diabodies and heterodimeric Fc diabodies that are capable of simultaneously binding B7-H3 and CD3.

Summary

[0021] The present disclosure is directed to bispecific monovalent diabodies that possess one binding site specific for an epitope of B7-H3 and one binding site specific for an epitope of CD3 (*i.e.*, a "B7-H3 x CD3 bispecific monovalent diabody"). Most preferably, such B7-H3 x CD3 bispecific monovalent diabodies are composed of three polypeptide chains and possess one binding site specific for an epitope of B7-H3 and one binding site specific for an epitope of CD3 and additionally comprise an immunoglobulin Fc Domain (*i.e.*, a "B7-H3 x CD3 bispecific monovalent Fc diabody"). The bispecific monovalent Fc diabodies of the present disclosure are capable of simultaneous binding to B7-H3 and CD3. The disclosure is directed to pharmaceutical compositions that contain such bispecific monovalent Fc diabodies. The disclosure is additionally directed to methods for the use of such diabodies in the treatment of cancer and other diseases and conditions.

[0022] The present disclosure is particularly directed to B7-H3 x CD3 bispecific monovalent Fc diabodies. The B7-H3 x CD3 bispecific monovalent Fc diabodies of the disclosure comprise polypeptide chains that associate with one another in a heterodimeric manner to form one binding site specific for an epitope of B7-H3 and one binding site specific for an epitope of CD3. The B7-H3 x CD3 bispecific monovalent Fc diabodies of the disclosure are thus monovalent in that they are capable of binding to only one copy of an epitope of B7-H3 and to only one copy of an epitope of CD3, but bispecific in that a single diabody is able to bind simultaneously to the epitope of B7-H3 and to the epitope of CD3.

[0023] The preferred B7-H3 x CD3 bispecific monovalent Fc diabodies of the disclosure comprise three polypeptide chains (a "first," "second" and "third" polypeptide chain), wherein the first and second polypeptide chains are covalently bonded to one another and the first and third polypeptide chains are covalently bonded to one another.

[0024] In detail, the disclosure provides a B7-H3 x CD3 bispecific monovalent Fc diabody, wherein the bispecific monovalent Fc diabody is capable of specific binding to an epitope of B7-H3 and to an epitope of CD3, and possesses an IgG Fc Domain, wherein the bispecific monovalent Fc diabody comprises a first polypeptide chain, a second polypeptide chain and a third polypeptide chain, wherein the first and second polypeptide chains are covalently bonded

[0025] The disclosure further concerns the embodiments of such B7-H3 x CD3 bispecific monovalent Fc diabodies, which are capable of cross-reacting with both human and primate B7-H3 and CD3.

[0026] The disclosure particularly concerns the embodiments of such B7-H3 x CD3 bispecific monovalent Fc diabodies wherein:

(A) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:53**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:55**, and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**; or

(B) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:59**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:60** and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**; or

(C) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:61**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:62**, and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**

(D) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:63**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:64**, and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**.

[0026a] In a first aspect, there is provided a B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

(A) said first polypeptide chain comprises the amino acid sequence of SEQ ID NO:53;

(B) said second polypeptide chain comprises the amino acid sequence of SEQ ID NO:55; and

(C) said third polypeptide chain comprises the amino acid sequence of SEQ ID NO:57.

[0026b] In a second aspect, there is provided a B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

(A) said first polypeptide chain comprises the amino acid sequence of SEQ ID NO:59;

(B) said second polypeptide chain comprises the amino acid sequence of SEQ ID NO:60; and

(C) said third polypeptide chain comprises the amino acid sequence of SEQ ID NO:57.

[0026c] In a third aspect, there is provided a B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

(A) said first polypeptide chain comprises the amino acid sequence of SEQ ID NO:61;

(B) said second polypeptide chain comprises the amino acid sequence of SEQ ID NO:62; and

(C) said third polypeptide chain comprises the amino acid sequence of SEQ ID NO:57

[0026d] In a fourth aspect, there is provided a B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

(A) said first polypeptide chain comprises the amino acid sequence of SEQ ID NO:63;

(B) said second polypeptide chain comprises the amino acid sequence of SEQ ID NO:64; and

(C) said third polypeptide chain comprises the amino acid sequence of SEQ ID NO:57.

