

SUMMARY

[0017] The present disclosure is directed to molecules (*e.g.*, an antibody, a diabody, an scFv, an antibody, a TandAb, *etc.*) capable of binding an epitope of human CD16 (a “**CD16 Binding Molecule**”). The present disclosure is further directed to CD16 Binding Molecules that are capable of binding an epitope of human CD16 and one or more epitope(s) of a Disease Antigen (“**DA**”) (*e.g.*, a “**CD16 x DA Binding Molecule**”). The present disclosure is particularly directed to such **CD16 x DA Binding Molecules** that are antibodies, or that comprise an Epitope Binding Domain thereof, or are diabodies (including DART® diabodies), bispecific antibodies, TandAbs, other multispecific binding molecules (*e.g.*, trivalent TRIDENT™ molecules), *etc.* The disclosure particularly concerns **CD16 x DA Binding Molecules** that are capable of binding a Disease Antigen that is a Cancer Antigen or a Pathogen-Associated Antigen in addition to being able to bind CD16. The disclosure particularly concerns the use of such **CD16** and **CD16 x DA Binding Molecules** in the treatment of cancer and pathogen-associated diseases. The present disclosure is also directed to pharmaceutical compositions that comprise such molecule(s).

[0018] In one aspect, the disclosure refers to a **CD16 x Disease Antigen (CD16 x DA) Binding Molecule** comprising a CD16 Binding Domain capable of binding an epitope of CD16 and also a Disease Antigen-Binding Domain capable of binding an epitope of a Disease Antigen, wherein said CD16 Binding Domain comprises one or more of:

- (I)
 - (A) a CDR_H1 Domain comprising the amino acid sequence of **SEQ ID NO:66**;
 - (B) a CDR_H2 Domain comprising the amino acid sequence of **SEQ ID NO:67**;
 - (C) a CDR_H3 Domain comprising the amino acid sequence of **SEQ ID NO:68** or **SEQ ID NO:60**;
 - (D) a CDR_L1 Domain comprising the amino acid sequence of **SEQ ID NO:69** or **SEQ ID NO:74**;
 - (E) a CDR_L2 Domain comprising the amino acid sequence of **SEQ ID NO:70**; and
 - (F) a CDR_L3 Domain comprising the amino acid sequence of **SEQ ID NO:71** or **SEQ ID NO:61**;
- (II)
 - (A) a CDR_H1 Domain comprising the amino acid sequence of **SEQ ID NO:77**;
 - (B) a CDR_H2 Domain comprising the amino acid sequence of **SEQ ID NO:78**;
 - (C) a CDR_H3 Domain comprising the amino acid sequence of **SEQ ID NO:79**;
 - (D) a CDR_L1 Domain comprising the amino acid sequence of **SEQ ID NO:80**;

- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:81**;
and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:82**;
- (III) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72**, **SEQ ID NO:83**, **SEQ ID NO:84**, or **SEQ ID NO:58**;
- (IV) a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**, **SEQ ID NO:85**, or **SEQ ID NO:59**;
- (V) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**; and
- (VI) a VH Domain comprising the amino acid sequence of **SEQ ID NO:83** or **SEQ ID NO:84** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:85**
- (VII) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**;
- (VIII) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**; and
- (IX) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**.

[0019] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecule, wherein the Molecule is a bispecific antibody, a bispecific diabody, a bispecific TandAb, a bispecific trivalent molecule, or a bispecific CAR.

[0020] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the Molecule is capable of binding more than one Disease Antigen and/or more than one epitope of CD16.

[0021] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the CD16 Binding Domain comprises:

- (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:66**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:67**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:68**,
or **SEQ ID NO:60**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:69**
or **SEQ ID NO:74**;

- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:70**;
and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:71**,
or **SEQ ID NO:61**.

[0022] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the CD16 Binding Domain comprises:

- (A) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72**, or **SEQ ID NO:58**;
- (B) a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**, or **SEQ ID NO:59**; or
- (C) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**
- (D) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**;
- (E) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**; or
- (F) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**.

[0023] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the CD16 Binding Domain comprises:

- (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:77**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:78**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:79**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:80**;
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:81**;
and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:82**.

