

additionally directed to the example of such method in which a cellular sample from the patient evidences an expression of one or more target genes that is increased relative to a baseline level of expression of such genes, for example, a baseline level of expression of such genes in a reference population of individuals who are suffering from the hematologic malignancy, or with respect to the level of expression of a reference gene.

[0018] In detail, the disclosure provides a method of treating a chemo-refractory hematologic malignancy in a patient, wherein the method comprises administering to the patient a treatment dosage of a **CD123 x CD3** bispecific molecule, the dosage being effective to stimulate the killing of cells of the hematologic malignancy in the patient and thereby treat such malignancy.

[0018a] In one aspect, the present disclosure refers to the use of a CD123 x CD3 bispecific molecule in the manufacture of a medicament for treating a chemo-refractory hematologic malignancy in a patient in need thereof, wherein:

(A) said CD123 x CD3 bispecific molecule is to be administered prior to and/or subsequent to an evaluation, via an *ex vivo* method, of:

(i) the expression of said one or more target gene(s), wherein said one or more target gene(s) comprise: CXCL9, CXCL10, CXCL11, and STAT1; and

(ii) the expression of one or more reference gene(s) which expression is not characteristically associated with said hematologic malignancy, wherein said one or more reference gene(s) comprise: ABCF1, G6PD, NRDE2, OAZ1, and POLR2; and

(B) said CD123 x CD3 bispecific molecule is to be administered at a dosage effective to stimulate the killing of cells of said hematologic malignancy in said patient; wherein said CD123 x CD3 bispecific molecule is JNJ-63709178, XmAb14045 or APVO436, or is a covalently bonded bispecific diabody comprising:

(a) a first polypeptide chain having the amino acid sequence of SEQ ID NO:21;
and

(b) a second polypeptide chain having the amino acid sequence of SEQ ID NO:23;
and

wherein said first and second polypeptide chains are covalently bonded to one another by a disulfide bond.

[0019] The disclosure further provides the example of such methods that additionally comprises evaluating the expression of one or more target and/or reference genes in a cellular sample from the patient, prior to and/or subsequent to the administration of the **CD123 x CD3** bispecific molecule. The disclosure further provides, the example of such methods wherein the method comprises evaluating the expression of such one or more target and/or such one or more reference genes prior to the administration of the **CD123 x CD3** bispecific molecule. The disclosure also provides the example of such methods wherein the method comprises evaluating the expression of such one or more target and/or such one or more reference genes subsequent to the administration of the **CD123 x CD3** bispecific molecule.

[0020] The disclosure further provides a method of determining whether a patient would be a suitable responder to the use of a **CD123 x CD3** bispecific molecule to treat a hematologic malignancy, wherein the method comprises:

- (a) evaluating the expression of one or more target genes in a cellular sample from the patient prior to the administration of the **CD123 x CD3** bispecific molecule, relative to the expression of one or more target and/or reference genes; and
- (b) identifying the patient as a suitable responder for treatment with a **CD123 x CD3** bispecific molecule if the expression of the one or more target genes is

found to be increased relative to the expression of the one or more target and/or reference genes.

~~[0020a] In another aspect, the present disclosure refers to an *ex vivo* method of determining whether a patient would be a suitable responder to the use of a CD123 x CD3 bispecific molecule to treat a hematologic malignancy, wherein said method comprises:~~

- ~~(a) evaluating the expression of one or more target gene(s) in a cellular sample obtained from said patient, relative to the expression of one or more target and/or reference gene(s); and~~
- ~~(b) identifying said patient as a suitable responder for treatment with a CD123 x CD3 bispecific molecule if the expression of said one or more target gene(s) is found to be increased relative to said expression of said one or more target and/or reference gene(s).~~

[0021] The disclosure further provides the example of such methods wherein the method evaluates: (i) the expression of one or more target gene; and (ii) one or more reference gene whose expression is not characteristically associated with the hematologic malignancy.

[0022] The disclosure further provides the example of such methods that comprises evaluating the expression of the one or more target genes relative to the baseline expression of the one or more reference genes of the patient.

