

59:2909-2916; WO 2006/113665; WO 2008/157379; WO 2010/080538; WO 2012/018687; WO 2012/162068). Normally, effector cell activation is triggered by the binding of an antigen-bound antibody to an effector cell via Fc-FcγR interaction; thus, in this regard, diabody molecules may exhibit Ig-like functionality independent of whether they comprise an Fc Domain (e.g., as assayed in any effector function assay known in the art or exemplified herein (e.g., ADCC assay)). By cross-linking tumor and effector cells, the diabody not only brings the effector cell within the proximity of the tumor cell, but leads to effective tumor killing (see e.g., Cao *et al.* (2003) “*Bispecific Antibody Conjugates In Therapeutics*,” Adv. Drug. Deliv. Rev. 55:171-197).

[0016] Several bispecific molecules targeting CD123 and CD3 capable of mediating T cell redirected cell killing of CD123-expressing malignant cells are in development (see, e.g., Vey, N., *et al.* (2017) “*Interim Results From A Phase I First-In-Human Study Of Flotetuzumab, a CD123 x CD3 Bispecific DART Molecule In AML/MDS*,” Annals of Oncology, 28(S5)5, mdx373.001; Godwin, C.D., *et al.* (2017) “*Bispecific Anti-CD123 x Anti-CD3 Adaptir™ Molecules APVO436 and APVO437 Have Broad Activity Against Primary Human AML Cells In Vitro*” Blood. 130(S1): 2639; Forslund, A., *et al.* (2016) “*Ex Vivo Activity Profile of the CD123xCD3 Duobody® Antibody JNJ-63709178 Against Primary Acute Myeloid Leukemia Bone Marrow Samples*” Blood 128(22):2875.). However, efforts to employ bispecific binding molecules that are capable of targeting a T cell to the location of a hematologic malignancy have not been fully successful. Hence, an unmet need remains to develop new strategies for the treatment of hematologic malignancies with **CD123 x CD3** bispecific binding molecules. The present invention directly addresses this need and others, as described below.

SUMMARY:

[0017] The present disclosure is directed to a method of treating a hematologic malignancy such as acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS), including hematologic malignancies that are refractive to chemotherapeutic and/or hypomethylating agents. The method concerns administering a **CD123 x CD3** bispecific binding molecule to a patient in an amount effective to stimulate the killing of cells of the hematologic malignancy in the patient. The present disclosure is

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 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.

[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

gene(s) of such individual or of such population of unsuccessfully treated individuals. The disclosure further provides the example of such methods wherein the expression of the one or more target genes of such patient has a log₂-fold change of at least about 0.4, at least about 0.5, at least about 0.6, or higher relative to the expression levels of such target gene(s) of such individual or such population of unsuccessfully treated individuals.

[0025] 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 successfully treated for a hematologic malignancy using the methods and compositions of the present disclosure (*e.g.*, an individual who successfully responded 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 within the first quartile (*i.e.*, within the bottom 25%) of the expression levels of such target gene(s), within the second quartile (*i.e.*, between the bottom 25% and 50%), or within the third quartile (*i.e.*, between the bottom 50% and 75%) of the expression levels of such target gene(s) of such individual or such population of successfully treated individuals.

[0026] The disclosure further provides the example of such methods wherein the relative expression level of the one or more target genes in the population is established by averaging the gene expression level in cellular samples obtained from the population of individuals.

[0027] The disclosure further provides the example of such methods wherein such patient exhibits an expression level of at least one of such target genes:

- (a) that is greater than the first quartile of the expression levels of such target gene in a population of individuals who are suffering from the hematologic malignancy; or
- (b) that is greater than the first quartile of the expression levels of such target gene in a population of individuals who did not successfully respond to a treatment

for the 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 such target gene in a population of individuals who did not successfully respond to a treatment for the 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 such target gene in a population of individuals who successfully responded to a treatment for a hematologic malignancy that used a **CD123 x CD3** bispecific molecule.

[0028] The disclosure further provides the example of such methods wherein such patient exhibits an expression level of at least one of such target genes:

- (a) that is greater than the second quartile of the expression levels of such target gene in a population of individuals who are suffering from the hematologic malignancy; or
- (b) that is greater than the second quartile of the expression levels of such target gene in a population of individuals who did not successfully respond to a treatment for the 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 such target gene in a population of individuals who did not successfully respond to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule; or
- (d) that is within at least the second quartile of the expression levels of such target gene in a population of individuals who successfully responded to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule.

