immunospecific binding to an epitope of a molecule present on the surface of an effector cell.

[0024] The disclosure additionally concerns the example of such B7-H3 binding molecules wherein the molecule is a trivalent binding molecule, and particularly concerns the examples wherein the molecule comprises, one epitope-binding site capable of immunospecific binding to an epitope of B7-H3, one epitope-binding site capable of immunospecific binding to an epitope of a first molecule present on the surface of an effector cell; and one epitope-binding site capable of immunospecific binding to an epitope of a second molecule present on the surface of an effector cell, wherein such first and second molecules are not B7-H3.

[0025] The disclosure further concerns the example of such a B7-H3-binding molecule, wherein the molecule is capable of simultaneously binding to B7-H3 and to a second epitope, and particularly concerns the example wherein the second epitope is an epitope of a second molecule present on the surface of an effector cell (especially wherein the second epitope is an epitope of CD2, CD3, CD8, CD16, TCR, or NKG2D, and most particularly wherein the second epitope is an epitope of CD3). The disclosure additionally concerns the example of such B7-H3-binding molecules, wherein the effector cells is a cytotoxic T-cell or a Natural Killer (NK) cell. The disclosure additionally concerns the example of such B7-H3-binding molecules, wherein the molecule is also capable of binding a third epitope, and particularly concerns the example wherein the third epitope is an epitope of CD8. The disclosure further concerns the examples of such molecules wherein molecule mediates coordinated binding of a cell expressing B7-H3 and a cytotoxic T cell.

[0026] The disclosure further provides pharmaceutical compositions comprising an effective amount of any of the above-described B7-H3-binding molecules and a pharmaceutically acceptable carrier, excipient or diluent.
[0026a] In another aspect, the present disclosure refers to a pharmaceutical composition that comprises an effective amount of the **B7-H3-ADC** as described herein, or the B7-H3 binding molecule as described herein, and a pharmaceutically acceptable carrier, excipient or diluent.
[0026b] In another aspect, the present disclosure refers to the **B7-H3-ADC** as described herein, the B7-H3 binding molecule as described herein, or the pharmaceutical composition as described herein for use as a medicament.
[0026c] In another example, the present disclosure refers to the **B7-H3-ADC** as described herein, the B7-H3 binding molecule as described herein, or the pharmaceutical composition as described herein for use in the treatment of cancer.

[0026d] In another aspect, the present disclosure refers to use of the **B7-H3-ADC** as described herein, the B7-H3 binding molecule as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating cancer.

[0027] The disclosure is additionally directed to the use of any of the above-described B7-H3-binding molecules 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

Example 1
Generation, Humanization and Characterization of Anti-B7-H3 Antibodies

[00348] Monoclonal antibodies were generated via immunization of mice with viable human fetal progenitor cells or tumor initiating/cancer step-like cells (CSLCs) as previously described (Loo *et al.* (2007) "The glycotope-specific RAV12 monoclonal antibody induces oncosis in vitro and has antitumor activity against gastrointestinal adenocarcinoma tumor xenografts in vivo" Mol Cancer Ther; 6: 856-65). An IHC screen for cancer-specific mAbs identified a panel of anti-B7-H3 (CD276) reactive mAbs with highly differential tumor-versus-normal tissue binding. A subset of anti-B7-H3 antibodies that were efficiently internalized was identified using an internalization assay performed in a 5 day assay using a saporin-conjugated anti-mouse Fab at 1:1 or 10:1 Fab-ZAP:Test mAb ratio according to the manufacturers protocol (Advanced Targeting Systems). As shown in **Figure 7**, a number of anti-B7-H3 antibodies including the anti-B7-H3 antibodies designated "**mAb-C**," and "**mAb-D**" were efficiently internalized.

[00349] The above-described murine anti-B7-H3 mAbs: **mAb-B**, **mAb-C** and **mAb-D** are used to form humanized VL and VH Domains in which the CDR_Ls and CDR_Hs of their domains are fused to human framework domains. The humanized VH and VL domains are then used to generate humanized light chains having a kappa light chain constant region (*i.e.*, **SEQ ID NO:1**) and IgG1 CH1, hinge, and Fc Domains (*i.e.*, **SEQ ID NOs:3, 7, 12**). The humanized antibodies were designated "**hmAb-B**," "**hmAb-C**," and "**hmAb-D**."

[00350] The amino acid sequences of the humanized VL and VH Domains are provided above. It will be noted that the CDRs of **hmAb-B** may be modified to generate alternative humanized VL and VH domains as described above. The amino acid sequence of the entire humanized light and heavy chains of **hmAb-C** and **hmAb-D** are provided above.

