very efficacious (Polson AG, Williams M et al., Anti-CD22-MCC-DM1: an antibody-drug conjugate with a stable linker for the treatment of non-Hodgkin's lymphoma. Leukemia. 2010 Sep;24(9):1566-73. Epub 2010 Jul 1). Monomethyl Auristatin E (MMAE), a synthetic analog of the natural product dolastin 10, is a microtubule inhibitor. This cytotoxic drug is conjugated to the anti-CD22-Antibody. After binding of the ADC to the tumor cell, it is internalized and degraded releasing MMAE and therefore inducing cell death.

[018] There exists a need in the art for additional drugs to treat various B cell-related cancers such as lymphomas such as non-Hodgkin's lymphoma and other B cell proliferative disorders. Particularly useful drugs for this purpose include B cell targeted anti-CD22 antibody-drug conjugates having a significantly lower toxicity, yet useful therapeutic efficiency. These and other limitations and problems of the past are addressed by the present invention.

[019] The recitation of any reference in this application is not an admission that the reference is prior art to this application. All references cited herein, including patents, patent applications and publications, are incorporated by reference in their entirety.

Summary

[020] We have now found out that the combination of an afucosylated anti-CD20 antibody with a CD22 antibody-drug conjugate showed significantly enhanced antiproliferative effects.

[021] The disclosure relates to an afucosylated anti-CD20 antibody with an amount of fucose of 60% or less of the total amount of oligosaccharides (sugars) at Asn297, for the treatment of cancer in combination with a CD22 antibody-drug conjugate.

[022] The disclosure relates to the use of an afucosylated anti-CD20 antibody with an amount of fucose of 60% or less of the total amount of

oligosaccharides (sugars) at Asn297, for the manufacture of a medicament for the treatment of cancer in combination with a CD22 antibody-drug conjugate.

[023] The disclosure relates to a method of treatment of patient suffering from cancer by administering an afucosylated anti-CD20 antibody with an amount of fucose of 60% or less of the total amount of oligosaccharides (sugars) at Asn297, in combination with a CD22 antibody-drug conjugate, to a patient in the need of such treatment.

[024] In one embodiment, the amount of fucose is between 40% and 60% of the total amount of oligosaccharides (sugars) at Asn297. In another embodiment, the amount of fucose is 0% of the total amount of oligosaccharides (sugars) at Asn297.

[025] In one embodiment, the afucosylated anti-CD20 antibody is an IgG1 antibody. In another embodiment, said cancer is a CD20 expressing cancer, preferably a lymphoma or lymphocytic leukemia. In one embodiment said afucosylated anti-CD20 antibody is a humanized B-Ly1 antibody. In a specific embodiment, the anti-CD20 antibody is obinutuzumab (recommended INN, WHO Drug Information, Vol. 26, No. 4, 2012, p. 453). As used herein, obinutuzumab is synonymous for GA101 or RO5072759. This replaces all previous versions (e.g. Vol. 25, No. 1, 2011, p.75-76), and is formerly known as afutuzumab (recommended INN, WHO Drug Information, Vol. 23, No. 2, 2009, p. 176;Vol. 22, No. 2, 2008, p. 124).

[026] In one embodiment, the CD22 antibody in the CD22 antibody-drug conjugate comprises (a) an HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs:9, 10, 19-23, 32 and 33, and (b) at least one, two, three, four, or five HVRs selected from:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2;
(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;
(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6;

(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and

(5) an HVR-L3 comprising an amino acid sequence of SEQ ID NO:14.

[027] In one embodiment, the CD22 antibody-drug conjugate having the formula Ab-(L-D)p, wherein

(a) Ab is the CD22 antibody as defined herein;

(b) L is a linker;

(c) D is a drug moiety.

[028] In one embodiment of the CD22 antibody-drug conjugate having the formula Ab-(L-D)p, L is selected from 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB). In one embodiment of the CD22 antibody-drug conjugate having the formula Ab-(L-D)p, D is selected from the group consisting of auristatin, dolostantin, DM1, DM3, DM4, MMAE and MMAF.

[029] In one embodiment, said CD22 antibody-drug conjugate is anti-CD22-MC-vc-PAB-MMAE. In a specific embodiment, the anti-CD22 antibody in said conjugate is 10F4.v3.

