(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 September 2015 (11.09.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/134603 A2

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2015/018724

(22) International Filing Date:
4 March 2015 (04.03.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/948,499	5 March 2014 (05.03.2014)	US
61/951,622	12 March 2014 (12.03.2014)	US
61/990,477	8 May 2014 (08.05.2014)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

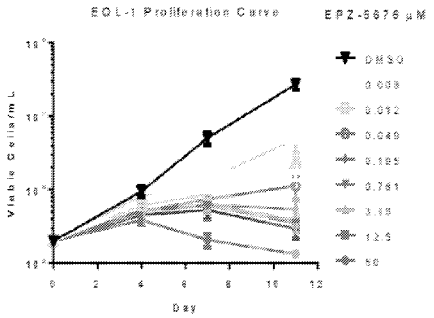
Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: METHODS FOR TREATING CANCER



Compound	K_i (nM)	Prolif. IC_{50} (µM)
EPZ004777	0.3	0.012
EPZ-5676	< 0.08	0.002

FIG. 2

(57) Abstract: The disclosure relates to methods that include diagnosing if a subject has MLL-PTD and, if the subject has MLL-PTD, administering a therapeutically effective amount of a DOT1L inhibitor to the subject.

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METHODS FOR TREATING CANCER

RELATED APPLICATIONS

[001] This application claims priority to, and the benefit of, U.S. provisional application Nos. 61/948,499 filed March 5, 2014; 61/951,622, filed March 12, 2014; and 61/990,477, filed May 8, 2014, the entire contents of each of which are incorporated herein by reference in their entireties.

FIELD OF INVENTION

[002] The present invention relates generally to the field of cancer treatment, and in particular, the treatment of leukemia.

BACKGROUND OF THE INVENTION

[003] Disease-associated chromatin-modifying enzymes (*e.g.*, DOT1L) play a role in diseases such as proliferative disorders, metabolic disorders, and blood disorders. Thus, there is a need for the development of small molecules that are capable of modulating the activity of DOT1L.

SUMMARY OF THE INVENTION

[004] The disclosure relates to methods that include diagnosing if a subject has a partial tandem duplication (PTD) of the *MLL* gene (MLL-PTD) and, if the subject has MLL-PTD, administering a therapeutically effective amount of a DOT1L inhibitor to the subject.

[005] The present invention provides a method that includes steps of (i) providing a nucleic acid sample from a biological sample obtained from a subject; (ii) detecting the presence of a chromosomal rearrangement, wherein the chromosomal rearrangement is MLL-PTD; (iii) identifying the subject as a candidate for treatment; and selecting a therapy that includes the administration of a therapeutically effective amount of a DOT1L inhibitor to the subject identified in step (iii).

[006] The present invention also provides a method that includes the steps of (i) providing a nucleic acid sample from a biological sample obtained from a subject; (ii) contacting the nucleic acid sample with at least one primer that specifically hybridizes to a nucleic acid sequence of the *MLL* gene SEQ ID NO:61 and/or SEQ ID NO:62, a complement or a fragment thereof; (iii) amplifying at least a portion of the nucleic acid sequence of the *MLL* gene, a complement or a

fragment thereof; (iv) detecting the presence of the chromosomal rearrangement MLL-PTD by detecting the presence of an amplified nucleic acid with molecular weight larger than the molecular weight of an amplified nucleic acid of the corresponding wild type *MLL* gene; (v) identifying the subject as a candidate for treatment; and (vi) selecting a therapy that includes the administration of a therapeutically effective amount of a DOT1L inhibitor to the subject identified in step (v).

[007] In some embodiments, the method further comprises administering a therapeutically effective amount of a DOT1L inhibitor to the subject.

[008] In some embodiments, the detecting step is carried out by polymerase chain reaction (PCR), nested PCR, real-time PCR or multiplex ligation dependent probe amplification.

[009] In some embodiments, the detecting step comprises contacting the nucleic acid sample with at least one primer that specifically hybridizes to a nucleic acid sequence of the *MLL* gene SEQ ID NO:61 and/or SEQ ID NO:62, a complement or a fragment thereof.

[010] In some embodiments, the detecting step further comprises amplifying at least a portion of the nucleic acid sequence of the *MLL* gene, a complement or a fragment thereof. For example, the amplification is carried out by polymerase chain reaction (PCR), nested polymerase chain reaction (PCR), real-time PCR or multiplex ligation dependent probe amplification.

[011] In some embodiments, the primer is any one of SEQ ID NOs: 1-60.

[012] In some embodiments, the subject has a hematological cancer.

[013] In some embodiments, the subject had at least one prior therapy to treat a hematological cancer.