[0029] The disclosure further provides the example of such methods wherein such patient exhibits an expression level of at least one of such target genes:

- (a) that is greater than the third quartile of the expression levels of such target gene in a population of individuals who are suffering from said hematologic malignancy; or

- (b) that is greater than the third quartile of the expression levels of such 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 0.6 relative to the expression levels of such 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.

[0030] The disclosure further provides a method of treating a hematologic malignancy, wherein the method comprises:

- (a) employing the method of any one of the above examples to determine whether a patient would be a suitable responder to the use of a **CD123 x CD3** bispecific molecule to treat the hematologic malignancy;
- (b) administering a treatment dosage of the **CD123 x CD3** bispecific molecule to the patient if the patient is determined to be a suitable responder to such treatment;

wherein the administration of the **CD123 x CD3** bispecific molecule stimulates the killing of cells of the hematologic malignancy in the patient.

[0031] The disclosure further provides the example of such methods that additionally comprises evaluating the expression of such one or more target genes in a cellular sample obtained from the patient one or more times after the initiation of the treatment.

[0032] The disclosure further provides the example of such methods wherein the cellular sample is a bone marrow or a blood sample. Particularly, the example of such methods wherein the cellular sample is a bone marrow sample.

[0033] The disclosure further provides the example of such methods that further comprises detecting the expression level of one or more target genes in a sample of the patient's bone marrow. The disclosure further provides the example of such methods that further comprises detecting the expression level of one or more reference genes.

[0034] The disclosure further provides the example of such methods that comprise detecting the expression level of such one or more target genes and/or such one or more reference genes in a sample of the patient's bone marrow, particularly prior to administration of a **CD123 x CD3** bispecific molecule.

[0035] The disclosure further provides the example of such methods wherein the evaluation of expression or the determination of whether the patient would be a suitable responder to the use of a **CD123 x CD3** bispecific molecule to treat a hematologic malignancy is performed by:

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

[0036] The disclosure further provides the example of such methods wherein the evaluation of expression or the determination of whether the patient would be a suitable responder to the use of a **CD123 x CD3** bispecific molecule to treat a hematologic malignancy is performed by:

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

[0037] The disclosure further provides the example of such methods wherein the one or more target genes comprise:

- (a) one or more of: CXCL9, CXCL10, CXCL11, and STAT1; and or
- (b) 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

- (c) one or more of: AREG, CSF3, CXCL1, CXCL2, CXCL3, CCL20, FOSL1, IER3 (NM_003897.4), IL6 and PTGS2; and/or
- (d) one or more of: CCL2, CCL3/L1, CCL4, CCL7 and CCL8; and/or
- (e) one or more of: MAGEA3/A6, MAGEA1, MAGEA12, MAGEA4, MAGEB2, MAGEC1 and MAGEC2; and/or
- (f) 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
- (g) one or more of: PSMB8, PSMB9 and PSMB10; and/or
- (h) IL-10; and or
- (i) CD274; and/or
- (j) PDCD1LG2.

[0038] The disclosure further provides the example of such methods wherein the one or more target genes further comprises IFNG (NM_000619.2).

[0039] The disclosure further provides the example of such methods wherein the one or more reference genes comprise one or more of: ABCF1, G6PD, NRDE2, OAZ1, POLR2A, SDHA, STK11IP, TBC1D10B, TBP, and UBB.

[0040] The disclosure further provides the example of such methods wherein a gene signature score is determined for the one or more target genes. In specific examples of the disclosure such gene signature score is determined from the raw RNA levels of each target gene by a process comprising:

- (a) measuring the raw RNA levels for each target gene in one more cellular sample using a gene expression platform comprising a reference gene set of housekeeping genes,
- (b) normalizing each of the measured raw RNA levels to the geometric mean of such 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 a single gene signature score.

[0041] Preferably, the gene signature is determined using the target genes provided in **Tables 6** and **12A-12G**. In certain examples of the disclosure, the weight factors are those provided in **Tables 6** and **12A-12G**. In certain examples of the disclosure, an adjustment factor is added to each score. In particular examples, the adjustment factors are those provided in **Tables 6** and **12B-12G**.

[0042] The disclosure particularly, provides the example of such methods wherein a 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.