[0027] The B7-H3 x CD3 bi-specific monovalent Fc diabodies of the present disclosure are preferably capable of mediating redirected killing of target tumor cells using human T cells in an assay employing a target human tumor cell line selected from the group consisting of: A498 (kidney cancer), JIMT-1/Luc (breast cancer), A375 (melanoma); 22Rv1 (prostate cancer), Detroit562 (pharyngeal cancer), DU145 (prostate cancer); BxPC3 (pancreatic cancer), SKMES-1 (lung cancer), and U87 (glioblastoma), and using purified human primary T cells as effector cells at an Effector cell to T cell ratio of 1:1, 5:1, or 10:1. In such an assay, target tumor cell killing is measured using a lactate dehydrogenase (LDH) release assay in which the enzymatic activity of LDH released from cells upon cell death is quantitatively measured, or by a luciferase assay in which luciferase relative light unit (RLU) is the read-out to indicate relative viability of target cells, which have been engineered to express both the green

fluorescent protein (GFP) and luciferase reporter genes. The observed EC_{50} of such redirected killing is about 1.5 μg/mL or less, about 1.0 μg/mL or less, about 500 ng/mL or less, about 300 ng/mL or less, about 200 ng/mL or less, about 100 ng/mL or less, about 50 ng/mL or less.

[0028] The B7-H3 x CD3 bi-specific monovalent Fc diabodies of the present disclosure are preferably capable of mediating the inhibition of human tumor growth in a co-mix xenograft in which such molecules are introduced into NOD/SCID mice along with 22Rv1 (human prostate cancer) or A498 (human kidney cancer) tumor cells and activated human T cells at a ratio of 5:1. Additionally, or alternatively the B7-H3 x CD3 bi-specific monovalent Fc diabodies of the present disclosure are capable of mediating the inhibition of human tumor growth and/or exhibiting anti-tumor activity in a in an xenograft model in female NSG B2m - / - mice:

(A) implanted with human PBMCs (1×10^7) by intraperitoneal (IP) injection on Day -1 and Detroit (human pharyngeal cancer tumor cells (5×10^6)) intradermally (ID) on Day 0, and administration of diabody on or Days 20, 22, 23, 26, 28, 30, 33, 35, and 37; or

(B) implanted with A498 (human kidney cancer tumor cells (5×10^6)) intradermally (ID) on Day 0, and human PBMCs (1×10^7) by intraperitoneal (IP) injection on Day 13 and administration of diabody on Days 33, 35, 36, 39, 41, 43, 46, 48, and 50.

[0029] Preferably, the B7-H3 x CD3 bi-specific monovalent Fc diabodies of the present disclosure are capable of inhibiting tumor growth in such xenograft models when provided at a concentration of greater than about 1.0 mg/kg, at a concentration of about 1 mg/kg, at a concentration of about 0.5 mg/kg, at a concentration of about 0.25 mg/kg, at a concentration of about 0.1 mg/kg, at a concentration of about 0.05 mg/kg, at a concentration of about 0.02 mg/kg, at a concentration of about 0.01 mg/kg, or at a concentration of about 0.005 mg/kg, or at a concentration less than 0.005 mg/kg.

[0030] The disclosure additionally provides any of the above-described B7-H3 x CD3 bi-specific monovalent Fc diabodies for use as a pharmaceutical.

[0030a] In a fifth aspect, there is provided a pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of the first aspect and a physiologically acceptable carrier.

[0030b] In a sixth aspect, there is provided a pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of the second aspect and a physiologically acceptable carrier.

[0030c] In a seventh aspect, there is provided a pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of the third aspect and a physiologically acceptable carrier.

[0030d] In an eighth aspect, there is provided a pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of the fourth aspecr and a physiologically acceptable carrier.