[014] In some embodiments, the hematological cancer is refractory to the prior therapy.

[015] In some embodiments, the hematological cancer shows recurrence following remission.

[016] In some embodiments, the subject received and failed all known effective therapies for the hematological cancer.

[017] In some embodiments, the hematological cancer is selected from the group consisting of acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, a myeloproliferative disorder, and chronic myelogenous leukemia.

[018] In some embodiments, the hematological cancer is leukemia.

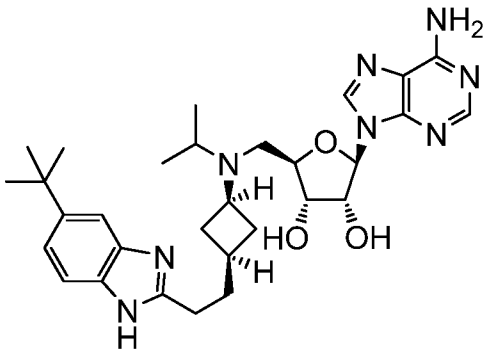
[019] In some embodiments, the subject has a leukemia characterized by MLL gene rearrangement. For example, the MLL gene rearrangement is a partial tandem duplication of the *MLL* gene.

[020] In some embodiments, the subject is simultaneously being treated with another therapy to treat acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, a myeloproliferative disorder, or chronic myelogenous leukemia.

[021] In some embodiments, another therapy is standard of care for the treatment of acute myeloid leukemia.

[022] In some embodiments, another therapy is standard of care for the treatment of acute lymphoblastic leukemia.

[023] In some embodiments, the DOT1L inhibitor used in any methods described herein is Compound A2 (also called EPZ-5676) having the formula:



(A2).

[024] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the specification, the singular forms also include the plural unless the context clearly dictates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents and other references mentioned herein are incorporated by reference. The references cited herein are not admitted to be prior art to the claimed invention. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods and examples are illustrative only and are not intended to be limiting.

[025] Other features and advantages of the invention will be apparent from the following detailed description and claims.

DESCRIPTION OF THE FIGURES

[026] Figures 1A and 1B are diagrams showing *mixed-lineage leukemia* (*MLL*) chromosome: Figure 1A is wild type and Figure 1B is rearranged chromosome indicating a partial tandem

duplication (PTD) of the *MLL* gene. "Old" and "new" indicate old and new numbering of exons, respectively.

[027] Figure 2 is a graph showing that EOL-1 (MLL-PTD$^+$) cells are sensitive to DOT1L inhibitors. Inhibition of EOL-1 proliferation (graph to the left) correlates with DOT1L inhibitor biochemical potency (table to the right).

[028] Figure 3 is a graph demonstrating that DOT1L inhibitor EPZ-5676 (*i.e.*, Compound A2) inhibits EOL-1 tumor growth treated by vehicle or by EPZ-5676 at various dosages (70mg/kg/day continuous infusion for 21 days; 35mg/kg/day continuous infusion for 21 days and 17.5mg/kg/day continuous infusion for 21 days).

[029] Figures 4A-4D are multiple graphs showing results of each individual experiment of each group shown in Figure 3. Figure 4A shows results of each individual experiment for vehicle treated animals. Figure 4B shows results of each individual experiment for EPZ-5676 treated animals (70mg/kg/day continuous infusion for 21 days). Figure 4C shows results of each individual experiment for EPZ-5676 treated animals (35mg/kg/day continuous infusion for 21 days). Figure 4D shows results of each individual experiment for EPZ-5676 treated animals (17.5mg/kg/day continuous infusion for 21 days).

[030] Figure 5 is a graph showing body weight change of animals treated by vehicle or EPZ-5676 at various dosages.

[031] Figure 6 includes tables showing mean or median EPZ-5676 plasma levels in the treated animals at different time points.

[032] Figures 7A-7D are multiple graphs demonstrating inhibition of H3K79 methylation in EOL-1 surrogate tissue. EOL-1 tumored nude rats were treated with vehicle or with EPZ-5676 for 7 days at 17.5, 35, and 70 mg/kg/day by continuous infusion. At day 7, tumors, PBMCs and bone marrow were harvested for analysis. Figure 7A shows H3K79me2 quantitative ELISA results of tumor tissue. Figure 7B shows H3K79me2 quantitative ELISA results of bone marrow. Figure 7C shows H3K79me2 quantitative Western Blot results of PBMCs. Figure 7D shows EPZ-5676 plasma concentration.

[033] Figures 8A-8F are graphs showing tumor target gene expression in EOL-1 tumors. Figure 8A shows expression of HOXA9. Figure 8B shows expression of MEIS2. Figure 8C shows expression of TBP. Figure 8D shows expression of BCL. Figure 8E shows expression of MEIS1. Figure 8F shows expression of FLT3.