[0023] The disclosure further provides the example of such methods that comprises evaluating the expression of the one or more target genes of a patient relative to the expression of the one or more target genes of an individual who is suffering from the hematologic malignancy or of a population of such individuals. The disclosure further provides the example of such methods wherein the expression of the one or more target genes of such patient is greater than the first quartile (*i.e.*, greater than the bottom 25%), greater than the second quartile (*i.e.*, greater than the bottom 50%), or greater than the third quartile (*i.e.*, greater than the bottom 75%) of the expression levels of such target gene(s) of such individual or of such population of individuals who are suffering from the hematologic malignancy.

[0024] The disclosure further provides the example of such methods that comprises evaluating the expression of the one or more target genes of a patient relative to the expression of the one or more target genes of an individual who had previously been unsuccessfully treated for a hematologic malignancy using the methods and compositions of the present disclosure (*e.g.*, an individual who did not successfully respond to a treatment for a hematologic malignancy using a **CD123 x CD3** bispecific molecule), or a population of such individuals. The disclosure further provides the example of such methods wherein the expression of the one or more target genes of such patient is greater than the first quartile (*i.e.*, greater than the bottom 25%), greater than the second quartile (*i.e.*, greater than the bottom 50%), or greater than the third quartile (*i.e.*, greater than the bottom 75%) of the expression levels of such target

WHAT IS CLAIMED IS:

Claim 1. Use of a CD123 x CD3 bispecific molecule in the manufacture of a medicament for treating a chemo-refractory hematologic malignancy in a patient in need thereof, wherein:

(A) said CD123 x CD3 bispecific molecule is to be administered prior to and/or subsequent to an evaluation, via an *ex vivo* method, of:

(i) the expression of said one or more target gene(s), wherein said one or more target gene(s) comprise: CXCL9, CXCL10, CXCL11, and STAT1; and

(ii) the expression of one or more reference gene(s) which expression is not characteristically associated with said hematologic malignancy, wherein said one or more reference gene(s) comprise: ABCF1, G6PD, NRDE2, OAZ1, and POLR2; and

(B) said CD123 x CD3 bispecific molecule is to be administered at a dosage effective to stimulate the killing of cells of said hematologic malignancy in said patient;

wherein said CD123 x CD3 bispecific molecule is JNJ-63709178, XmAb14045 or APVO436, or is a covalently bonded bispecific diabody comprising:

(a) a first polypeptide chain having the amino acid sequence of SEQ ID NO:21; and

(b) a second polypeptide chain having the amino acid sequence of SEQ ID NO:23; and

wherein said first and second polypeptide chains are covalently bonded to one another by a disulfide bond.

~~Claim 2. The use of claim 1, wherein said CD123 x CD3 bispecific molecule is to be administered prior to and/or subsequent to an evaluation, via an *ex vivo* method, of the expression of one or more target and/or reference gene(s) in a cellular sample obtained from said patient.~~

~~Claim 3. An *ex vivo* method of determining whether a patient would be a suitable responder to the use of a CD123 x CD3 bispecific molecule to treat a hematologic malignancy, wherein said method comprises:~~

- ~~(a) evaluating the expression of one or more target gene(s) in a cellular sample obtained from said patient, relative to the expression of one or more target and/or reference gene(s); and~~
- ~~(b) identifying said patient as a suitable responder for treatment with a CD123 x CD3 bispecific molecule if the expression of said one or more target gene(s) is found to be increased relative to said expression of said one or more target and/or reference gene(s).~~

Claim 42. The use of claim 21, ~~or the method of claim 3~~, wherein said evaluation method comprises evaluating:

- (i) the expression of said one or more target gene(s); and
- (ii) the expression of one or more reference gene(s) which expression is not characteristically associated with said hematologic malignancy.

Claim 53. The use of ~~claim 2~~ any one of claims 1-2, ~~or the method of claim 3~~, wherein said method comprises evaluating the expression of said one or more target genes relative to a baseline expression of said one or more reference genes of said patient.