[0024] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the CD16 Binding Domain comprises:

- (A) a VH Domain comprising the amino acid sequence of **SEQ ID NO:83**, or **SEQ ID NO:84**;
- (B) a VL Domain comprising the amino acid sequence of or **SEQ ID NO:85**;
or

- (C) a VH Domain comprising the amino acid sequence of **SEQ ID NO:83** or **SEQ ID NO:84** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:85**.

[0025] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the Disease Antigen is a Cancer Antigen and the disease is cancer.

[0026] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the cancer is selected from the group consisting of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, hairy cell leukemia, Burkett's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, non-Hodgkin's lymphoma, small lymphocytic lymphoma, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, testicular cancer, and uterine cancer.

[0027] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the Cancer Antigen is selected from the group consisting of the Cancer Antigens: 19.9, 4.2, A33, ADAM-9, AH6, ALCAM, B1, B7-H3, BAGE, beta-catenin, blood group ALe^b/Le^y, Burkitt's lymphoma antigen-38.13, C14, CA125, Carboxypeptidase M, CD5, CD19, CD20, CD22, CD23, CD25, CD27, CD28, CD33, CD36, CD40/CD154, CD45, CD56, CD46, CD52, CD56, CD79a/CD79b, CD103, CD123, CD317, CDK4, CEA, CEACAM5/CEACAM6, CO17-1A, CO-43, CO-514, CTA-1, CTLA-4, Cytokeratin 8, D1.1, D156-22, DR5, E₁ series, EGFR, an Ephrin receptor, EphA2, Erb, GAGE, a GD2/GD3/GM2 ganglioside, GICA 19-9, gp100, Gp37, gp75, gpA33, HER2/neu, HMFG, human papillomavirus-E6/human papillomavirus-E7, HMW-MAA, I antigen, IL13R α 2, Integrin β 6, JAM-3, KID3, KID31, KS 1/4 pan-carcinoma antigen, L6/L20, LEA, LUCA-2, M1:22:25:8, M18, M39, MAGE, MART, mesothelin, MUC-1, MUM-1, Myl, N-acetylglucosaminyltransferase, neoglycoprotein, NS-10, OFA-1, OFA-2, Oncostatin M, p15, p97, PEM, PEMA, PIPA, PSA, PSMA, prostatic acid phosphate, R₂₄, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T cell receptor derived peptide, T₅A₇, TAG-72, TL5, TNF-receptor, TNF- γ receptor, TRA-1-85, a

Transferrin Receptor, 5T4, TSTA, VEGF, a VEGF Receptor, VEP8, VEP9, VIM-D5, and Y hapten, Le^y.

[0028] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the Disease Antigen is 5T4, B7-H3, CEACAM5/CEACAM6, CD19, CD123, EGRF, EphA2, HER2/neu, IL13R α 2 or VEGF.

[0029] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the Disease Antigen is a Pathogen-Associated Antigen.

[0030] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the Pathogen-Associated Antigen is selected from the group consisting of the Pathogen-Associated Antigens: Herpes Simplex Virus infected cell protein (ICP)47, Herpes Simplex Virus gD, Epstein-Barr Virus LMP-1, Epstein-Barr Virus LMP-2A, Epstein-Barr Virus LMP-2B, Human Immunodeficiency Virus gp160, Human Immunodeficiency Virus gp120, Human Immunodeficiency Virus gp41, *etc.*), Human Papillomavirus E6, Human Papillomavirus E7, human T-cell leukemia virus gp64, human T-cell leukemia virus gp46, and human T-cell leukemia virus gp21.

[0031] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the Disease Antigen is an HIV env antigen.

[0032] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the molecule is:

- (A) a diabody, said diabody being a covalently bonded complex that comprises two, or three, four or five polypeptide chains; or
- (B) a trivalent binding molecule, said trivalent binding molecule being a covalently bonded complex that comprises three, four or five polypeptide chains, or
- (C) a bispecific antibody.

[0033] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the molecule comprises an Fc Region.

[0034] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the Fc Region, is of the IgG1, IgG2, IgG3, or IgG4 isotype.

[0035] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules, wherein the Fc Region is a variant Fc Region that comprises:

- (A) one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR; and/or
- (B) one or more amino acid modifications that enhances the serum half-life of the variant Fc Region.