[034] Figures 9A-9C show examples of the MLL-PTD variants including their relative

occurrence frequency. Exemplary PCR primer landing sites to detect the MLL-PTD variants are indicated in Figures 9A-9C as well. Figure 9A shows exemplary primer landing sites for MLL-PTD variant MLL-PTD 9-3. Figure 9B shows exemplary primer landing sites for MLL-PTD variant MLL-PTD 10-3. Figure 9C shows exemplary primer landing sites for MLL-PTD variant MLL-PTD 11-3.

DETAILED DESCRIPTION OF THE INVENTION

[035] The present invention is based upon the discovery that cells having a partial tandem duplication (PTD) chromosome rearrangement of the *mixed-lineage leukemia* (*MLL*) gene are sensitive to DOT1L inhibitors.

[036] Mixed lineage leukemia (MLL) is a genetically distinct form of acute leukemia that constitutes over 70% of infant leukemia and approximately 10% of adult acute myeloid leukemia (AML) (Hess, J. L. (2004), Trends Mol Med 10, 500-507; Krivtsov, A. V., and Armstrong, S. A. (2007), Nat Rev Cancer 7, 823-833). MLL represents a particularly aggressive form of leukemia and patients with this disease generally have poor prognoses; these patients often suffer from early relapse after treatment with current chemotherapies. MLL can be characterized by the genetic lesions of the *mixed-lineage leukemia* (*MLL*) gene. Such genetic lesions include chromosomal rearrangements, such as translocations, deletions, and/or duplications of the MLL gene. MLL has been categorized or characterized as having a chimeric fusion of MLL, partial tandem duplication of the MLL gene (MLL-PTD), or nonrearranged MLL.

[037] Genetic alterations of the *MLL* gene are commonly identified in acute leukemias. In acute myeloid leukemia (AML), a partial tandem duplication (PTD) of *MLL* occurs in about 5-10% of AML patients and is associated with adverse prognosis. The mutation leads to an in-frame duplication of exons 5 to 11 resulting in the production of an aberrant MLL protein. Myeloid leukemia cells with MLL-PTD are sensitive to pharmacological inhibition of the H3K79 methytransferase DOT1L. *See e.g.,* Kuhn et al., ASH Annual Meeting 2013; Poster Abstract, incorporated herein by reference. There is thus a great and present need for diagnosis and treatment modalities for patient suffering with MLL-PTD.

[038] Accordingly, the present invention provides methods for treating or alleviating a symptom of cancer in a subject in need of by first detecting the presence of chromosomal rearrangement *MLL*-PTD in the subject.

[039] In some embodiments, the present invention provides methods for identifying a subject who is responsive to DOT1L inhibitor treatment by (i) providing a nucleic acid sample from a biological sample obtained from the subject; (ii) detecting the presence of a chromosomal rearrangement, wherein the chromosomal rearrangement is MLL-PTD; and (iii) identifying the subject as a candidate for treatment if MLL-PTD is detected.

[040] In some embodiments, the present invention provides methods that include steps of (i) providing a nucleic acid sample from a biological sample obtained from a subject; (ii) detecting the presence of a chromosomal rearrangement, wherein the chromosomal rearrangement is MLL-PTD; (iii) identifying the subject as a candidate for treatment; and (iv) selecting a therapy that includes the administration of a therapeutically effective amount of a DOT1L inhibitor to the subject identified in step (iii).

[041] In some embodiments, the methods further comprise administering a therapeutically effective amount of a DOT1L inhibitor to the subject identified in step (iii).

[042] In some embodiments, the nucleic acid sequence of the *MLL* gene (wild type) is SEQ ID NO: 61 (NM_001197104.1). In some embodiments, the nucleic acid sequence of the *MLL* gene (wild type) is SEQ ID NO: 62 (NM_005933.3).

Homo sapiens lysine (K)-specific methyltransferase 2A (KMT2A), transcript variant 1, mRNA (SEQ ID NO: 61)

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Homo sapiens lysine (K)-specific methyltransferase 2A (KMT2A), transcript variant 2, mRNA (SEQ ID NO: 62)