[00351] The binding kinetics of the humanized antibodies was investigated using Biacore analysis in which a soluble human or cyno B7-H3 (4Ig)-His tag fusion protein (shB7-H3-His or scB7-H3-His, respectively) was passed over immobilized antibodies. Briefly, each humanized antibody was captured on immobilized a Fab2 goat anti-human Fc surface and was incubated with shB7-H3-His or scB7-H3-his (6.25-100Nm), and the kinetics of binding were determined via Biacore analysis. The calculated k_a, k_d and K_D from these studies using a bivalent binding fit are presented in **Table 6.** The results demonstrate

WHAT IS CLAIMED IS:

Claim 1. An anti-B7-H3 antibody drug conjugate (**B7-H3-ADC**) that comprises the formula:

$$\mathbf{Ab\text{-}(LM)_m\text{-}(D)_n,}$$

wherein:

Ab is a humanized B7-H3 antibody or B7-H3 binding fragment thereof that binds to B7-H3 and comprises:

(i) the CDR_L1 sequence RASESIYSYLA, the CDR_L2 sequence NTKTLPE and the CDR_L3 sequence QHHYGTPPWT in its Variable Light Chain (VL) domain, and

(ii) the CDR_H1 sequence SYGMS, the CDR_H2 sequence TINSGGSNTYY PDSLKG and the CDR_H3 sequence HDGGAMDY in its Variable Heavy Chain (VH) domain;

D is a cytotoxic drug moiety comprising a duocarmycin cytotoxin;

LM comprises at least one bond or a Linker Molecule that covalently links Ab and D;

m is an integer between 0 and n and denotes the number of Linker Molecules of the **B7-H3-ADC**;

and

n is an integer between 1 and 10 and denotes the number of cytotoxic drug moieties covalently linked to the **B7-H3-ADC** molecule.

Claim 2. The **B7-H3-ADC** of claim 1, wherein said Ab comprises:

(i) a humanized Variable Light Chain (VL) domain comprising the amino acid sequence of **SEQ ID NO:20**; and

(ii) a humanized Variable Heavy Chain (VH) domain comprising the amino acid sequence of **SEQ ID NO:21**.

Claim 3. The **B7-H3-ADC** of claim 1 or claim 2, wherein said **Ab** further comprises an Fc domain of a human IgG.

Claim 4. The **B7-H3-ADC** of claim 3, wherein said human IgG is a human IgG1, IgG2, IgG3, or IgG4.

Claim 5. The **B7-H3-ADC** of claim 3 or claim 4, wherein said Fc Domain is a variant Fc Domain that comprises:

(a) one or more amino acid modifications that reduces the affinity of the variant Fc Domain for an FcγR; and/or

(b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Domain.

Claim 6. The **B7-H3-ADC** of claim 5, wherein said modifications that reduce the affinity of the variant Fc Domain for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein the numbering is that of the EU index as in Kabat.

Claim 7. The **B7-H3-ADC** of claim 5 or claim 6, wherein said modifications that enhance the serum half-life of the variant Fc Domain comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein the numbering is that of the EU index as in Kabat.

Claim 8. The **B7-H3-ADC** of any one of claims 1-7, wherein at least one of said **LM** moieties is a Linker Molecule.

Claim 9. The **B7-H3-ADC** of claim 8, wherein said **LM** Linker Molecule comprises a cleavable linker.

Claim 10. The **B7-H3-ADC** of claim 9, wherein said cleavable linker is a peptidic linker.

Claim 11. The **B7-H3-ADC** of claim 10, wherein said peptidic linker is a valine-citrulline dipeptide linker.

Claim 12. The **B7-H3-ADC** of any one of claims 9-11, wherein said **LM** Linker Molecule further comprises a self-eliminating spacer between the cleavable linker and **D**.

Claim 13. The **B7-H3-ADC** of claim 12, wherein said self-eliminating spacer comprises a para-aminobenzyloxycarbonyl moiety.

Claim 14. The **B7-H3-ADC** of any one of claims 9-13, wherein said Linker Molecule further comprises a maleimide linker moiety between the cleavable linker and **Ab**.

Claim 15. The **B7-H3-ADC** of claim 8, wherein said conjugate comprises the formula:

Ab – [V-(W)$_k$-(X)$_1$-A] – D, and **LM** comprises the formula:

[V-(W)$_k$-(X)$_1$-A],

wherein:

V is a cleavable linker,

(W)$_k$-(X)$_1$-A is an elongated, self-eliminating spacer system, that self-eliminates via a l,(4+2n)-elimination,

W and **X** are each a l,(4+2n) electronic cascade spacer, being the same or different,

A is either a spacer group of formula (**Y**)$_m$, wherein **Y** is a l,(4+2n) electronic cascade spacer, or a group of formula **U**, being a cyclisation elimination spacer,

k, 1 and m are independently an integer of 0 (included) to 5 (included),

n is an integer of 0 (included) to 10 (included),

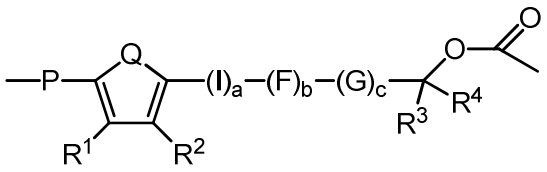
with the provisos that:

when **A** is (**Y**)$_m$: then k+l+m ≥ 1, and

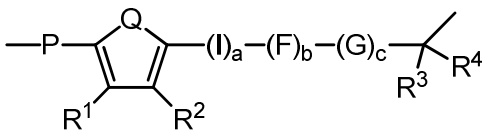
if k+l+m=l, then n>l;

when **A** is **U**: then k+1 ≥ 1.

