ANTITUMOR COMBINATIONS CONTAINING ANTIBODIES RECOGNIZING SPECIFICALLY CD38 AND BORTEZOMIB.

The present invention relates to combinations of monoclonal antibodies directed against CD38 and bortezomib 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 up regulated 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, herein incorporated by reference in its entirety. 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 cytoxicity (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.

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 (or 81), 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 herein incorporated by reference in its entirety).

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 herein incorporated by reference in its entirety. Humanized chimeric antibodies may 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 herein incorporated by reference in its entirety), chain shuffling (U.S. Pat. No. 5,565,332 herein incorporated by reference in its entirety), and identification of flexible residues (PCT/US2008/074381herein incorporated by reference in its entirety).. 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.

An 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 modeling 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, 36 and 81. In an 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 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 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 (or 81), 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 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 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 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 an 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 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 an 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 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, 10 and 100 mg/kg or 50 and 100 mg/kg administered orally or between 1 and 150 mg/kg, 10 and 100 mg/kg or 50 and 100 mg/kg administered intravenously.

Bortezomib is a boronic acid dipeptide ([(1*R*)-3-methyl-1-({(2*S*)-3-phenyl-2-[(pyrazin-2-ylcarbonyl)amino]propanoyl}amino)butyl]boronic acid; brand name: Velcade™), with antitumoral activity (Richardson et al., *N. Engl. J. Med.*, **352**: 2487-2498, 2005). Bortezomib is a highly specific, reversible inhibitor of the 26 S proteasome. In normal cells, the proteasome regulates protein expression and function by degradation of ubiquitinylated proteins; in addition, it is involved in the elimination of misfolded proteins. The mechanism of action of bortezomib involves stabilization of NF-κB, p21, p27, p53, Bid, and Bax, inhibition of caveolin-1 activation, and activation of JNK as well as the endoplasmic reticulum stress response (Boccadoro et al. *Cancer Cell Int.*, **5**(1): 18, 2005). While multiple mechanisms are likely to be involved, proteasome inhibition may thus permit activation of programmed cell death in neoplastic cells dependent upon suppression of pro-apoptotic pathways. Specifically, the agent inhibits nuclear factor (NF)-kappaB, thereby interfering with NF-kappaB-mediated cell survival, tumor growth, and angiogenesis. Bortezomib is thus used for treating multiple myeloma and mantle cell lymphoma. Bortezomib is usually administered intravenously.

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 bortezomib.

Moreover, since the activity of the products depends on the doses used, it is 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.

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This efficacy may be quantified, for example, by the $\log_{10}$ cell 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 have reached 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 or equal to 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 administered alone 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 unilaterally or bilaterally with tumor cells or 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 750 mg, or until the animal dies (if this occurs before the tumor reaches 750 mg). 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 bortezomib 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.
The timing between at least one administration of antibody specifically recognizing CD38, and at least one administration of bortezomib is approximately 1 month or less or approximately 2 weeks or less, or approximately 1 day or less.

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 may be 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: carcinoma and adenocarcinoma, 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) ; 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.

In an embodiment the antitumor efficacy of the combination is about 11-42% T/C. T/C(%) is the ratio Median tumor volume of the treated / Median tumor volume of the control x 100.

In another embodiment the antitumor efficacy of the combination is about 0-10% T/C.

Thus, an aspect of the invention is the above combinations for use in the treatment of cancer. In another aspect, the invention also encompasses the use of the above combinations for the manufacture of a medicament for the treatment of cancer.

Another embodiment of the invention is an article of manufacture comprising:

a) a packaging material

b) a combination of an antibody specifically recognizing CD38 and at least bortezomib, wherein said antibody is capable of killing a $CD38^+$ cell by apoptosis, antibody-dependent cell-mediated cytotoxicity (ADCC), and complement-dependent cytoxicity (CDC), and

c) a label or package insert contained within said packaging material indicting that said combination thereof is effective for treating cancer.

Example:

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

Test Article Administration:

Hu38SB19 and Bortezomib treatments were started thirty four days after cell inoculation and doses were calculated based on individual animal body weights determined from the last measurement taken before dosing. Administration of PBS and hu38SB19 antibody was carried out through intraperitoneal (IP) injection and bortezomib by intravenous (IV) injection via the tail vein, using a 1.0 mL syringe fitted with a 27 gauge, ½ inch needle.