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16561 ttaattaata aacatgtcaa gtttaaaaaa aaaaaaaaa
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[043] Detection of a MLL-PTD chromosomal rearrangement can be performed by any method known in the art. For example, PCR, reverse transcriptase PCR, real-time PCR, nested PCR, multiplex ligation dependent probe amplification (DNA-MLPA), FISH (fluorescence in situ hybridization) or DNA sequencing methods known in the art such as Sanger sequencing, de novo sequencing, shotgun sequencing, or next generation sequencing methods or any standard method for detecting translocations (such as utilizing sequence specific probes) may be used. In some embodiments, MLL-PTD is detected by real-time PCR. For example, PCR has been described in Dohner et al. J. Clin Oncol. 2002; 20: 3254-61, which is incorporated herein by reference. For example, nested PCR has been described in Caligiuri et al., Cancer Res 1996; 56: 1418-25; Schnittger et al., Blood 1998; 92(5):1728-34; Schnittger et al., Leukemia 2000; 14: 796-804; Shiah et al., Leukemia 2002; 16: 196-202; Dohner et al., Journal of Clinical Oncology 2002; 20: 3254-61; Weisser et al., Haematologica 2005; 90: 881-9; Fermandez et al., The New England Journal of Medicine 2009; 361: 1249-59; and Patel et al., The New England Journal of Medicine 2012; 366: 1079-89, all of which are incorporated herein by reference. For example, real-time PCR has been described in Ross et al., Blood 2004; 104: 3679-87; Whitman et al., Blood 2005; 106: 345-52; and Santamaria et al., Blood 2009; 114: 148-52, all of which are incorporated herein by reference. DNA-MLPA has been described in Balgobind et al., European Journal of Cancer 2010; 46: 1892-9, which is incorporated herein by reference.

[044] Exemplary primers or probes that can be used in detection are listed in the tables A-C below.

Table A. Forward Primer

Forward Primer (5'-3')	SEQ ID NO	Start NM_001197104.1	Stop NM_001197104.1
CCACCACCAGAATCAGGTCC	1	4020	4039
AGTCAAGCAAGCAGGTCTCC	3	3901	3920
AAGCCCGTCGAGGAAAAGAG	5	3816	3835

CAGCACTCTCTCCAATGGCA	7	4163	4182
TAGTGAGCCTCCTCCACGAA	9	3797	3816
GCCCAAGAAAAAGCAGCCTC	11	4001	4020
GAGCCCAAGAAAAAGCAGCC	13	3999	4018
TGTGGGAGATGGGAGGCTTA	15	4264	4283
GGGAGATGGGAGGCTTAGGA	17	4267	4286
CAGCAGATGGAGTCCACAGG	19	4204	4223
CTGGGACCAAACTACCCCAC	21	4551	4570
TGAATCCAAACAGGCCACCA	23	3866	3885
GGATCCACAACTCCAGGCAA	25	4632	4651
ACCACTCCTAGTGAGCCCAA	27	3987	4006
TCTGTTGTGAGCCCTTCCAC	29	4372	4391
GTCTGTTGTGAGCCCTTCCA	31	4371	4390
AGAAAAAGCAGCCTCCACCA	33	4006	4025
CCAAGTCTGTTGTGAGCCCT	35	4367	4386
TTTAGAGGAGAACGAGCGCC	37	4400	4419
TCAGCACTCTCTCCAATGGC	39	4162	4181
GCCGAAACAGCTATCACCCT	41	4525	4544
GTGCCAGTAGTGGGCATGTA	43	4333	4352
CCTGGGCCTGAATCCAAACA	45	3858	3877
CCACCTCCGGTCAATAAGCA	47	4116	4135
ACCTACTACAGGACCGCCAA	49	3950	3969

[045]

Table B. Reverse Primer			
Reverse Primer (5'-3')	**SEQ ID NO**	**Start NM_001197104.1**	**Stop NM_001197104.1**
TACTCCAGGGAAGGTGGGAG	2	476	457
GTGGCTTGCTGAAACGTAGC	4	586	567
CCGGACTTTCTGGGGCTTTT	6	776	757
CGGTCAGAGCCACTTCTAGG	8	337	318
AGCCACTTCTAGGTCTCCCA	10	330	311
TTACTCCAGGGAAGGTGGGA	12	477	458
TGGGGCTTTTCCAGTTCAGA	14	766	747
TACTCCAGGGAAGGTGGGAG	16	697	678
TCTGGGGCTTTTCCAGTTCA	18	768	749
GCCACTTCTAGGTCTCCCAC	20	329	310
CGGACTTTCTGGGGCTTTTC	22	775	756
CTTTCCGGACTTTCTGGGGC	24	780	761
GTGGCTTGCTGAAACGTAGC	26	807	788
AATGAGGAGACCCGAAGGGG	28	743	724
TCTTTGTGGCTTGCTGAAACG	30	591	571