[030] In one embodiment, the afucosylated anti-CD20 antibody binds CD20 with an KD of 10^{-8} M to 10^{-13} M.

[031] One embodiment of the disclosure is a composition comprising an afucosylated anti-CD20 antibody with an amount of fucose of 60% or less of the total amount of oligosaccharides (sugars) at Asn297, (in one embodiment an afucosylated humanized B-Ly1 antibody), and a CD22 antibody-drug conjugate. In one embodiment of the composition according to the disclosure, said anti-CD20 antibody is obinutuzumab. In one embodiment of the composition according to the

disclosure, said CD22 antibody in the CD22 antibody-drug conjugate comprises (a) an HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs:9, 10, 19-23, 32 and 33, and (b) at least one, two, three, four, or five HVRs selected from:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2;
(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;
(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6;
(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
(5) an HVR-L3 comprising an amino acid sequence of SEQ ID NO:14.

[032] In one embodiment of the composition according to the disclosure, said CD22 antibody binds to the same epitope as an antibody selected from ATCC PTA-7621 (10F4.4.1).

[033] In one embodiment of the composition according to the disclosure, one or more additional other cytotoxic, chemotherapeutic or anti-cancer agents, or compounds or ionizing radiation that enhance the effects of such agents are administered.

[034] In one embodiment of the composition according to the disclosure, the CD22 antibody-drug conjugate is having the formula Ab-(L-D)p, wherein

(a) Ab is the CD22 antibody as defined herein;

(b) L is a linker;
(c) D is a drug moiety.

[035] In one embodiment of the composition according to the disclosure, the CD22 antibody-drug conjugate is having the formula Ab-(L-D)p, wherein L is selected from 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-

maleimidomethyl) cyclohexane-1 carboxylate (SMCC), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB).

[036] In one embodiment of the composition according to the disclosure, the CD22 antibody-drug conjugate is having the formula Ab-(L-D)p, wherein D is selected from the group consisting of auristatin, dolostantin, DM1, DM3, DM4, MMAE and MMAF.

[037] In one embodiment of the composition according to the disclosure, the CD22 antibody-drug conjugate is anti-CD22-MC-vc-PAB-MMAE for the treatment of cancer. In a specific embodiment, the anti-CD22 antibody in said conjugate is 10F4.v3. An afucosylated anti-CD20 antibody with an amount of fucose of 60% or less of the total amount of oligosaccharides (sugars) at Asn297, for the treatment of cancer in combination with a CD22 antibody-drug conjugate.

[038] One embodiment of the disclosure is a method of treatment of patient suffering from cancer by administering an afucosylated anti-CD20 antibody with an amount of fucose of 60% or less of the total amount of oligosaccharides (sugars) at Asn297, in combination with a CD22 antibody-drug conjugate, to a patient in the need of such treatment.

[039] In one embodiment according to the disclosure, the method is characterized in that said cancer is a CD20 expressing cancer.

[040] In one embodiment according to the disclosure, the method is characterized in that said CD20 expressing cancer is a lymphoma or lymphocytic leukemia.

[041] In one embodiment according to the disclosure, the method is characterized in that said anti-CD20 antibody is a humanized B-Ly1 antibody.

[042] In one embodiment according to the disclosure, the method is characterized in that said anti-CD20 antibody is obinutuzumab.

[043] In one embodiment according to the disclosure, the method is characterized in that one or more additional other cytotoxic, chemotherapeutic or anti-cancer agents, or compounds or ionizing radiation that enhance the effects of such agents are administered.

[044] In one embodiment according to the disclosure, the method is characterized in that said CD22 antibody in the CD22 antibody-drug conjugate comprises (a) an HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs:9, 10, 19-23, 32 and 33, and (b) at least one, two, three, four, or five HVRs selected from:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2;
(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;
(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6;
(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
(5) an HVR-L3 comprising an amino acid sequence of SEQ ID NO:14.

[045] In one embodiment according to the disclosure, the method is characterized in that said CD22 antibody binds to the same epitope as an antibody selected from ATCC PTA-7621 (10F4.4.1).

[046] In one embodiment of the method according to the disclosure, the CD22 antibody-drug conjugate is having the formula Ab-(L-D)p, wherein

(a) Ab is the CD22 antibody as disclosed herein;
(b) L is a linker;
(c) D is a drug moiety.