[0031] The disclosure additionally provides any of the above-described B7-H3 x CD3 bi-specific monovalent Fc diabodies for use in the treatment of a disease or condition associated with or characterized by the expression of B7-H3, or in a method of treating a disease or

condition characterized by the expression of B7-H3, particularly wherein the disease or condition associated with or characterized by the expression of B7-H3 is cancer, and more particularly, wherein the cancer is selected from the group consisting of: an acute myeloid leukemia, an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, a glioblastoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a malignant mesothelioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a pharyngeal cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[0031a] In a ninth aspect, there is provided the B7-H3 x CD3 bispecific monovalent Fc diabody of the first aspect, or the pharmaceutical composition of the fifth aspect, for use in treating a disease or condition associated with or characterized by the expression of B7-H3.

[0031b] In a tenth aspect, there is provided use of the B7-H3 x CD3 bispecific monovalent Fc diabody of the first aspect, or the pharmaceutical composition of the fifth aspect, in the manufacture of a medicament for treating a disease or condition associated with or characterized by the expression of B7-H3.

[0031c] In an eleventh aspect, there is provided the B7-H3 x CD3 bispecific monovalent Fc diabody of the second aspect, or the pharmaceutical composition of the sixth aspect, for use in treating a disease or condition associated with or characterized by the expression of B7-H3.

[0031d] In a twelfth aspect, there is provided use of the B7-H3 x CD3 bispecific monovalent Fc diabody of the second aspect, or the pharmaceutical composition of the sixth aspect, in the manufacture of a medicament for treating a disease or condition associated with or characterized by the expression of B7-H3.

[0031e] In a thirteenth aspect, there is provided the B7-H3 x CD3 bispecific monovalent Fc diabody of the third aspect, or the pharmaceutical composition of the seventh aspect, for use in treating a disease or condition associated with or characterized by the expression of B7-H3.

[0031f] In a fourteenth aspect, there is provided use of the B7-H3 x CD3 bispecific monovalent Fc diabody of the third aspect, or the pharmaceutical composition of the seventh aspect, in the manufacture of a medicament for treating a disease or condition associated with or characterized by the expression of B7-H3.

[0031g] In a fifteenth aspect, there is provided the B7-H3 x CD3 bispecific monovalent Fc diabody of the fourth aspect, or the pharmaceutical composition of the eighth aspect, for use in treating a disease or condition associated with or characterized by the expression of B7-H3.

[0031h] In a sixteenth aspect, there is provided use of the B7-H3 x CD3 bispecific monovalent Fc diabody of the fourth aspect, or the pharmaceutical composition of the eighth aspect, in the manufacture of a medicament for treating a disease or condition associated with or characterized by the expression of B7-H3.

Brief Description Of The Drawings

[0032] **Figure 1** illustrates the structure of a covalently associated bispecific monovalent diabody composed of two polypeptide chains, which does not comprise an Fc Region. The polypeptide chains are covalently associated to one another via disulfide bonds that form between cysteine ("**C**") residues.

[0033] **Figures 2A and 2B** illustrate the structures of two versions of the first, second and third polypeptide chains of a three chain bispecific monovalent Fc diabody of the present invention (Version 1, **Figure 2A**; Version 2, **Figure 2B**). The polypeptide chains are

covalently associated to one another via disulfide bonds that form between cysteine ("**C**") residues

[0034] **Figures 3A-3J** show FACS histograms of A498 (kidney cancer) (**Figure 3A**), JIMT-1/Luc (breast cancer) (**Figure 3B**), A375 (melanoma) (**Figure 3C**), 22Rv1 (prostate cancer) (**Figure 3D**), Detroit562 (pharyngeal cancer) (**Figure 3E**), DU145 (prostate cancer) (**Figure 3F**), BxPC-3 (pancreatic cancer) (**Figure 3G**), SKMES-1 (lung cancer) (**Figure 3H**), U87 (glioblastoma) (**Figure 3I**), and Raji (B-lymphoma) (**Figure 3J**) cell lines. Dashed lines represent cells stained with an isotype control PE-labeled antibody and solid lines represent cells stained with anti-B7-H3-PE antibody.