[0036] The disclosure additionally concerns the example of such **CD16 x Disease Antigen** Binding Molecules:

- (A) said modifications that reduces the affinity of the variant Fc Region for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A; and
- (B) said modifications that that enhances the serum half-life of the variant Fc Region comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein said numbering is that of the EU index as in Kabat.

[0037] In another aspect, the disclosure additionally refers to a CD16 Binding Molecule, that comprises:

- (A) a CDR_H1 Domain comprising the amino acid sequence of **SEQ ID NO:66**;
- (B) a CDR_H2 Domain comprising the amino acid sequence of **SEQ ID NO:67**;
- (C) a CDR_H3 Domain comprising the amino acid sequence of **SEQ ID NO:68**, or **SEQ ID NO:60**;
- (D) a CDR_L1 Domain comprising the amino acid sequence of **SEQ ID NO:69** or **SEQ ID NO:74**;
- (E) a CDR_L2 Domain comprising the amino acid sequence of **SEQ ID NO:70**; and
- (F) a CDR_L3 Domain comprising the amino acid sequence of **SEQ ID NO:71**, or **SEQ ID NO:61**.

[0038] The disclosure additionally concerns the example of such a CD16 Binding Molecule wherein the molecule comprises:

- (A) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72**, or **SEQ ID NO:58**;
- (B) a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**, or **SEQ ID NO:59**;
- (C) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**; or
- (D) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**;
- (E) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**; or
- (F) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**.

[0039] In another aspect, the disclosure additionally refers to a CD16 Binding Molecule that comprises:

- (A) a CDR_H1 Domain comprising the amino acid sequence of **SEQ ID NO:77**;
- (B) a CDR_H2 Domain comprising the amino acid sequence of **SEQ ID NO:78**;
- (C) a CDR_H3 Domain comprising the amino acid sequence of **SEQ ID NO:79**;
- (D) a CDR_L1 Domain comprising the amino acid sequence of **SEQ ID NO:80**;
- (E) a CDR_L2 Domain comprising the amino acid sequence of **SEQ ID NO:81**;
- and
- (F) a CDR_L3 Domain comprising the amino acid sequence of **SEQ ID NO:82**.

[0040] The disclosure additionally concerns the example of such CD16 Binding Molecule wherein the molecule comprises:

- (A) a VH Domain comprising the amino acid sequence of **SEQ ID NO:83** or **SEQ ID NO:84**;
- (B) a VL Domain comprising the amino acid sequence of or **SEQ ID NO:85**;
- or
- (C) a VH Domain comprising the amino acid sequence of **SEQ ID NO:83** or **SEQ ID NO:84** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:85**.

[0041] The disclosure additionally concerns the example of such CD16 Binding Molecules wherein the molecule is selected from the group consisting of: an antibody, a

multispecific antibody, a Fab' fragment, a F(ab')₂ fragment, a (Fv) fragment, a single-chain (scFv), a single-chain antibody, a disulfide-linked bispecific Fv (sdFv), a diabody, a trivalent binding molecule, and a CAR-T molecule.

[0042] The disclosure additionally concerns a pharmaceutical composition that comprises any of the above-described **CD16 x Disease Antigen** Binding Molecules, or CD16 Binding Molecules, and a pharmaceutically acceptable carrier. In one aspect, the present disclosure refers to a pharmaceutical composition that comprises any of the above-described **CD16 x Disease Antigen** Binding Molecules and a pharmaceutically acceptable carrier. In another aspect, the present disclosure refers to a pharmaceutical composition that comprises any of the above-described CD16 Binding Molecules and a pharmaceutically acceptable carrier.

[0043] The disclosure additionally concerns the use of the above-described pharmaceutical composition in the treatment of a disease characterized by the expression of the Disease Antigen. In one aspect, the present disclosure refers to use of any of the above-described CD16 x Disease Antigen Binding Molecules, or the above-described pharmaceutical composition, in the manufacture of a medicament for the treatment of a disease characterized by the expression of said Disease Antigen. In another aspect, the present disclosure refers to any of the above-described CD16 x Disease Antigen Binding Molecules, or the above-described pharmaceutical composition, for use in the treatment of a disease in a subject in need thereof characterized by the expression of said Disease Antigen.