[047] In one embodiment of the method according to the disclosure, the CD22 antibody-drug conjugate is having the formula Ab-(L-D)p, wherein L is selected from 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-

maleimidomethyl) cyclohexane-1 carboxylate (SMCC), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB).

[048] In one embodiment of the method according to the disclosure, the CD22 antibody-drug conjugate is having the formula Ab-(L-D)p, wherein D is selected from the group consisting of auristatin, dolostantin, DM1, DM3, DM4, MMAE and MMAF.

[049] In one embodiment of the method according to the disclosure, the CD22 antibody-drug conjugate is anti-CD22-MC-vc-PAB-MMAE.

[050] In one embodiment of the method according to the disclosure, the anti-CD22 antibody in said CD22 antibody-drug conjugate is 10F4.v3.

[051] One embodiment according to the disclosure is the use of an afucosylated anti-CD20 antibody with an amount of fucose of 60% or less of the total amount of oligosaccharides (sugars) at Asn297, for the manufacture of a medicament for the treatment of cancer in combination with a CD22 antibody-drug conjugate.

[052] One embodiment of the use according to the disclosure is characterized in that said cancer is a CD20 expressing cancer.

[053] One embodiment of the use according to the disclosure is characterized in that said CD20 expressing cancer is a lymphoma or lymphocytic leukemia.

[054] One embodiment of the use according to the disclosure is characterized in that said anti-CD20 antibody is a humanized B-Ly1 antibody.

[055] One embodiment of the use according to the disclosure is characterized in that said anti-CD20 antibody is obinutuzumab.

[056] One embodiment of the use according to the disclosure is characterized in that one or more additional other cytotoxic, chemotherapeutic or

anti-cancer agents, or compounds or ionizing radiation that enhance the effects of such agents are administered.

[057] One embodiment of the use according to the disclosure is characterized in that said CD22 antibody in the CD22 antibody-drug conjugate comprises (a) an HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs:9, 10, 19-23, 32 and 33, and (b) at least one, two, three, four, or five HVRs selected from:

(1) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:2;
(2) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:4;
(3) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:6;
(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:12; and
(5) an HVR-L3 comprising an amino acid sequence of SEQ ID NO:14.

[058] One embodiment of the use according to the disclosure is characterized in that said CD22 antibody binds to the same epitope as an antibody selected from ATCC PTA-7621 (10F4.4.1).

[059] One embodiment of the use according to the disclosure is characterized in that the CD22 antibody-drug conjugate is having the formula Ab-(L-D)p, wherein

(a) Ab is the CD22 antibody as disclosed herein;
(b) L is a linker;
(c) D is a drug moiety.

[060] One embodiment of the use according to the disclosure is characterized in that the CD22 antibody-drug conjugate is having the formula Ab-(L-D)p, wherein L is selected from 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit), alanine-phenylalanine (ala-phe), p-aminobenzyloxycarbonyl (PAB), N-Succinimidyl 4-(2-pyridylthio)

pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB).

[061] One embodiment of the use according to the disclosure is characterized in that the CD22 antibody-drug conjugate is having the formula Ab-(L-D)p, wherein D is selected from the group consisting of auristatin, dolostantin, DM1, DM3, DM4, MMAE and MMAF.

[062] One embodiment of the use according to the disclosure is characterized in that the CD22 antibody-drug conjugate is anti-CD22-MC-vc-PAB-MMAE.

[063] One embodiment of the use according to the disclosure is characterized in that the anti-CD22 antibody in said CD22 antibody-drug conjugate is 10F4.v3.

[064] One embodiment of the use according to the disclosure is characterized in that one or more additional other cytotoxic, chemotherapeutic or anti-cancer agents, or compounds or ionizing radiation that enhance the effects of such agents are administered.

[064a] One aspect of the disclosure is obinutuzumab, for use in the treatment of cancer in combination with a CD22 antibody-drug conjugate anti-CD22-MC-vc-PAB-MMAE, wherein the anti-CD22 antibody in said CD22 antibody-drug conjugate comprises the light chain variable domain SEQ ID NO: 50 and the heavy chain variable domain SEQ ID NO: 51.