TGCTGAAACGTAGCAGAAGGT	32	580	560
TTTCCGGACTTTCTGGGGC	34	779	761
CCGGACTTTCTGGGGCTTT	36	776	758
GCTGAATTTCGGTCAGAGCC	38	346	327
TTTACTCCAGGGAAGGTGGGA	40	478	458
GACCCGAAGGGGTCTTTATCC	42	735	715
TACTCCAGGGAAGGTGGGAG	44	697	678
CGGTCAGAGCCACTTCTAGG	46	558	539
AGCCACTTCTAGGTCTCCCA	48	551	532
TTACTCCAGGGAAGGTGGGA	50	698	679

[046]

Table C. Other Primers	SEQ ID NO
AGGAGAGAGTTTACCTGCTC	51
GGAAGTCAAGCAAGCAGGTC	52
ACACAGATGGATCTGAGAGG	53
GTCCAGAGCAGAGCAAACAG	54
GCCTTGTTTCTAGTGACAGG	55
GGAAGTCAAGCAAGCAGGTC	56
GGAGCAAGAGGTTCAGCATC	57
GTCCAGAGCAGAGCAAACAG	58
CGCACTCTGACTTCTTCATC	59
GTCCAGAGCAGAGCAAACAG	60

[047] In some embodiments, MLL-PTD variants include any partial tandem duplication (PTD) of *MLL* gene, including, but are not limited to, Exon9/Exon 3 duplication, Exon10/Exon 3 duplication, Exon 11/Exon 3 duplication, Exon 12/Exon 3, Exon 9/Exon 5, Exon 8/Exon 3 and Exon 8+ (*See e.g.,* Balgobind et al., European J. of Cancer 2010; 46: 1892-199; Caligiuri et al., Cancer Res 1996; 56: 1418-1425 (with old numbering), Dohner et al. 2002; 20: 3254-3261; Schnittger et al., Leukemia 2000; 14: 796-804 and Shiah et al. Leukemia 2002; 16: 196-202, which are all incorporated herein by reference). Figures 9A-9C show examples of the MLL-PTD variants including their relative occurrence (*See also* the references cited in this paragraph). Exemplary PCR primer landing sites to detect the MLL-PTD variants are indicated in Figure 9 as well. As indicated in Figure 1, there are old and new two numbering systems for the exons of the *MLL* gene.

[048] In some embodiments, the detecting step may comprise contacting the nucleic acid sample with at least one primer that specifically hybridizes to a nucleic acid sequence of the *MLL* gene, a complement or a fragment thereof.

[049] In some embodiments, the hybridization step is followed by an amplification step where the nucleic acid sequence of the *MLL* gene, a complement or a fragment thereof is amplified. The presence of an amplified nucleic acid with molecular weight larger than the molecular weight of an amplified nucleic acid of the corresponding wild type *MLL* gene indicates the presence of the chromosomal rearrangement MLL-PTD. In other words, the presence of an amplified nucleic acid that comprises a duplicate of at least a portion of one or more exons of the *MLL* gene indicates the presence of the chromosomal rearrangement MLL-PTD. For example, the presence of an amplified nucleic acid that includes (from 5' to 3') at least a portion of a higher numbered exon followed by at least a portion of a lower numbered exon of the *MLL* gene (such as a portion of Exon 9 followed by a portion of Exon 3 for Exon9/Exon 3 duplication variant) indicates the presence of the chromosomal rearrangement MLL-PTD.

[050] In another aspect, the present invention provides a method that includes the steps of (i) providing a nucleic acid sample from a biological sample obtained from a subject; (ii) contacting the nucleic acid sample with at least one primer that specifically hybridizes to a nucleic acid sequence of *MLL* gene, a complement or a fragment thereof; (iii) amplifying the nucleic acid sequence of *MLL* gene, a complement or a fragment thereof; (iv) detecting the presence of the chromosomal rearrangement MLL-PTD by detecting the presence of an amplified nucleic acid with molecular weight larger than the molecular weight of an amplified nucleic acid of the corresponding wild type *MLL* gene; and (vi) selecting a therapy that includes the administration of a therapeutically effective amount of a DOT1L inhibitor to the subject identified in step (v). In other words, the presence of an amplified nucleic acid that comprises a duplicate of at least a portion of one or more exons of the *MLL* gene indicates the presence of the chromosomal rearrangement MLL-PTD in step (iv). For example, the presence of an amplified nucleic acid that include (from 5' to 3') at least a portion of a higher numbered exon followed by at least a portion of a lower numbered exon of the *MLL* gene (such as a portion of Exon 9 followed by a portion of Exon 3 for Exon9/Exon 3 duplication variant) indicates the presence of the chromosomal rearrangement MLL-PTD. In some embodiments, the method further comprises administering a therapeutically effective amount of a DOT1L inhibitor to the subject identified in step (v).