[0044] The disclosure additionally concerns a method for the treatment of a disease characterized by the expression of the Disease Antigen, comprising administering to a subject in need thereof a therapeutically effective amount of the above-described pharmaceutical composition.

[0045] The disclosure additionally concerns the example of such use or method wherein the **CD16 x Disease Antigen** Binding Molecule is capable of binding more than one Disease Antigen and/or more than one epitope of CD16.

[0046] The disclosure additionally concerns such use or method wherein the **CD16 x Disease Antigen** Binding Molecule, wherein the Disease Antigen is a Cancer Antigen, and the disease is cancer.

[0047] The disclosure additionally concerns such use or method wherein the cancer is selected from the group consisting of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, hairy cell leukemia, Burkett's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, non-Hodgkin's lymphoma, small lymphocytic lymphoma, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, testicular cancer, and uterine cancer.

[0048] The disclosure additionally concerns such use or method wherein the Cancer Antigen is selected from the group consisting of the Cancer Antigens: 19.9, 4.2, A33, ADAM-9, AH6, ALCAM, B1, B7-H3, BAGE, beta-catenin, blood group ALe^b/Le^y, Burkitt's lymphoma antigen-38.13, C14, CA125, Carboxypeptidase M, CD5, CD19, CD20, CD22, CD23, CD25, CD27, CD28, CD33, CD36, CD40/CD154, CD45, CD56, CD46, CD52, CD56, CD79a/CD79b, CD103, CD123, CD317, CDK4, CEA, CEACAM5/CEACAM6, CO17-1A, CO-43, CO-514, CTA-1, CTLA-4, Cytokeratin 8, D1.1, D156-22, DR5, E₁ series, EGFR, an Ephrin receptor, EphA2, Erb, GAGE, a GD2/GD3/GM2 ganglioside, GICA 19-9, gp100, Gp37, gp75, gpA33, HER2/neu, HMFG, human papillomavirus-E6/human papillomavirus-E7, HMW-MAA, I antigen, IL13R α 2, Integrin β 6, JAM-3, KID3, KID31, KS 1/4 pan-carcinoma antigen, L6/L20, LEA, LUCA-2, M1:22:25:8, M18, M39, MAGE, MART, mesothelin, MUC-1, MUM-1, Myl, N-acetylglucosaminyltransferase, neoglycoprotein, NS-10, OFA-1, OFA-2, Oncostatin M, p15, p97, PEM, PEMA, PIPA, PSA, PSMA, prostatic acid phosphate, R₂₄, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T cell receptor derived peptide, T_{5A7}, TAG-72, TL5, TNF-receptor, TNF- γ receptor, TRA-1-85, a Transferrin Receptor, 5T4, TSTA, VEGF, a VEGF Receptor, VEP8, VEP9, VIM-D5, and Y hapten, Le^y.

[0049] The disclosure additionally concerns such use or method wherein the Disease Antigen is 5T4, B7-H3, CEACAM5/CEACAM6, CD19, CD123, EGFR, EphA2, HER2/neu, IL13R α 2 or VEGF.

[0050] The disclosure additionally concerns such use or method wherein the **CD16 x Disease Antigen** Binding Molecule, wherein the Disease Antigen is a Pathogen-Associated Antigen.

[0051] The disclosure additionally concerns such use or method wherein the Pathogen-Associated Antigen is selected from the group consisting of the Pathogen-Associated Antigens: Herpes Simplex Virus infected cell protein (ICP)47, Herpes Simplex Virus gD, Epstein-Barr Virus LMP-1, Epstein-Barr Virus LMP-2A, Epstein-Barr Virus LMP-2B, Human Immunodeficiency Virus gp160, Human Immunodeficiency Virus gp120, Human Immunodeficiency Virus gp41, *etc.*), Human Papillomavirus E6, Human Papillomavirus E7, human T-cell leukemia virus gp64, human T-cell leukemia virus gp46, and human T-cell leukemia virus gp21.

[0052] The disclosure additionally concerns such use or method wherein the Disease Antigen is an HIV env antigen.