[0043] The disclosure further provides the example of such methods wherein a patient gene signature score that:

- (a) is greater than the first quartile of scores for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who are suffering from the hematologic malignancy; or
- (b) is greater than the first quartile of scores for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who did not successfully respond to a treatment for the 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 the gene signature calculated from the expression levels of one or more of the target

genes in a population of individuals who did not successfully respond to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule; or

(d) is within at least the first quartile of the scores for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who successfully responded to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule,

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

[0044] The disclosure further provides the example of such methods wherein a patient gene signature score that:

- (a) is greater than the second quartile for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who are suffering from the hematologic malignancy; or
- (b) is greater than the second quartile for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who did not successfully respond to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule; or
- (c) has a log₂-fold change of at least about 0.5 relative to scores for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who did not successfully respond to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule; or
- (d) is within at least the second quartile of the scores for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who successfully responded to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule,

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

[0045] The disclosure further provides the example of such methods wherein a patient gene signature score that:

- (a) is greater than the third quartile of scores for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who are suffering from the hematologic malignancy; or
- (b) is greater than the third quartile of scores for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who did not successfully respond to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule; or
- (c) has a log₂-fold change of at least about 0.6 relative to scores for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who did not successfully respond to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule,

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

[0046] The disclosure further provides the example of such methods wherein:

- (a) the gene signature is the IFN Gamma Signaling Signature, and a patient gene signature score of at least about 2.5 is indicative of a more favorable patient response to treatment with the **CD123 x CD3** bispecific molecule, and/or
- (b) the gene signature is the Tumor Inflammation Signature, and a patient gene signature score of at least about 5.5 is indicative of a more favorable patient response to treatment with the **CD123 x CD3** bispecific molecule; and/or
- (c) the gene signature is the IFN Downstream Signaling Signature, and a patient gene signature score of at least about 4.5 is indicative of a more favorable patient response to treatment with the **CD123 x CD3** bispecific molecule.

[0047] The disclosure further provides the example of such methods wherein the gene signature is the IFN Gamma Signaling Signature, the Tumor Inflammation Signature, or the IFN Downstream Signaling Signature, and a patient gene signature score that:

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

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

[0048] The disclosure further provides the example of such methods wherein the gene signature is the IFN Gamma Signaling Signature, the Tumor Inflammation Signature, or the IFN Downstream Signaling Signature, and a patient gene signature score that:

- (a) is greater than the second quartile of scores of the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who are suffering from the hematologic malignancy; or
- (b) is greater than the second quartile of scores for the gene signature calculated from the expression levels of one or more of the target genes in a population of

- individuals who did not successfully respond to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule; or
- (c) has a log₂-fold change of at least about 0.5 relative to scores for the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who did not successfully respond to a treatment for the hematologic malignancy that used a **CD123 x CD3** bispecific molecule; or
 - (d) is within at least the second quartile of the scores of the gene signature calculated from the expression levels of one or more of the target genes in a population of individuals who successfully responded to a treatment for a hematologic malignancy that used a **CD123 x CD3** bispecific molecule
- is indicative of a more favorable patient response to treatment with the **CD123 x CD3** bispecific molecule.

[0049] The disclosure also provides the example of such methods 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 more favorable patient response to treatment with the **CD123 x CD3** bispecific molecule.

[0050] The disclosure further provides the example of such methods wherein the **CD123 x CD3** bispecific molecule is a bispecific antibody or a bispecific molecule comprising an scFv.

[0051] The disclosure further provides the example of such methods wherein the **CD123 x CD3** bispecific molecule is JNJ-63709178, XmAb14045 or APVO436.

[0052] The disclosure further provides the example of such methods wherein the **CD123 x CD3** bispecific molecule is a covalently bonded bispecific diabody having two, three, or four polypeptide chains.

[0053] The disclosure further provides the example of such methods wherein the **CD123 x CD3** bispecific molecule is a diabody that comprises:

- (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 the first and the second polypeptide chains are covalently bonded to one another by a disulfide bond.

[0054] The disclosure further provides the example of such methods wherein the hematologic malignancy of such 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), including mantle cell lymphoma (MCL) and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, and Burkitt's lymphoma.

[0055] The disclosure further provides the examples of such methods wherein the hematologic malignancy of such patient is AML, MDS, BPDCN, or T-ALL.

[0056] The disclosure further provides the example of such methods wherein the hematologic malignancy of such patient is refractory to chemotherapy (**CTX**), such as being refractory to cytarabine/anthracycline-based cytotoxic chemotherapy or refractory to hypomethylating agents (**HMA**) chemotherapy.

[0057] The disclosure further provides the example of such methods that further comprises determining the level expression of CD123 of blast cells (cancer cells) as compared to a corresponding baseline level CD123 expressed by normal peripheral blood mononuclear cells (PBMCs).

