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".

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/US2008/074381~~herein 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

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.

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.

Claims

1. A pharmaceutical combination comprising an antibody and at least bortezomib, wherein said antibody specifically recognizes CD38 and wherein said antibody is capable of killing a $CD38^+$ cell by apoptosis, antibody-dependent cell-mediated cytotoxicity (ADCC), and complement-dependent cytoxicity (CDC), and wherein said antibody comprises at least one heavy chain and at least one light chain, wherein

(i) 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;

(ii) 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;

(iii) 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;

(iv) 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;

(v) 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; or

(vi) 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.

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

3. The combination of claim 1 or 2 wherein said heavy chain comprises an amino acid sequence SEQ ID NO: 66 and wherein said light chain comprises an amino acid sequence selected from the group of SEQ ID NOS: 62 or 64.

4. The combination of claim 1 or 2 wherein said heavy chain comprises an amino acid sequence represented by SEQ ID NO: 72 and wherein said light chain comprises an amino acid sequence selected from the group of SEQ ID NOS: 68 and 70.

5. A pharmaceutical combination comprising an antibody and at least bortezomib, wherein said antibody specifically recognizes CD38 and wherein said antibody consists of:

- a light chain comprising an amino acid sequence of SEQ ID NOS: 62; and
- a heavy chain comprising an amino acid sequence of SEQ ID NO: 66.

6. The combination according to any one of claim 1 to 5 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 any one of claims 1 to 5 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 any one of claims 1 to 7 for use in the treatment of cancer.

9. The combination according to any one of claims 6 to 8 wherein the antibody specifically recognizing CD38 and bortezomib are to be administered separately.

10. The combination according to any one of claim 6 to 8 wherein the antibody specifically recognizing CD38 and bortezomib are to be administered semi-simultaneously.

11. The combination according to any one of claims 6 to 9 wherein the antibody specifically recognizing CD38 and bortezomib are to be administered spaced out over a period of time so as to obtain the maximum efficacy of the combination.

12. An article of manufacture comprising:

a) a packaging material

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

(i) 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;

(ii) 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;

(iii) 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;

(iv) 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;

(v) 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; or

(vi) 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, and

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