W, **X**, and **Y** are independently selected from compounds having the formula:



or the formula:

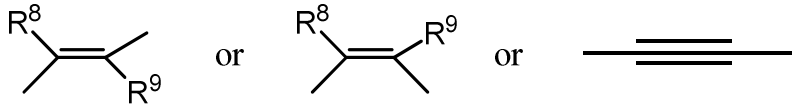


wherein: Q is $-R^5C=CR^6-$, S, O, NR^5, $-R^5C=N-$, or $-N=CR^5-$

P is NR^7, O or S

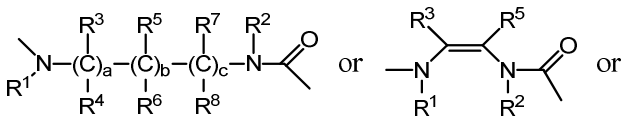
a, b, and c are independently an integer of 0 (included) to 5 (included);

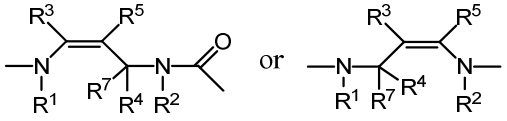
I, F and G are independently selected from compounds having the formula:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, and R^9 independently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are independently selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, or R^9 optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures;

U is selected from compounds having the formula:





wherein:

a, b and c are independently selected to be an integer of 0 or 1; provided that a + b + c = 2 or 3;

R^1 and/or R^2 independently represent H, C1-6 alkyl, the alkyl being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH_2), mono-substituted amino (NR_xH), disubstituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_X), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_X$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate (S(=O)ORx), sulphinyl (S(=O)Rx), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group; and

R^3, R^4, R^5, R^6, R^7 and R^8 independently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), disubstituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, and two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, or R^8 are optionally connected to one another to form one or more aliphatic or aromatic cyclic structures.

Claim 16. The **B7-H3-ADC** of claim 15, wherein said **LM** linker molecule comprises:

(1) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl;

(2) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl;

(3) p-ammocinnamyloxycarbonyl;

(4) p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl;

(5) p-amino-benzyloxycarbonyl-p-aminocinnamyloxycarbonyl;

(6) p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl;

(7) p-aminophenylpentadienyloxycarbonyl;

(8) p-aminophenylpentadienyloxycarbonyl-p-arninocinnamyloxycarbonyl;

(9) p-aminophenylpentadienyloxycarbonyl-paminobenzyloxycarbonyl;

(10) p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyloxycarbonyl;

(11) p-aminobenzyloxycarbonyl(methylamino)ethyl(methylamino) carbonyl;

(12) p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino) carbonyl;

(13) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl(methylamino) ethyl(methylamino)carbonyl;

(14) p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl (methylamino)ethyl(methylamino)carbonyl;

(15) p-aminobenzyloxycarbonyl-p-arninocinnamyloxycarbonyl (methylamino)ethyl(methylamino)-carbonyl;

(16) p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl (methylamino)ethyl(methylamino)carbonyl;

(17) p-aminobenzyloxycarbonyl-p-aminobenzyl;

(18) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl -p-aminobenzyl;

(19) p-aminocinnamyl;

(20) p-aminocinnamyloxycarbonyl-p-aminobenzyl;

(21) p-aminobenzyloxycarbonyl-p-aminocinnamyl;

(22) p-amino-cinnamyloxycarbonyl-p-aminocinnamyl;

(23) p-aminophenylpentadienyl;

(24) p-aminophenylpentadienyloxycarbonyl-p-aminocinnamyl;

(25) p-aminophenylpentadienyloxycarbonyl-p-aminobenzyl;

or

(26) p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyl.

Claim 17. The **B7-H3-ADC** of any one of claims 8-16, wherein said **LM** Linker Molecule is conjugated to the side chain of an amino acid of a polypeptide chain of **Ab** and binds the **Ab** to a molecule of the cytotoxic drug moiety **D**.