[0035] **Figures 4A-4E** show FACS histograms of anti-EK-coil antibody fluorescence on B7-H3-expressing target cancer cell lines (**Figures 4A-4D**) or human primary T cells (**Figure 4E**). DART-A at a concentration of 10 μg/mL was added to B7-H3-expressing cancer cell lines (A498 (**Figure 4A**), JIMT-1/Luc (**Figure 4B**), Detroit562 (**Figure 4C**), or 22Rv1 (**Figure 4D**)) or human primary T cells (**Figure 4E**) and incubated for 30 minutes. Cells were analyzed by FACS for DART-A cell surface binding after further incubation with biotin-conjugated anti-EK-coil antibody mixed with APC-streptavidin. Solid lines represent DART-A binding profile on cells. Non-specific staining on cells from biotin-conjugated anti-EK-coil antibody is shown by the dashed lines.

[0036] **Figures 5A-5L** show dose-response curves for DART-A-mediated cytotoxicity on B7-H3-expressing cell lines (A498 (**Figure 5A**), JIMT-1/Luc (**Figures 5B-5C**), A375 (**Figure 5D**), U87 (**Figure 5E**), DU145 (**Figure 5F**), BxPC-3 (**Figure 5G**), SKMES-1 (**Figure 5H**), Detroit562 (**Figure 5I**) and 22Rv1 (**Figure 5J**)) and B7-H3-negative cell lines (CHO (**Figure 5K**) and Raji (**Figure 5L**)). DART-A or control DART was incubated *in vitro* with the different tumor cell lines and primary human T cells at an effector cell:target cell (E:T) ratio of 5:1 for about 24 hours. Percent cytotoxicity was evaluated using the LDH release assay for all cell lines (**Figures 5A-5B** and **5D-5L**). In addition, cytotoxicity was measured using the LUM assay for the JIMT-1/Luc cell line (**Figure 5C**). Representative data are shown from multiple experiments using T cells from multiple donors. **DART-A: ●; Control DART: ■.**

[0037] **Figures 6A-6F** show DART-A-mediated redirected killing of A498 cells (**Figures 6A, 6C and 6E**) and A375 cells (**Figures 6B, 6D and 6F**) at E:T ratios of 10:1 (**Figures 6A**

H3 expression levels based on the fluorescence intensity of anti-B7-H3-PE antibody binding. The cell lines with the highest B7-H3 expression were: A498 (kidney cancer) (**Figure 3A**), JIMT-1/Luc (breast cancer) (**Figure 3B**), and A375 (melanoma) (**Figure 3C**); medium B7-H3 expression: 22Rv1 (prostate cancer) (**Figure 3D**), Detroit562 (pharyngeal cancer) (**Figure 3E**), and DU145 (prostate cancer) (**Figure 3F**); and low B7-H3 expression: BxPC3 (pancreatic cancer) (**Figure 3G**), SKMES-1 (lung cancer) (**Figure 3H**), and U87 (glioblastoma) (**Figure 3I**). Raji cells, a B-lymphoma cell line that is known to be negative for B7-H3 expression, did not show any fluorescence with the anti-B7-H3-PE antibody used (**Figure 3J**). The range of B7-H3 expression on the panel of cell lines evaluated provides a basis to characterize the biological activity of B7-H3 X CD3 bispecific monovalent Fc diabodies (*e.g.*, DART-A) on tumor cell lines with various levels of target density and derived from different human tissues.

Example 3
Binding to B7-H3-Positive Cancer Cell Lines and CD3-Expressing T Cells

[00209] The illustrative B7-H3 x CD3 bispecific monovalent Fc diabodies were examined for their bispecific binding capacity. Four B7-H3-expressing tumor cell lines (A948, JIMT-1/Luc, Detroit562, and 22Rv1) and human primary T cells were evaluated for DART-A cell surface binding by FACS analysis. Since DART-A binds with CD3, instead of using CD3 as a marker for T cells, the combination of CD4 and CD8 was used as a T cell marker. Therefore, in this study when primary human leukocytes were used, the combined CD4+ plus CD8+ gated events represent the T cell population.