BRIEF DESCRIPTION OF THE DRAWINGS:

[0053] **Figures 1A-1B** provide a schematic of a representative covalently bonded diabody having two epitope-binding sites composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain (alternative Heterodimer-Promoting Domains are provided below). A cysteine residue may be present in a linker (**Figure 1A**) and/or in the Heterodimer-Promoting Domain (**Figure 1B**). VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The wavy line (WWW) in this and all of the Figures providing schematic presentations of binding molecule domains represents one or more optional Heterodimer-Promoting Domains, that is/are preferably present.

[0054] **Figure 2** provides a schematic of a representative covalently bonded diabody molecule having two epitope-binding sites composed of two polypeptide chains, each having a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0055] **Figures 3A-3E** provide schematics showing representative covalently bonded tetravalent diabodies having four epitope-binding sites composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide chain of each pair possesses a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The two pairs of polypeptide chains may be same. In such embodiments, wherein the two pairs of polypeptide chains are the same and the VL and VH Domains recognize different epitopes (as shown in **Figures 3A-3B**), the resulting molecule possesses four epitope-binding sites and is bispecific and bivalent with respect to each bound epitope. In such embodiments, wherein the VL and VH Domains recognize the same epitope (*e.g.*, the same VL Domain CDRs and the same VH Domain CDRs are used on both chains) the resulting molecule possesses four epitope-binding sites and is monospecific and tetravalent with respect to a single epitope. Alternatively, the two pairs of polypeptides may be different. In such embodiments, wherein the two pairs of polypeptide chains are different and the VL and VH Domains of each pair of polypeptides recognize different epitopes (as

WHAT IS CLAIMED IS:

Claim 1. A **CD16 x Disease Antigen (CD16 x DA)** Binding Molecule comprising a CD16 Binding Domain capable of binding an epitope of CD16 and also a Disease Antigen-Binding Domain capable of binding an epitope of a Disease Antigen, wherein said CD16 Binding Domain comprises one or more of:

- (I) (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:66;**
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:67;**
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:68 or SEQ ID NO:60;**
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:69 or SEQ ID NO:74;**
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:70;** and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:71 or SEQ ID NO:61;**
- (II) (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:77;**
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:78;**
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:79;**
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:80;**
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:81;** and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:82;**
- (III) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72, SEQ ID NO:83, SEQ ID NO:84, or SEQ ID NO:58;**
- (IV) a VL Domain comprising the amino acid sequence of **SEQ ID NO:73, SEQ ID NO:85, or SEQ ID NO:59;**

- (V) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**;
- (VI) a VH Domain comprising the amino acid sequence of **SEQ ID NO:83** or **SEQ ID NO:84** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:85**;
- (VII) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**;
- (VIII) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**; and
- (IX) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**.

Claim 2. The **CD16 x Disease Antigen** Binding Molecule of claim 1, wherein said Molecule is a bispecific antibody, a bispecific diabody, a bispecific TandAb, a bispecific trivalent molecule, or a bispecific CAR.

Claim 3. The **CD16 x Disease Antigen** Binding Molecule of any one of claims 1-2, wherein said Molecule is capable of binding more than one Disease Antigen and/or more than one epitope of CD16.

Claim 4. The **CD16 x Disease Antigen** Binding Molecule of any one of claims 1-3, wherein said CD16 Binding Domain comprises:

- (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:66**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:67**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:68**, or **SEQ ID NO:60**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:69** or **SEQ ID NO:74**;

- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:70**; and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:71**, or **SEQ ID NO:61**.

Claim 5. The **CD16 x Disease Antigen** Binding Molecule of any one of claims 1-3, wherein said CD16 Binding Domain comprises:

- (A) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72**, or **SEQ ID NO:58**;
- (B) a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**, or **SEQ ID NO:59**;
- (C) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**;
- (D) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**;
- (E) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**; or
- (F) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**.

Claim 6. The **CD16 x Disease Antigen** Binding Molecule of any one of claims 1-3, wherein said CD16 Binding Domain comprises:

- (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:77**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:78**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:79**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:80**;

- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:81**; and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:82**.