[0058] The disclosure further provides the example of such methods wherein the level of expression is determined by measuring the cell surface expression of CD123. The disclosure further provides the example of such methods wherein the cell surface expression of CD123 is increased by at least about 20% relative to a baseline level of expression. The disclosure further provides the example of such methods

wherein the increase in CD123 expression renders the patient more responsive to treatment with the **CD123 x CD3** bispecific molecule.

[0059] The disclosure further provides the example of such methods wherein the effective dosage of the **CD123 x CD3** bispecific molecule is selected from the group consisting of 30, 100, 300, and 500 ng/kg patient weight/day.

[0060] The disclosure further provides the example of all of the above-described methods wherein the treatment dosage is administered as a continuous infusion. The disclosure further provides the example of such methods wherein the treatment dosage is 30 ng/kg/day administered by continuous infusion for 3 days followed by a treatment dosage of 100 ng/kg/day administered by continuous infusion for 4 days. The disclosure further provides the example of such methods wherein the treatment dosage further comprises administration of 500 ng/kg/day administered by continuous infusion.

[0061] The disclosure further provides the example of all of the above-described methods wherein the patient is a human patient.

BRIEF DESCRIPTION OF THE DRAWINGS:

[0062] **Figures 1A-1C** illustrate the overall structure of exemplary diabody molecules. **Figure 1A** provides the structure of the first and second polypeptide chains of a two chain **CD123 x CD3** bispecific diabody (“**DART-A**” also known as flotetuzumab) having two epitope-binding domains, Heterodimer-Promoting Domains and a cysteine containing linker. **Figures 1B-1C** provide the overall structure of a **CD123 x CD3** bispecific diabody having two epitope-binding domains composed of three polypeptide chains. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Domain. The polypeptide chains comprising the VL and VH Domain further comprise a Heterodimer-Promoting Domain and a linker. A cysteine residue may be present in a linker (**Figures 1A and 1B**) and/or in the Heterodimer-Promoting Domain (**Figure 1C**). VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

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 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.
- 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 4. The use of claim 2, or the method of claim 3, wherein said 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 5. The use of claim 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 6. The use of claim 2, 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 7. The use or method of claim 6, 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 8. The use of claim 2, or the method of any one of claims 3-7, 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 9. The use of claim 2, or the method of any one of claims 3-8, wherein said cellular sample is a bone marrow sample.
- Claim 10. The use of claim 2, or the method of any one of claims 3-9, 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 11. The use of claim 2, or the method of any one of claims 3-10, 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 12. The use of claim 2, or the method of any one of claims 3-11, wherein said one or more target gene(s) comprise:
- (a) one or more of: CXCL9, CXCL10, CXCL11, and STAT1; and/or
 - (b) 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
 - (c) one or more of: AREG, CSF3, CXCL1, CXCL2, CXCL3, CCL20, FOSL1, IER3 (NM_003897.4), IL6 and PTGS2; and/or
 - (d) one or more of: CCL2, CCL3/L1, CCL4, CCL7 and CCL8; and/or
 - (e) one or more of: MAGEA3/A6, MAGEA1, MAGEA12, MAGEA4, MAGEB2, MAGEC1 and MAGEC2; and/or

- (f) 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
- (g) one or more of: PSMB8, PSMB9 and PSMB10; and/or
- (h) IL-10; and/or
- (i) CD274; and/or
- (j) PDCD1LG2.

Claim 13. The use of claim 2, or the method of any one of claims 3-12, wherein said one or more reference gene(s) comprise one or more of: ABCF1, G6PD, NRDE2, OAZ1, POLR2A, SDHA, STK11IP, TBC1D10B, TBP, and UBB.

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

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

Claim 17. The use or method of any one of claims 14-16, 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. The use or method of any one of claims 14-17, 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. The use or method of any one of claims 17-18, 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. The use or method of any one of claims 17-19, wherein said gene signature is the IFN Gamma Signaling Signature, the Tumor Inflammation Signature, or the IFN Downstream Signaling Signature.
- Claim 21. The use or method of any one of claims 16-20, 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. The use or method of claim 22, wherein said CD123 x CD3 bispecific molecule is JNJ-63709178, XmAb14045 or APVO436.
- Claim 24. 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 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. The use of any one of claims 1-2, 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. The use or method of claim 25, wherein said hematologic malignancy of said patient is AML.
- Claim 27. The method of any one of claims 3-26, wherein said hematologic malignancy of said patient is refractory to chemotherapy.
- Claim 28. The use of any one of claims 1-2, 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. The use of any one of claims 1-2, 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. The use of any one of claims 1-2, or the method of any one of claims 3-29, wherein said patient is a human patient.