[051] As used herein, a "subject" or "subject in need thereof" is a subject having cancer, hematologic cancer or leukemia or a subject having an increased risk of developing such disorder relative to the population at large. For example, the leukemia is acute myeloid leukemia, acute

lymphoblastic leukemia, acute mixed lineage leukemia, myelodysplastic syndrome, a myeloproliferative disorder, or chronic myelogenous leukemia. For example, the subject has a leukemia involving MLL-PTD. In certain embodiments the subject is the subject is unable to receive other therapy to treat the cancer due to age or intercurring illness. In certain embodiments the subject is at least 50 years old, or at least 60 years old, or at least 65 years old, or at least 70 years old or older.

[052] In some embodiments, the subject in need thereof had at least one prior therapy to treat acute myeloid leukemia, acute lymphoblastic leukemia, acute mixed lineage leukemia, myelodysplastic syndrome, a myeloproliferative disorder, or chronic myelogenous leukemia.

[053] In some embodiments, the subject has refractory cancer. Refractory cancer is a malignancy for which surgery is ineffective, which is either initially unresponsive to chemo- or radiation therapy, or which becomes unresponsive over time.

[054] In some embodiments, the subject has hematological cancer recurrence following remission.

[055] In some embodiments, the subject is not a candidate for allogeneic stem cell transplantation.

[056] In some embodiments, the subject is simultaneously being treated with another therapy to treat acute myeloid leukemia, acute lymphoblastic leukemia, acute mixed lineage leukemia, myelodysplastic syndrome, a myeloproliferative disorder, or chronic myelogenous leukemia. Additional therapies and agents that can be used to treat these cancers are known to the skilled artisan; and are described in U.S. Provisional Application No. 61/785,446, the contents of which are incorporated herein by reference in their entireties. In certain embodiments the patient is treated with the standard of care treatment as described in the most current National Comprehensive Cancer Network (NCCN) guidelines. For example, for the treatment of AML such therapy may include all-trans retinoic acid (ATRA), cytaribine (Ara-C), daunorubicin, idarubicine, arsenic trioxide (ATO, 6-mercaptopurine and/or methotrexate. For example, for the treatment of ALL the standard of care may include vincristine, corticosteroids such as prednisone, and/or methotrexate.

[057] In some embodiments, the subject received and failed all known effective therapies for the hematological cancer that the subject has or is suffering from.

[058] In some embodiments, a subject in need thereof may have a secondary cancer as a result of a previous therapy. “Secondary cancer” means cancer that arises due to or as a result from

previous carcinogenic therapies, such as chemotherapy. In some embodiments, the secondary cancer is a hematologic cancer, such as leukemia.

[059] In some embodiments, the subject has increased mRNA, protein, and/or activity level of at least one protein selected from the group consisting of HOXA9, FLT3, MEIS1, MEIS2, TBP, BCL, and DOT1L.

[060] A "subject" includes a mammal. The mammal can be *e.g.*, any mammal, *e.g.*, a human, primate, bird, mouse, rat, fowl, dog, cat, cow, horse, goat, camel, sheep or a pig. Preferably, the mammal is a human.

[061] As used herein, the term "responsiveness" is interchangeable with terms "responsive", "sensitive", and "sensitivity", and it is meant that a subject is showing therapeutic responses when administered an DOT1L inhibitor, *e.g.*, tumor cells or tumor tissues of the subject undergo apoptosis and/or necrosis, and/or display reduced growing, dividing, or proliferation. This term is also meant that a subject will or has a higher probability, relative to the population at large, of showing therapeutic responses when administered an DOT1L inhibitor, *e.g.*, tumor cells or tumor tissues of the subject undergo apoptosis and/or necrosis, and/or display reduced growing, dividing, or proliferation.

[062] As used herein, the term "increase in activity" refers to increased or a gain of function of a gene product/protein compared to the wild type. In one aspect of the present invention, increased activity can be caused by increased mRNA and/or increased protein levels. Increased mRNA levels can be caused by gene amplification and increased transcription, for example. Increased protein levels can be caused by increased stability, inhibition of degradation pathways, or increased transcription. Alternatively, increased activity levels can be caused by a gain of function mutation resulting from a point mutation (*e.g.*, a substitution, a missense mutation, or a nonsense mutation), an insertion, and/or a deletion, or a rearrangement in a polypeptide selected from the group consisting of HOXA9, FLT3, MEIS1, MEIS2, TBP, BCL, and DOT1L, or a nucleic acid sequence encoding a polypeptide selected from the group consisting of HOXA9, FLT3, MEIS1, MEIS2, TBP, BCL, and DOT1L. The mutations referred herein are somatic mutations. The term "somatic mutation" refers to a deleterious alteration in at least one gene allele that is not found in every cell of the body, but is found only in isolated cells. A characteristic of the somatic mutations as used herein is, that they are restricted to particular tissues or even parts of tissues or cells within a tissue and are not present in the whole organism harboring the tissues or cells. The term "wild-type" refers to a gene or gene product that has the