Claim 7. The **CD16 x Disease Antigen** Binding Molecule of any one of claims 1-3, wherein said CD16 Binding Domain comprises:

- (A) a VH Domain comprising the amino acid sequence of **SEQ ID NO:83**, or **SEQ ID NO:84**;
- (B) a VL Domain comprising the amino acid sequence of or **SEQ ID NO:85**; or
- (C) a VH Domain comprising the amino acid sequence of **SEQ ID NO:83** or **SEQ ID NO:84** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:85**.

Claim 8. The **CD16 x Disease Antigen** Binding Molecule of any one of claims 1-7, wherein said Disease Antigen is a Cancer Antigen and said disease is cancer.

Claim 9. The **CD16 x Disease Antigen** Binding Molecule of claim 8, wherein said cancer is selected from the group consisting of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, hairy cell leukemia, Burkett's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, non-Hodgkin's lymphoma, small lymphocytic lymphoma, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, testicular cancer, and uterine cancer.

Claim 10. The **CD16 x Disease Antigen** Binding Molecule of claim 8 or 9, wherein said Cancer Antigen is selected from the group consisting of: 19.9, 4.2, A33, ADAM-9, AH6, ALCAM, B1, B7-H3, BAGE, beta-catenin, blood group ALe^b/Le^y, Burkitt's lymphoma antigen-38.13, C14, CA125,

Carboxypeptidase M, CD5, CD19, CD20, CD22, CD23, CD25, CD27, CD28, CD33, CD36, CD40/CD154, CD45, CD56, CD46, CD52, CD56, CD79a/CD79b, CD103, CD123, CD317, CDK4, CEA, CEACAM5/CEACAM6, CO17-1A, CO-43, CO-514, CTA-1, CTLA-4, Cytokeratin 8, D1.1, D156-22, DR5, E₁ series, EGFR, an Ephrin receptor, EphA2, Erb, GAGE, a GD2/GD3/GM2 ganglioside, GICA 19-9, gp100, Gp37, gp75, gpA33, HER2/neu, HMFG, human papillomavirus-E6/human papillomavirus-E7, HMW-MAA, I antigen, IL13R α 2, Integrin β 6, JAM-3, KID3, KID31, KS 1/4 pan-carcinoma antigen, L6/L20, LEA, LUCA-2, M1:22:25:8, M18, M39, MAGE, MART, mesothelin, MUC-1, MUM-1, Myl, N-acetylglucosaminyltransferase, neoglycoprotein, NS-10, OFA-1, OFA-2, Oncostatin M, p15, p97, PEM, PEMA, PIPA, PSA, PSMA, prostatic acid phosphate, R₂₄, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T cell receptor derived peptide, T_{5A7}, TAG-72, TL5, TNF-receptor, TNF- γ receptor, TRA-1-85, a Transferrin Receptor, 5T4, TSTA, VEGF, a VEGF Receptor, VEP8, VEP9, VIM-D5, and Y hapten, Le^y.

- Claim 11. The **CD16 x Disease Antigen** Binding Molecule of claim 10, wherein said Disease Antigen is 5T4, B7-H3, CEACAM5/CEACAM6, CD19, CD123, EGRF, EphA2, HER2/neu, IL13R α 2 or VEGF.
- Claim 12. The **CD16 x Disease Antigen** Binding Molecule of any one of claims 1-7, wherein said Disease Antigen is a Pathogen-Associated Antigen.
- Claim 13. The **CD16 x Disease Antigen** Binding Molecule of claim 12, wherein said Pathogen-Associated Antigen is selected from the group consisting of: Herpes Simplex Virus infected cell protein (ICP)47, Herpes Simplex Virus gD, Epstein-Barr Virus LMP-1, Epstein-Barr Virus LMP-2A, Epstein-Barr Virus LMP-2B, Human Immunodeficiency Virus gp160, Human Immunodeficiency Virus gp120, Human Immunodeficiency Virus gp41, *etc.*), Human Papillomavirus E6, Human Papillomavirus E7, human T-cell leukemia virus gp64, human T-cell leukemia virus gp46, and human T-cell leukemia virus gp21.