[00210] After incubation with 10 µg/mL of DART-A, cell-bound DART-A on target cancer cell lines and T cells was detected using an anti-EK-coil antibody, which recognizes the E-coil/K-coil (EK) Heterodimer-Promoting Domain of the DART-A protein. DART-A showed binding to both human B7-H3-expressing tumor cells (**Figures 4A-4D**) and CD3-expressing T cells (**Figure 4E**). DART-B, DART-C, and DART-D were also found to have bispecific binding capacity in similar studies.

Example 4
CTL Activity On Multiple Target Cancer Cell Lines With Purified Human T Cells As Effector Cells

[00211] After confirming binding to both CD3-expressing T cells and B7-H3-expressing tumor cells (see **Example 3**), B7-H3 x CD3 bispecific monovalent Fc diabody-mediated redirected T cell killing of B7-H3-expressing target cells was evaluated *in vitro* using 9 human tumor cell lines (A498, JIMT-1/Luc, A375, U87, DU145, BxPC-3, SKMES-1, Detroit562, and

What Is Claimed Is:

Claim 1. A B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

(A) said first polypeptide chain comprises the amino acid sequence of **SEQ ID NO:53**;

(B) said second polypeptide chain comprises the amino acid sequence of **SEQ ID NO:55**; and

(C) said third polypeptide chain comprises the amino acid sequence of **SEQ ID NO:57**.

Claim 2. A pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 1 and a physiologically acceptable carrier.

Claim 3. The B7-H3 x CD3 bispecific monovalent Fc diabody of claim 1, or the pharmaceutical composition of claim 2, for use in treating a disease or condition associated with or characterized by the expression of B7-H3.

Claim 4. Use of the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 1, or the pharmaceutical composition of claim 2, in the manufacture of a medicament for treating a disease or condition associated with or characterized by the expression of B7-H3.

Claim 5. The B7-H3 x CD3 bispecific monovalent Fc diabody or the pharmaceutical composition of claim 3, or the use of claim 4, wherein said disease or condition associated with or characterized by the expression of B7-H3 is cancer.

Claim 6. The B7-H3 x CD3 bispecific monovalent Fc diabody, or the pharmaceutical composition, or the use of claim 5, wherein said cancer is selected from the group consisting of: an acute myeloid leukemia, an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, a glioblastoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a

liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a malignant mesothelioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a pharyngeal cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

Claim 7. The B7-H3 x CD3 bispecific monovalent Fc diabody, or the pharmaceutical composition, or the use of claim 6, wherein said cancer is selected from the group consisting of: bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, lung cancer, melanoma, neuroblastoma, ovarian cancer, pancreatic cancer, pharyngeal cancer, prostate cancer, renal cell carcinoma, rhabdomyosarcoma, and squamous cell cancer of the head and neck (SCCHN).

Claim 8. A B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

(A) said first polypeptide chain comprises the amino acid sequence of **SEQ ID NO:59**;

(B) said second polypeptide chain comprises the amino acid sequence of **SEQ ID NO:60**; and

(C) said third polypeptide chain comprises the amino acid sequence of **SEQ ID NO:57**.

Claim 9. A pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 8 and a physiologically acceptable carrier.

Claim 10. The B7-H3 x CD3 bispecific monovalent Fc diabody of claim 8, or the pharmaceutical composition of claim 9, for use in treating a disease or condition associated with or characterized by the expression of B7-H3.

Claim 11. Use of the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 8, or the pharmaceutical composition of claim 9, in the manufacture of a medicament for treating a disease or condition associated with or characterized by the expression of B7-H3.

Claim 12. The B7-H3 x CD3 bispecific monovalent Fc diabody or the pharmaceutical composition of claim 10, or the use of claim 11, wherein said disease or condition associated with or characterized by the expression of B7-H3 is cancer.