Claim 18. The **B7-H3-ADC** of any one of claims 1-17, wherein said duocarmycin cytotoxin is selected from the group consisting of: duocarmycin A, duocarmycin B1, duocarmycin B2, duocarmycin C1, duocarmycin C2, duocarmycin D, duocarmycin SA, CC-1065, adozelesin, bizelesin, carzelesin (U-80244), *seco*-duocarmycin and spiro-duocarmycin (DUBA).

Claim 19. The **B7-H3-ADC** of claim 18, wherein said duocarmycin cytotoxin is *seco*-duocarmycin.

Claim 20. The **B7-H3-ADC** of any of claims 1-19, wherein said LM Linker Molecule is covalently linked to the **Ab** via reduced inter-chain disulfides.

Claim 21. The **B7-H3-ADC** of any of claims 1-14 and 17-20, wherein said **Ab** comprises:

(i) a light chain comprising the Variable Light Chain (VL) domain comprising the amino acid sequence of **SEQ ID NO:20** and a CL Kappa Domain of **SEQ ID NO:1**; and

(ii) a heavy chain comprising the Variable Heavy Chain (VH) domain comprising the amino acid sequence of **SEQ ID NO:21**, a CH1 Domain of **SEQ ID NO:3**, a Hinge Domain of **SEQ ID NO:7** and a Fc Domain comprising a CH2-CH3 Domain of **SEQ ID NO:12**;

said **D** comprises *seco*-duocarmycin; and

said **LM** comprises a Linker Molecule comprising a maleimide linker moiety, a valine-citrulline dipeptide linker, and a para-aminobenzyloxycarbonyl moiety.

Claim 22. A B7-H3 binding molecule, wherein said B7-H3 binding molecule is an antibody, a diabody, or an epitope-binding fragment thereof, which is capable of binding to B7-H3, wherein said B7-H3 binding molecule comprises:

(i) a humanized Variable Light Chain (VL) domain comprising the amino acid sequence of **SEQ ID NO:20**; and

(ii) a humanized Variable Heavy Chain (VH) domain comprising the amino acid sequence of **SEQ ID NO:21**.

Claim 23. The B7-H3 binding molecule of claim 22, which further comprises an Fc Domain of a human IgG.

Claim 24. The B7-H3 binding molecule of claim 23, wherein said human IgG is a human IgG1, IgG2, IgG3, or IgG4.

Claim 25. The B7-H3 binding molecule of claim 23 or claim 24, wherein said Fc Domain is a variant Fc Domain that comprises:

(a) one or more amino acid modifications that reduces the affinity of the variant Fc Domain for an FcγR; and/or

(b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Domain.

Claim 26. The B7-H3 binding molecule of claim 25, wherein said modifications that reduce the affinity of the variant Fc Domain for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein the numbering is that of the EU index as in Kabat.

Claim 27. The B7-H3 binding molecule of claim 25 or claim 26, wherein said modifications that enhance the serum half-life of the variant Fc Domain comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein the numbering is that of the EU index as in Kabat.

Claim 28. A pharmaceutical composition that comprises an effective amount of the **B7-H3-ADC** of any one of claims 1-21, or the B7-H3 binding molecule of

any one of claims 22-27, and a pharmaceutically acceptable carrier, excipient or diluent.

Claim 29. The **B7-H3-ADC** of any one of claims 1-21, the B7-H3 binding molecule of any one of claims 22-27, or the pharmaceutical composition of claim 28 for use as a medicament.

Claim 30. Use of the **B7-H3-ADC** of any one of claims 1-21, the B7-H3 binding molecule of any one of claims 22-27, or the pharmaceutical composition of claim 28, in the manufacture of a medicament for treating cancer.

Claim 31. The use of claim 30, wherein said cancer is selected from the group consisting of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, an adrenal cancer, a bladder cancer, a bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a B-cell cancer, 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, a gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, a glioblastoma, a hematological malignancy, a hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, an acute myeloid leukemia, a chronic lymphocytic leukemia, a chronic myeloid leukemia, a hairy cell leukemia, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a Burkett's lymphoma, a diffuse large B cell lymphoma, a follicular lymphoma, a mantle cell lymphoma, a marginal zone lymphoma, a non-Hodgkin's lymphoma, a small lymphocytic lymphoma, a lung cancer, a non-small-cell lung cancer (NSCLC), a medulloblastoma, a melanoma, a meningioma, a mesothelioma pharyngeal cancer, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic 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 renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a small round blue cell tumor of childhood, a soft-tissue sarcoma, a squamous cell cancer, a squamous cell cancer of the head and neck (SCCHN), a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid cancer, a thyroid metastatic cancer, and a uterine cancer.

Claim 32. The use of claim 30, wherein said cancer is selected from the group consisting of: an acute myeloid leukemia, a non-small cell lung cancer, a neuroblastoma, a squamous cell cancer of the head and neck, a prostate cancer and a thyroid metastatic cancer.

Claim 33. The use of claim 30, wherein the cancer is a prostate cancer.