- Claim 14. The **CD16 x Disease Antigen** Binding Molecule of claim 12 or 13, wherein said Disease Antigen is an HIV env antigen.
- Claim 15. The **CD16 x Disease Antigen** Binding Molecule of any one of claims 1-14, wherein said molecule is:
- (A) a diabody, said diabody being a covalently bonded complex that comprises two, three, four or five polypeptide chains; or
 - (B) a trivalent binding molecule, said trivalent binding molecule being a covalently bonded complex that comprises three, four or five polypeptide chains, or
 - (C) a bispecific antibody.
- Claim 16. The **CD16 x Disease Antigen** Binding Molecule of claim 15, wherein said molecule comprises an Fc Region.
- Claim 17. The **CD16 x Disease Antigen** Binding Molecule of claim 16, wherein said Fc Region is of the IgG1, IgG2, IgG3, or IgG4 isotype.
- Claim 18. The **CD16 x Disease Antigen** Binding Molecule of claim 16 or 17, wherein said Fc Region is a variant Fc Region that comprises:
- (A) one or more amino acid modifications that reduces the affinity of the variant Fc Region for an Fc γ R; and/or
 - (B) one or more amino acid modifications that enhances the serum half-life of the variant Fc Region.
- Claim 19. The **CD16 x Disease Antigen** Binding Molecule of claim 18, wherein:
- (A) said one or more amino acid modifications that reduces the affinity of the variant Fc Region for an Fc γ R comprise the substitution of L234A; L235A; or L234A and L235A; and/or
 - (B) said one or more amino acid modifications that enhances the serum half-life of the variant Fc Region comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K,
- wherein said numbering is that of the EU index as in Kabat.

- Claim 20. A CD16 Binding Molecule, that comprises:
- (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:66**;
 - (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:67**;
 - (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:68**, or **SEQ ID NO:60**;
 - (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:69** or **SEQ ID NO:74**;
 - (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:70**; and
 - (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:71**, or **SEQ ID NO:61**.
- Claim 21. The CD16 Binding Molecule of claim 20, wherein said molecule comprises:
- (A) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72**, or **SEQ ID NO:58**;
 - (B) a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**, or **SEQ ID NO:59**;
 - (C) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**;
 - (D) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:73**;
 - (E) a VH Domain comprising the amino acid sequence of **SEQ ID NO:72** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**; or
 - (F) a VH Domain comprising the amino acid sequence of **SEQ ID NO:58** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:59**.

- Claim 22. A CD16 Binding Molecule that comprises:
- (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:77**;
 - (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:78**;
 - (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:79**;
 - (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:80**;
 - (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:81**; and
 - (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:82**.
- Claim 23. The CD16 Binding Molecule of claim 22, wherein said molecule comprises:
- (A) a VH Domain comprising the amino acid sequence of **SEQ ID NO:83** or **SEQ ID NO:84**;
 - (B) a VL Domain comprising the amino acid sequence of or **SEQ ID NO:85**; or
 - (C) a VH Domain comprising the amino acid sequence of **SEQ ID NO:83** or **SEQ ID NO:84** and a VL Domain comprising the amino acid sequence of **SEQ ID NO:85**.
- Claim 24. The CD16 Binding Molecule of any one of claims 20-23, wherein said molecule is selected from the group consisting of: an antibody, a multispecific antibody, a Fab' fragment, a F(ab')₂ fragment, a (Fv) fragment, a single-chain (scFv), a single-chain antibody, a disulfide-linked bispecific Fv (sdFv), a diabody, a trivalent binding molecule, and a CAR-T molecule.
- Claim 25. A pharmaceutical composition that comprises the **CD16 x Disease Antigen** Binding Molecule of any one of claims 1-19 and a pharmaceutically acceptable carrier.