Specific Study Design:

The NCI-H929 multiple myeloma subcutaneous xenograft model was initiated in female CB.17 SCID mice by subcutaneous injection of 1 x 10^7 cells per mouse, suspended in 0.1 ml serum-free culture medium.

Thirty-three days after tumor cell inoculation, sixty-four mice were randomized into 8 groups (n=8) by tumor volume. The tumor volumes ranged from 86.7 to 355.2 (189.0 ± 74.3, Median ± SD) mm^3. Body weights of the mice ranged from 17.2 to 22.4 (19.4 ± 1.1, Median ± SD) grams. Mice in each group were identified by a puncture in the ear.

Treatment groups included PBS control (200 µL/mouse/treatment), hu38SB19 antibody (5.0 mg/kg/treatment), bortezomib (1.0, 0.6 and 0.4 mg/kg/treatment) and three combination groups with hu38SB19 (5.0 mg/kg/treatment) and bortezomib (1.0, 0.6 and 0.4 mg/kg/treatment). PBS, hu38SB19 and bortezomib were administered twice per week for one week (days 34 and 37 for a total of 2 treatments). Treatments were started on day 34 post inoculation of tumor cells.

Tumor volumes were measured one to two times weekly in three dimensions using a caliper. The tumor volume was expressed in mm^3 (or mg) using the formula:
V = Length x Width x Height x ½.

Body weights were measured twice per week as a rough index of test agent toxicity. Tumor volume and body weight were determined and recorded by Labcat software.

Results:

The anti-tumor activity of the hu38SB19 antibody and bortezomib alone and in combination was evaluated in female SCID mice bearing subcutaneous NCI-H929 tumor xenografts, a human multiple myeloma model.

The following end points were used:

Toxicity was declared at dosages inducing ≥ 20% body weight loss or ≥ 10 % drug death,

Antitumor efficacy was determined by calculating:

T/C(%) = Median tumor volume of the treated / Median tumor volume of the control x 100,

wherein a T/C ≤ 42% is the minimum level of anti-tumor activity. A T/C <10% is considered a high anti-tumor activity level;

$\log_{10}$ cell kill = (T-C value in days) / (3.32 x Td in days)]

wherein T, C, and Td are as defined above. No antitumor activity was declared for log cell kill < 0.7.

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.

Hu38SB19 antibody alone at 5.0 mg/kg/injection had marginal anti-tumor activity with a %T/C of 41% and a $\log_{10}$ cell kill of 0.5 (see Table 1).

Likewise, bortezomib alone was inactive for doses of 0.4 and 0.6 mg/kg/injection, with T/C of 45 and 75%, and $\log_{10}$ cell kill of 0.1 and 0.3, respectively. No TFS was observed at 0.6 mg/kg/injection, whereas a single TFS was obtained with 0.4 mg/kg/injection. Only, the highest dosed tested of bortezomib (1.0 mg/kg/injection) was somewhat active with a %T/C of 15% and 2 TFS obtained; however, the $\log_{10}$ cell kill was only of 0.6.

On the other hand, the combination of hu38SB19 antibody (5.0 mg/kg/injection) and bortezomib is active even at the lowest doses. Indeed, when huSB3819 is administered with 0.4 and 0.6 mg/kg/injection, the %T/C is 8.7 and 11 %, respectively. In both cases, the $\log_{10}$ cell kill was around 1 (0.9 and 1.5.respectively), and 3 TFS, out of 8 mice, obtained with the combinations.

Thus, the combination of hu38SB19 antibody (5 mg/kg/injection) and bortezomib (0.4, 0.6 and 1.0 mg/kg/injection) demonstrated greater activity than the best single agent of the study (bortezomib).

We conclude that the combination of the hu38SB19 antibody and bortezomib show therapeutic synergism.

Table I: Combination of hu38SB19 and bortezomib against multiple myeloma NCI-H929 implanted in SCID mice.