Claim 64. The use of ~~claim 2~~ any one of claims 1-3, ~~or the method of any one of claims 3-5~~, wherein said method comprises evaluating the expression of said one or more target genes of said patient relative to the expression of said one or more target genes in a cellular sample(s) obtained from:

- (a) an individual who is suffering from said hematologic malignancy, or from a population of such individuals; or
- (b) an individual who did not successfully respond to the use of a CD123 x CD3 bispecific molecule to treat said hematologic malignancy, or from a population of such individuals; or
- (c) an individual who successfully responded to the use of a CD123 x CD3 bispecific molecule to treat said hematologic malignancy, or from a population of such individuals.

Claim ~~75~~. The use ~~or method of claim 6~~ of any one of claims ~~1-4~~, wherein a relative expression level of said one or more target genes in said population of individuals is established by averaging the gene expression levels in cellular samples obtained from said population of individuals.

Claim ~~86~~. The use of ~~claim 2, or the method of~~ any one of claims ~~3-71-5~~, wherein said patient exhibits an expression level of at least one of said target genes:

- (a) that is greater than the first quartile of the expression levels of said target gene in a population of individuals who are suffering from said hematologic malignancy; or
- (b) that is greater than the first quartile of the expression levels of said target gene in a population of individuals who did not successfully respond to a treatment for said hematologic malignancy that used a CD123 x CD3 bispecific molecule; or
- (c) that has a log₂-fold change of at least about 0.4 relative to the expression levels of said target gene in a population of individuals who did not successfully respond to a treatment for said hematologic malignancy that used a CD123 x CD3 bispecific molecule; or
- (d) that is within at least the first quartile of the expression levels of said target gene in a population of individuals who successfully responded to a treatment for said hematologic malignancy that used a CD123 x CD3 bispecific molecule.

Claim ~~97~~. The use of ~~claim 2, or the method of~~ any one of claims ~~3-81-6~~, wherein said cellular sample is a bone marrow sample.

Claim ~~108~~. The use of ~~claim 2, or the method of~~ any one of claims ~~3-91-7~~, wherein said evaluation of expression or said determination of whether said patient would be a suitable responder to the use of a CD123 x CD3 bispecific molecule to treat a hematologic malignancy comprises the steps of:

- (a) determining the expression level(s) for each of said one or more target gene(s) in one or more cellular sample(s) using a gene expression platform; and
- (b) comparing said expression level(s) of each of said one or more target gene(s) to an expression level(s) of said one or more reference gene(s).

Claim ~~119~~. The use of ~~claim 2, or the method of~~ any one of claims ~~3-101-8~~, wherein said evaluation of expression or said determination of whether said patient would be a suitable responder to the use of a CD123 x CD3 bispecific molecule to treat a hematologic malignancy comprises the steps of:

- (a) measuring raw RNA levels for each of said one or more target gene(s) in one or more cellular sample(s) using a gene expression platform comprising a reference gene set of housekeeping genes; and
- (b) assigning a relative expression value for each of the measured raw RNA levels for each of said one or more target gene(s) using the measured RNA levels of internal reference genes.

Claim ~~1210~~. The use of ~~claim 2, or the method of~~ any one of claims ~~3-111-9~~, wherein said one or more target gene(s) additionally comprise:

- ~~(a) — one or more of: CXCL9, CXCL10, CXCL11, and STAT1; and/or~~
- ~~(ba)~~ one or more of: CCL5, CD27, CD274, CD276, CD8A, CMKLR1, CXCL9, CXCR6, HLA-DQA1, HLA-DRB1, HLA-E, IDO1, LAG3, NKG7, PDCD1LG2, PSMB10, STAT1, and TIGIT; and/or
- ~~(eb)~~ one or more of: AREG, CSF3, CXCL1, CXCL2, CXCL3, CCL20, FOSL1, IER3 (NM_003897.4), IL6 and PTGS2; and/or
- ~~(ec)~~ one or more of: CCL2, CCL3/L1, CCL4, CCL7 and CCL8; and/or
- ~~(ed)~~ one or more of: MAGEA3/A6, MAGEA1, MAGEA12, MAGEA4, MAGEB2, MAGEC1 and MAGEC2; and/or
- ~~(fe)~~ one or more of: APOL6, DTX3L, GBP1, IFI16, IFI27, IFI35, IFI6, IFIH1, IFIT1, IFIT2, IFIT3, IFITM1, IFITM2, IRF1, IRF9, ISG15, MX1, OAS1, OAS2, PARP9, PSMB9, STAT2, TMEM140 and TRIM21; and/or
- ~~(gf)~~ one or more of: PSMB8, PSMB9 and PSMB10; and/or
- ~~(hg)~~ IL-10; and/or
- ~~(ih)~~ CD274; and/or
- ~~(ji)~~ PDCD1LG2.

Claim ~~1311~~. The use of ~~claim 2, or the method of~~ any one of claims ~~3-121-10~~, wherein said one or more reference gene(s) additionally comprise one or more of: ~~ABCF1,~~

~~G6PD, NRDE2, OAZ1, POLR2A,~~ SDHA, STK11IP, TBC1D10B, TBP, and UBB.

Claim ~~14~~12. The use of ~~claim 2, or the method of~~ any one of claims ~~3-13~~1-11, wherein a gene signature score is determined for said one or more target gene(s).

Claim ~~15~~13. The use ~~or method~~ of claim ~~14~~12, wherein said gene signature score is determined by a process comprising:

- (a) measuring raw RNA levels for each of said one or more target gene(s) in one or more cellular sample(s) using a gene expression platform comprising a reference gene set of housekeeping genes,
- (b) normalizing each of the measured raw RNA levels to a geometric mean of said housekeeping genes, and optionally further normalizing each RNA value to a standard,
- (c) log transforming each normalized RNA value,
- (d) multiplying each log transformed RNA value by a corresponding weight factor to generate a weighted RNA value, and
- (e) adding the weighted RNA values, and optionally adding an adjustment factor constant, to generate said gene signature score.

Claim ~~16~~14. The use ~~or method~~ of any one of claims ~~14-15~~12-13, wherein said gene signature score is determined using the target gene(s) and weights, scoring weights and optionally adjustment factors provided in Tables 6 ~~and or Tables~~ 12A-~~12F, or the target gene(s) and adjustment factors provided in Table~~ 12G.

Claim ~~17~~15. The use ~~or method~~ of any one of claims ~~14-16~~12-14, wherein said gene signature score is determined for one or more of:

- (a) the IFN Gamma Signaling Signature;
- (b) the Tumor Inflammation Signature;
- (c) the Myeloid Inflammation Signature;
- (d) the Inflammatory Chemokine Signature;
- (e) the MAGEs Signature;
- (f) the IFN Downstream Signaling Signature;
- (g) the Immunoproteasome Signature;
- (h) the IL-10 Signature;

- (i) the PD-L1 Signature; and/or
- (j) the PD-L2 Signature.

Claim ~~18~~16. The use ~~or method~~ of any one of claims ~~14-17~~12-15, wherein a patient gene signature score that:

- (a) is greater than the first quartile of scores for said gene signature score calculated from the expression levels of one or more of said target genes in a population of individuals who are suffering from said hematologic malignancy; or
- (b) is greater than the first quartile of scores for said gene signature score calculated from the expression levels of one or more of said target genes in a population of individuals who did not successfully respond to a treatment for said hematologic malignancy that used a CD123 x CD3 bispecific molecule; or
- (c) has a log₂-fold change of at least about 0.4 relative to scores for said gene signature score calculated from the expression levels of one or more of said target genes in a population of individuals who did not successfully respond to a treatment for said hematologic malignancy that used a CD123 x CD3 bispecific molecule; or
- (d) is within at least the first quartile of the scores for said gene signature score calculated from the expression levels of one or more of said target genes in a population of individuals who successfully responded to a treatment for said hematologic malignancy that used a CD123 x CD3 bispecific molecule,

is indicative of a favorable patient response to treatment with said CD123 x CD3 bispecific molecule.