[064b] Another aspect of the disclosure is a composition for use in the treatment of cancer comprising obinutuzumab and a CD22 antibody-drug conjugate anti-CD22-MC-vc-PAB-MMAE, wherein the anti-CD22 antibody in said CD22 antibody-drug conjugate comprises the light chain variable domain SEQ ID NO: 50 and the heavy chain variable domain SEQ ID NO: 51.

[064c] Another aspect of the disclosure is use of obinutuzumab in the manufacture of a medicament for the treatment of a CD20 expressing cancer in combination with a CD22 antibody-drug conjugate anti-CD22-MC-vc-PAB-MMAE, wherein the anti-CD22 antibody in said CD22 antibody-drug conjugate

comprises the light chain variable domain SEQ ID NO: 50 and the heavy chain variable domain SEQ ID NO: 51.

Description of the Figures

[065] **Figure 1:** Effect of obinutuzumab (GA101), rituximab, anti-CD22-ADC and the combinations of CD22 with GA101 or rituximab on a WSU-DLCL2 lymphoma model in SCID FcgR3a transgenic mice

[066] **Figure 2:** Statistical analysis of the data in Figure 1 by Pairwise Wilcoxon and Pairwise Log-Rank test

[067] **Figure 3:** **Figures 3A-3B**: **Figure 3A** depicts the amino acid sequence of the heavy chain variable region of murine 10F4 anti-CD22 antibody of

comprises the light chain variable domain SEQ ID NO: 50 and the heavy chain variable domain SEQ ID NO: 51.

Description of the Figures

[065] **Figure 1:** Effect of obinutuzumab (GA101), rituximab, anti-CD22-ADC and the combinations of CD22 with GA101 or rituximab on a WSU-DLCL2 lymphoma model in SCID FcgR3a transgenic mice

[066] **Figure 2:** Statistical analysis of the data in Figure 1 by Pairwise Wilcoxon and Pairwise Log-Rank test

[067] **Figure 3:** **Figures 3A-3B**: **Figure 3A** depicts the amino acid sequence of the heavy chain variable region of murine 10F4 anti-CD22 antibody of

Claims

1. Obinutuzumab, for use in the treatment of cancer in combination with a CD22 antibody-drug conjugate anti-CD22-MC-vc-PAB-MMAE, wherein the anti-CD22 antibody in said CD22 antibody-drug conjugate comprises the light chain variable domain SEQ ID NO: 50 and the heavy chain variable domain SEQ ID NO: 51.

2. Obinutuzumab according to claim 1, characterized in that said cancer is a CD20 expressing cancer.

3. Obinutuzumab according to any one of claims 1 to 2, characterized in that said CD20 expressing cancer is a lymphoma or lymphocytic leukemia.

4. Obinutuzumab according to any one of claims 1 to 3, characterized in that one or more additional other cytotoxic, chemotherapeutic or anti-cancer agents, or compounds or ionizing radiation that enhance the effects of such agents are administered.

5. A composition for use in the treatment of cancer comprising obinutuzumab and a CD22 antibody-drug conjugate anti-CD22-MC-vc-PAB-MMAE, wherein the anti-CD22 antibody in said CD22 antibody-drug conjugate comprises the light chain variable domain SEQ ID NO: 50 and the heavy chain variable domain SEQ ID NO: 51.

6. The composition according to claim 5, characterized in that one or more additional other cytotoxic, chemotherapeutic or anti-cancer agents, or compounds or ionizing radiation that enhance the effects of such agents are administered.

7. Use of obinutuzumab in the manufacture of a medicament for the treatment of a CD20 expressing cancer in combination with a CD22 antibody-drug conjugate anti-CD22-MC-vc-PAB-MMAE, wherein the anti-CD22 antibody in said CD22 antibody-drug conjugate comprises the light chain variable domain SEQ ID NO: 50 and the heavy chain variable domain SEQ ID NO: 51.

8. The use according to claim 7, characterized in that said CD20 expressing cancer is a lymphoma or lymphocytic leukemia.

9. The use according to claim 7 or 8, characterized in that one or more additional other cytotoxic, chemotherapeutic or anti-cancer agents, or compounds or ionizing radiation that enhance the effects of such agents are to be administered.