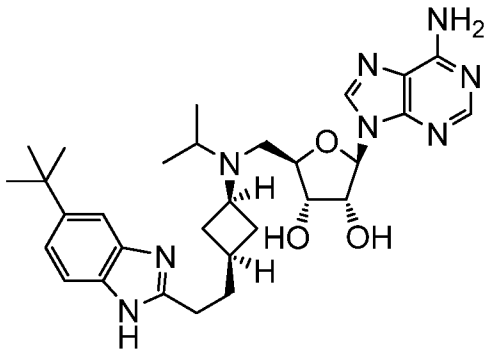
characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene.

[063] Accordingly, an increase in mRNA or protein expression and/or activity levels can be detected using any suitable method available in the art. For example, an increase in activity level can be detected by measuring the biological function of a gene product, such as the histone methyltransferase activity of DOT1L (*i.e.*, methylation of histone substrates such as H3K79 by immunoblot); transcriptional activity of HOXA9 or MEIS1 (*i.e.*, expression levels of HOXA9 or MEIS1 target genes by RT-PCR); or phosphorylation activity of FLT3 (*i.e.*, phosphorylation status of FLT3 targets by immunoblot or radioimmunoassay). Alternatively, a gain of function mutation can be determined by detecting any alternation in a nucleic acid sequence encoding a protein selected from the group consisting of HOXA9, FLT3, MEIS1 and DOT1L. For example, a nucleic acid sequence encoding HOXA9, FLT3, MEIS1 and DOT1L having a gain of function mutation can be detected by whole-genome resequencing or target region resequencing (the latter also known as targeted resequencing) using suitably selected sources of DNA and polymerase chain reaction (PCR) primers in accordance with methods well known in the art. The method typically and generally entails the steps of genomic DNA purification, PCR amplification to amplify the region of interest, cycle sequencing, sequencing reaction cleanup, capillary electrophoresis, and/or data analysis. Alternatively or in addition, the method may include the use of microarray-based targeted region genomic DNA capture and/or sequencing. Kits, reagents, and methods for selecting appropriate PCR primers and performing resequencing are commercially available, for example, from Applied Biosystems, Agilent, and NimbleGen (Roche Diagnostics GmbH). Detection of mRNA expression can be detected by methods known in the art, such as Northern blot, nucleic acid PCR, and quantitative RT-PCR. Detection of polypeptide expression (*i.e.*, wild-type or mutant) can be carried out with any suitable immunoassay in the art, such as Western blot analysis.

[064] By “sample” it means any biological sample derived from the subject, includes but is not limited to, cells, tissues samples, body fluids (including, but not limited to, mucus, blood, plasma, serum, urine, saliva, and semen), tumor cells, and tumor tissues. Preferably, the sample is selected from bone marrow, peripheral blood cells, blood, plasma and serum. Samples can be provided by the subject under treatment or testing. Alternatively samples can be obtained by the physician according to routine practice in the art.

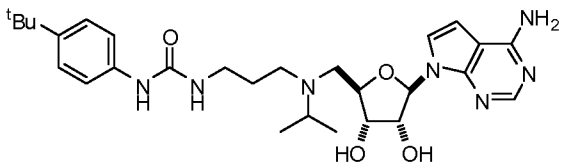
[065] "Risk" in the context of the present invention, relates to the probability that an event will occur over a specific time period and can mean a subject's "absolute" risk or "relative" risk. Absolute risk can be measured with reference to either actual observation post-measurement for the relevant time cohort, or with reference to index values developed from statistically valid historical cohorts that have been followed for the relevant time period. Relative risk refers to the ratio of absolute risks of a subject compared either to the absolute risks of low risk cohorts or an average population risk, which can vary by how clinical risk factors are assessed. Odds ratios, the proportion of positive events to negative events for a given test result, are also commonly used (odds are according to the formula p/(1-p) where p is the probability of event and (1- p) is the probability of no event) to no-conversion.

[066] In some embodiments, the DOT1L inhibitor used in any method described herein is Compound A2 (also called EPZ-5676) having the formula:



(A2).

[067] Alternatively, the DOT1L inhibitor used in any method described herein is Compound T (*i.e.*, Compound D16 or EPZ004777) having the formula



(D16).

