12, 15, 18, 21	-33.6 (22)	NTBA	NTBA	Toxic 4/5 deaths
-	10.0 (40.0)		-14.6 (24)	46.5	9.3	HNTD, highly active
-	6.2 (24.8)		-3.0 (24)	17.4	3.5	Highly active
-	3.8 (15.2)		+7.0 (22)	4.1	0.8	Moderately active
40.0 (120.0)[a]	16.1 (48.3)[a]	12, 15, 18, 21	-34.2 (22)	NTBA	NTBA	Toxic 5/5 deaths
40.0 (160.0)	10.0 (40.0)		-11.2 (23)	94.1	18.9	HNTD, highly active 1/5 TFS
40.0 (160.0)	6.2 (24.8)		-5.1 (25)	48.3	9.7	Highly active
40.0 (160.0)	3.8 (15.2)		-0.8 (14)	22.0	4.4	Highly active

Tumor doubling time = 1.5 days. Median tumor size at start of therapy = 111-127 mg. Time for median tumor to reach 1000 mg = 16.8 days. Formulation: hu38SB19= glucose 5 % in water, melphalan: 5 % ethanol, 5 % polysorbate 80, 90 % sodium chloride 0.9 % in water. BWC = body weight change, T-C= tumor growth delay, HNTD = highest nontoxic dose, HDT = highest dose tested, TFS = tumor free survivors, NTBA = Non tumor bearing animals, IV= intravenous.[a] treatments were stopped on day 18 when toxicity was reached.

Claims

1. A pharmaceutical combination comprising an antibody specifically recognizing CD38 and at least melphalan, wherein said antibody is capable of killing a $CD38^+$ cell by apoptosis, antibody-dependent cell-mediated cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC).

2. The combination of claim 1, wherein said antibody is a humanized antibody.

3. The combination of claim 2 wherein said antibody comprises one or more complementarity-determining region having an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36.

4. The combination of claim 3 wherein said antibody comprises at least one heavy chain and at least one light chain, wherein said heavy chain has an amino acid sequence represented by SEQ ID NO: 66 and said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 13, 14, and 15, and wherein said light chain has an amino acid sequence selected from the group of SEQ ID NOS: 62 and 64, and said light chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 16, 17, and 18.

5. The combination of claim 3 wherein said antibody comprises at least one heavy chain and at least one light chain, wherein said heavy chain has an amino acid sequence represented by SEQ ID NO: 72 and said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 25, 26, and 27, and wherein said light chain has an amino acid sequence selected from the group of SEQ ID NOS: 68 and 70, and said light chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 28, 29, and 30.

6. Use of an antibody specifically recognizing CD38 for the preparation of a pharmaceutical combination according to claim 1 for the manufacture of a medicament for the treatment of cancer.

7. The use of claim 6, wherein said antibody is a humanized antibody.

8. The use of claim 7 wherein said antibody comprises one or more complementarity-determining region having an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36.

9. The use of claim 8 wherein said antibody comprises at least one heavy chain and at least one light chain, wherein said heavy chain has an amino acid sequence represented by SEQ ID NO: 66 and said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 13, 14, and 15, and wherein said light chain has an amino acid sequence selected from the group of SEQ ID NOS: 62 and 64, and said light chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 16, 17, and 18.

10. The use of claim 8 wherein said antibody comprises at least one heavy chain and at least one light chain, wherein said heavy chain has an amino acid sequence represented by SEQ ID NO: 72 and said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 25, 26, and 27, and wherein said light chain has an amino acid sequence selected from the group of SEQ ID NOS: 68 and 70, and said light chain comprises three sequential complementarity-determining regions having amino acid sequences represented by SEQ ID NOS: 28, 29, and 30.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2009/055389

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 A61K31/00 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, Sequence Search, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 2010	06/04/2010

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2009/055389

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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