Claim ~~19~~17. The use ~~or method~~ of any one of claims ~~17-18~~15-16, wherein:

- (a) said gene signature score is determined for the IFN Gamma Signaling Signature, and a patient gene signature score of at least about 2.5 is indicative of a favorable patient response to treatment with said CD123 x CD3 bispecific molecule, and/or
- (b) said gene signature score is determined for the Tumor Inflammation Signature, and a patient gene signature score of at least about 5.5 is

indicative of a favorable patient response to treatment with said CD123 x CD3 bispecific molecule; and/or

- (c) said gene signature score is determined for the IFN Downstream Signaling Signature, and a patient gene signature score of at least about 4.5 is indicative of a favorable patient response to treatment with said CD123 x CD3 bispecific molecule.

Claim ~~20~~18. The use ~~or method~~ of any one of claims ~~17-19~~15-17, wherein said gene signature is the IFN Gamma Signaling Signature, the Tumor Inflammation Signature, or the IFN Downstream Signaling Signature.

Claim ~~21~~19. The use ~~or method~~ of any one of claims ~~16-20~~14-18, wherein a patient that exhibits a gene expression signature that is characteristic of an immune-enriched and IFN gamma-dominant tumor microenvironment is indicative of a favorable patient response to treatment with said CD123 x CD3 bispecific molecule.

~~Claim 22. The use of any one of claims 1-2, or the method of any one of claims 3-21, wherein said CD123 x CD3 bispecific molecule is a bispecific antibody or bispecific molecule comprising an scFv.~~

Claim ~~23~~20. The use ~~or method of claim 22~~ of any one of claims ~~1-19~~, wherein said CD123 x CD3 bispecific molecule is said JNJ-63709178, XmAb14045 or APVO436.

Claim ~~24~~21. The use of any one of claims 1-~~219~~, ~~or the method of any one of claims 3-21,~~ wherein said CD123 x CD3 bispecific molecule is ~~a~~-said covalently bonded bispecific diabody comprising:

- (a) a first polypeptide chain having the amino acid sequence of SEQ ID NO:21; and
- (b) a second polypeptide chain having the amino acid sequence of SEQ ID NO:23; and

wherein said first and second polypeptide chains are covalently bonded to one another by a disulfide bond.

Claim ~~25~~22. The use of any one of claims 1-~~21~~, ~~or the method of any one of claims 3-24,~~ wherein said hematologic malignancy of said patient is selected from the group

consisting of: acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), blastic crisis of CML, Abelson oncogene-associated with CML (Bcr-ABL translocation), myelodysplastic syndrome (MDS), acute B lymphoblastic leukemia (B-ALL), acute T lymphoblastic leukemia (T-ALL), chronic lymphocytic leukemia (CLL), Richter's syndrome, Richter's transformation of CLL, hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphoma (NHL), mantle cell lymphoma (MCL), small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, and Burkitt's lymphoma.

Claim ~~26~~23. The use ~~or method~~ of claim ~~25~~22, wherein said hematologic malignancy of said patient is AML.

Claim ~~27~~24. The ~~method-use~~ of any one of claims ~~3-26~~1-23, wherein said hematologic malignancy of said patient is refractory to chemotherapy.

Claim ~~28~~25. The use of any one of claims 1-~~24~~24, ~~or the method of any one of claims 3-27~~, wherein said CD123 x CD3 bispecific molecule is to be administered at a dosage of 30 ng/kg patient weight/day, 100 ng/kg patient weight/day, 300 ng/kg patient weight/day, or 500 ng/kg patient weight/day.

Claim ~~29~~26. The use of any one of claims 1-~~25~~25, ~~or the method of any one of claims 3-28~~, wherein said CD123 x CD3 bispecific molecule is to be administered by continuous infusion.

Claim ~~30~~27. The use of any one of claims 1-~~26~~26, ~~or the method of any one of claims 3-29~~, wherein said patient is a human patient.