- Claim 26. A pharmaceutical composition that comprises the CD16 Binding Molecule of any one of claims 20-24 and a pharmaceutically acceptable carrier.
- Claim 27. Use of the CD16 x Disease Antigen Binding Molecule of any one of claims 1-19, or the pharmaceutical composition of claim 25, in the manufacture of a medicament for the treatment of a disease characterized by the expression of said Disease Antigen.
- Claim 28. The CD16 x Disease Antigen Binding Molecule of any one of claims 1-19, or the pharmaceutical composition of claim 25, for use in the treatment of a disease in a subject in need thereof characterized by the expression of said Disease Antigen.
- Claim 29. The use of claim 27 or the CD16 x Disease Antigen Binding Molecule or pharmaceutical composition of claim 28, wherein said **CD16 x Disease Antigen** Binding Molecule is capable of binding more than one Disease Antigen and/or more than one epitope of CD16.
- Claim 30. The use of claim 27 or the CD16 x Disease Antigen Binding Molecule or pharmaceutical composition of claim 28, wherein said Disease Antigen is a Cancer Antigen, and said disease is cancer.
- Claim 31. The use or CD16 x Disease Antigen Binding Molecule or pharmaceutical composition of claim 30, wherein said cancer is selected from the group consisting of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer, acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, hairy cell leukemia, Burkett's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, non-Hodgkin's lymphoma, small lymphocytic lymphoma, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, testicular cancer, and uterine cancer.
- Claim 32. The use or CD16 x Disease Antigen Binding Molecule or pharmaceutical composition of claim 30, wherein said Cancer Antigen is selected from the

group consisting of: 19.9, 4.2, A33, ADAM-9, AH6, ALCAM, B1, B7-H3, BAGE, beta-catenin, blood group ALe^b/Le^y, Burkitt's lymphoma antigen-38.13, C14, CA125, Carboxypeptidase M, CD5, CD19, CD20, CD22, CD23, CD25, CD27, CD28, CD33, CD36, CD40/CD154, CD45, CD56, CD46, CD52, CD56, CD79a/CD79b, CD103, CD123, CD317, CDK4, CEA, CEACAM5/CEACAM6, CO17-1A, CO-43, CO-514, CTA-1, CTLA-4, Cytokeratin 8, D1.1, D156-22, DR5, E₁ series, EGFR, an Ephrin receptor, EphA2, Erb, GAGE, a GD2/GD3/GM2 ganglioside, GICA 19-9, gp100, Gp37, gp75, gpA33, HER2/neu, HMFG, human papillomavirus-E6/human papillomavirus-E7, HMW-MAA, I antigen, IL13R α 2, Integrin β 6, JAM-3, KID3, KID31, KS 1/4 pan-carcinoma antigen, L6/L20, LEA, LUCA-2, M1:22:25:8, M18, M39, MAGE, MART, mesothelin, MUC-1, MUM-1, Myl, N-acetylglucosaminyltransferase, neoglycoprotein, NS-10, OFA-1, OFA-2, Oncostatin M, p15, p97, PEM, PEMA, PIPA, PSA, PSMA, prostatic acid phosphate, R₂₄, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T cell receptor derived peptide, T_{5A7}, TAG-72, TL5, TNF-receptor, TNF- γ receptor, TRA-1-85, a Transferrin Receptor, 5T4, TSTA, VEGF, a VEGF Receptor, VEP8, VEP9, VIM-D5, and Y hapten, Le^y.

- Claim 33. The use or CD16 x Disease Antigen Binding Molecule or pharmaceutical composition of claim 32, wherein said Disease Antigen is 5T4, B7-H3, CEACAM5/CEACAM6, CD19, CD123, EGRF, EphA2, HER2/neu, IL13R α 2 or VEGF.
- Claim 34. The use of claim 27 or the CD16 x Disease Antigen Binding Molecule or pharmaceutical composition of claim 28, wherein said Disease Antigen is a Pathogen-Associated Antigen.
- Claim 35. The use or CD16 x Disease Antigen Binding Molecule or pharmaceutical composition of claim 34, wherein said Pathogen-Associated Antigen is selected from the group consisting of: Herpes Simplex Virus infected cell protein (ICP)47, Herpes Simplex Virus gD, Epstein-Barr Virus LMP-1, Epstein-Barr Virus LMP-2A, Epstein-Barr Virus LMP-2B, Human Immunodeficiency Virus gp160, Human Immunodeficiency Virus gp120,

Human Immunodeficiency Virus gp41, *etc.*), Human Papillomavirus E6, Human Papillomavirus E7, human T-cell leukemia virus gp64, human T-cell leukemia virus gp46, and human T-cell leukemia virus gp21.

Claim 36. The use of CD16 x Disease Antigen Binding Molecule or pharmaceutical composition of claim 35, wherein said Disease Antigen is an HIV env antigen.