Claim 13. The B7-H3 x CD3 bispecific monovalent Fc diabody, or the pharmaceutical composition, or the use of claim 12, wherein said cancer is selected from the group consisting of: an acute myeloid leukemia, an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, a glioblastoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a malignant mesothelioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a pharyngeal cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

Claim 14. The B7-H3 x CD3 bispecific monovalent Fc diabody, or the pharmaceutical composition, or the use of claim 13, wherein said cancer is selected from the group consisting of: bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, lung cancer, melanoma, neuroblastoma, ovarian cancer, pancreatic cancer, pharyngeal cancer, prostate cancer, renal cell carcinoma, rhabdomyosarcoma, and squamous cell cancer of the head and neck (SCCHN).

Claim 15. A B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

(A) said first polypeptide chain comprises the amino acid sequence of **SEQ ID NO:61**;

(B) said second polypeptide chain comprises the amino acid sequence of **SEQ ID NO:62**; and

(C) said third polypeptide chain comprises the amino acid sequence of **SEQ ID NO:57**.

Claim 16. A pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 15 and a physiologically acceptable carrier.

Claim 17. The B7-H3 x CD3 bispecific monovalent Fc diabody of claim 15, or the pharmaceutical composition of claim 16, for use in treating a disease or condition associated with or characterized by the expression of B7-H3.

Claim 18. Use of the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 15, or the pharmaceutical composition of claim 16, in the manufacture of a medicament for treating a disease or condition associated with or characterized by the expression of B7-H3.

Claim 19. The B7-H3 x CD3 bispecific monovalent Fc diabody or the pharmaceutical composition of claim 17, or the use of claim 18, wherein said disease or condition associated with or characterized by the expression of B7-H3 is cancer.

Claim 20. The B7-H3 x CD3 bispecific monovalent Fc diabody, or the pharmaceutical composition, or the use of claim 19, wherein said cancer is selected from the group consisting of: an acute myeloid leukemia, an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia

of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, a glioblastoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a malignant mesothelioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a pharyngeal cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

Claim 21. The B7-H3 x CD3 bispecific monovalent Fc diabody, or the pharmaceutical composition, or the use of claim 20, wherein said cancer is selected from the group consisting of: bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, lung cancer, melanoma, neuroblastoma, ovarian cancer, pancreatic cancer, pharyngeal cancer, prostate cancer, renal cell carcinoma, rhabdomyosarcoma, and squamous cell cancer of the head and neck (SCCHN).

Claim 22. A B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

(A) said first polypeptide chain comprises the amino acid sequence of **SEQ ID NO:63**;

(B) said second polypeptide chain comprises the amino acid sequence of **SEQ ID NO:64**; and

(C) said third polypeptide chain comprises the amino acid sequence of **SEQ ID NO:57**.

Claim 23. A pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 22 and a physiologically acceptable carrier.

Claim 24. The B7-H3 x CD3 bispecific monovalent Fc diabody of claim 22, or the pharmaceutical composition of claim 23, for use in treating a disease or condition associated with or characterized by the expression of B7-H3.

Claim 25. Use of the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 22, or the pharmaceutical composition of claim 23, in the manufacture of a medicament for treating a disease or condition associated with or characterized by the expression of B7-H3.

Claim 26. The B7-H3 x CD3 bispecific monovalent Fc diabody or the pharmaceutical composition of claim 24, or the use of claim 25, wherein said disease or condition associated with or characterized by the expression of B7-H3 is cancer.

Claim 27. The B7-H3 x CD3 bispecific monovalent Fc diabody, or the pharmaceutical composition, or the use of claim 26, wherein said cancer is selected from the group consisting of: an acute myeloid leukemia, an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, a glioblastoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a malignant mesothelioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a pharyngeal cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

Claim 28. The B7-H3 x CD3 bispecific monovalent Fc diabody, or the pharmaceutical composition, or the use of claim 27, wherein said cancer is selected from the group consisting of: bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, lung cancer, melanoma, neuroblastoma, ovarian cancer, pancreatic cancer, pharyngeal cancer, prostate cancer, renal cell carcinoma, rhabdomyosarcoma, and squamous cell cancer of the head and neck (SCCHN).