Agent, route	Dosage in mg/kg/ Injection (total dose)	Schedule in days	Average body weight loss in % per mouse at nadir (day of nadir)	Time for median tumor to reach 750 mg in days	%T/C	T-C in days	log cell kill	TFS at day 176	Comments
PBS			*	62					
Hu38SB19, IP	5 (10)	34, 37	*	92	41	30	0.5	2/8	Marginaly Active
	1.0 (2.0)	34, 37	7.0 (d36)	99	15	37	0.6	2/8	Active
Bortezomib, IV	0.6 (1.2)	34, 37	0.6 (d40)	80	45	18	0.3	0/8	Inactive
	0.4 (0.8)	34, 37	0.8 (d36)	69	74	7	0.1	1/8	Inactive
Hu38SB19, IP & bortezomib, IV	5 (10) & 1.0 (2.0)	34, 37	7.5 (d36)	142	0	80	1.2	6/8	Highly Active
	5 (10) & 0.6 (1.2)	34, 37	2.0 (d40)	156	11	94	1.5	3/8	Active
	5 (10) & 0.4 (0.8)	34, 37	4.7 (d40)	122	8.7	60	0.9	3/8	Highly Active

NCI-H929 tumor cells implanted sc. 1.0 $x10^7$ cells/mouse. Tumor doubling time = 19.5 days. Median tumor size at start of therapy = 153-201 mg., T-C = tumor growth delay, TFS = tumor free survivors,. Formulations: hu38SB19 = phosphate buffer saline without Ca^{2+} and Mg^{2+}, pH7.4, bortezomib = glucose 5% in water.

Claims

1. A pharmaceutical combination comprising an antibody specifically recognizing CD38 and at least bortezomib, wherein said antibody is capable of killing a $CD38^+$ cell by apoptosis, antibody-dependent cell-mediated cytotoxicity (ADCC), and complement-dependent cytoxicity (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, 36 and 81.

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 SEQ ID NO: 66 and said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences SEQ ID NOS: 13, 14 (or 81), and 15, and wherein said light chain amino acid sequence is SEQ ID NOS: 62 or 64, and said light chain comprises three sequential complementarity-determining regions having amino acid sequences SEQ ID NOS: 16, 17 and18.

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 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 SEQ ID NOS: 28, 29, and 30.

6. The combination according to claim 1 for use in the treatment of cancer, wherein the antitumor efficacy of the combination is from about 11 to about 42% T/C.

7. The combination according to claim 1 for use in the treatment of cancer, wherein the antitumor efficacy of the combination is from about 0 to about 10% T/C.

8. A pharmaceutical combination according to claim 1 for use in the treatment of cancer.

9. The combination of claim 8, wherein said antibody is a humanized antibody.

10. The combination of claim 9 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, 36 and 81.

11. The combination of claim 9 wherein said antibody comprises at least one heavy chain and at least one light chain, wherein said heavy chain has an amino acid sequence SEQ ID NO: 66 and said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences SEQ ID NOS: 13, 14 (or 81), 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 SEQ ID NOS: 16, 17, and 18.

12. The combination of claim 11 wherein said antibody comprises at least one heavy chain and at least one light chain, wherein said heavy chain has an amino acid sequence SEQ ID NO: 72 and said heavy chain comprises three sequential complementarity-determining regions having amino acid sequences SEQ ID NOS: 25, 26, and 27, and wherein said light chain has an

amino acid sequence of SEQ ID NOS: 68 or 70, and said light chain comprises three sequential complementarity-determining regions having amino acid sequences SEQ ID NOS: 28, 29, and 30.

13. The combination according to claims 8 to 12 wherein the antibody specifically recognizing CD38 and bortezomib are administered separately.

14. The combination according to claims 8 to 12 wherein the antibody specifically recognizing CD38 and bortezomib are administered semi-simultaneously.

15. The combination according to claims 8 to 12 wherein the administration of antibody specifically recognizing CD38 and bortezomib are spaced out over a period of time so as to obtain the maximum efficacy of the combination.

16. An article of manufacture comprising:

a) a packaging material

b) a combination of an antibody specifically recognizing CD38 and at least bortezomib, wherein said antibody is capable of killing a $CD38^+$ cell by apoptosis, antibody-dependent cell-mediated cytotoxicity (ADCC), and complement-dependent cytoxicity (CDC), and

c) a label or package insert contained within said packaging material indicting that said combination thereof is effective for treating cancer.