[068] As used herein, "monotherapy" refers to the administration of a single active or therapeutic compound to a subject in need thereof. Preferably, monotherapy will involve administration of a therapeutically effective amount of a single active compound. For example, cancer monotherapy with one of the compound of the present invention, or a pharmaceutically acceptable salt, to a subject in need of treatment of cancer. In one aspect, the single active compound is a compound of the present invention, or a pharmaceutically acceptable salt.

[069] As used herein, "treating" or "treat" describes the management and care of a patient for the purpose of combating a disease, condition, or disorder and includes the administration of a

compound of the present invention, or a pharmaceutically acceptable salt, to alleviate the symptoms or complications of a disease, condition or disorder, or to eliminate the disease, condition or disorder.

[070] A compound of the present invention, or a pharmaceutically acceptable salt, can also be used to prevent a disease, condition or disorder. As used herein, "preventing" or "prevent" describes reducing or eliminating the onset of the symptoms or complications of the disease, condition or disorder.

[071] As used herein, the term "alleviate" is meant to describe a process by which the severity of a sign or symptom of a disorder is decreased. Importantly, a sign or symptom can be alleviated without being eliminated. In a preferred embodiment, the administration of pharmaceutical compositions of the invention leads to the elimination of a sign or symptom, however, elimination is not required. Effective dosages are expected to decrease the severity of a sign or symptom. For instance, a sign or symptom of a disorder such as cancer, which can occur in multiple locations, is alleviated if the severity of the cancer is decreased within at least one of multiple locations.

[072] As used herein, the term "severity" is meant to describe the potential of cancer to transform from a precancerous, or benign, state into a malignant state. Alternatively, or in addition, severity is meant to describe a cancer stage, for example, according to the TNM system (accepted by the International Union Against Cancer (UICC) and the American Joint Committee on Cancer (AJCC)) or by other art-recognized methods. Cancer stage refers to the extent or severity of the cancer, based on factors such as the location of the primary tumor, tumor size, number of tumors, and lymph node involvement (spread of cancer into lymph nodes). Alternatively, or in addition, severity is meant to describe the tumor grade by art-recognized methods (see, National Cancer Institute, www.cancer.gov). Tumor grade is a system used to classify cancer cells in terms of how abnormal they look under a microscope and how quickly the tumor is likely to grow and spread. Many factors are considered when determining tumor grade, including the structure and growth pattern of the cells. The specific factors used to determine tumor grade vary with each type of cancer. Severity also describes a histologic grade, also called differentiation, which refers to how much the tumor cells resemble normal cells of the same tissue type (see, National Cancer Institute, www.cancer.gov). Furthermore, severity describes a nuclear grade, which refers to the size and shape of the nucleus in tumor cells and the percentage of tumor cells that are dividing (see, National Cancer Institute, www.cancer.gov).

[073] In another aspect of the invention, severity describes the degree to which a tumor has secreted growth factors, degraded the extracellular matrix, become vascularized, lost adhesion to juxtaposed tissues, or metastasized. Moreover, severity describes the number of locations to which a primary tumor has metastasized. Finally, severity includes the difficulty of treating tumors of varying types and locations. For example, inoperable tumors, those cancers which have greater access to multiple body systems (hematological and immunological tumors), and those which are the most resistant to traditional treatments are considered most severe. In these situations, prolonging the life expectancy of the subject and/or reducing pain, decreasing the proportion of cancerous cells or restricting cells to one system, and improving cancer stage/tumor grade/histological grade/nuclear grade are considered alleviating a sign or symptom of the cancer.

[074] As used herein the term "symptom" is defined as an indication of disease, illness, injury, or that something is not right in the body. Symptoms are felt or noticed by the individual experiencing the symptom, but may not easily be noticed by others. Others are defined as non-health-care professionals.

[075] As used herein the term "sign" is also defined as an indication that something is not right in the body. But signs are defined as things that can be seen by a doctor, nurse, or other health care professional.

[076] Treating cancer can result in a reduction in size of a tumor. A reduction in size of a tumor may also be referred to as “tumor regression”. Preferably, after treatment, tumor size is reduced by 5% or greater relative to its size prior to treatment; more preferably, tumor size is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75% or greater. Size of a tumor may be measured by any reproducible means of measurement. The size of a tumor may be measured as a diameter of the tumor.

[077] Treating cancer can result in a reduction in tumor volume. Preferably, after treatment, tumor volume is reduced by 5% or greater relative to its size prior to treatment; more preferably, tumor volume is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75% or greater. Tumor volume may be measured by any reproducible means of measurement.

[078] Treating cancer results in a decrease in number of tumors. Preferably, after treatment, tumor number is reduced by 5% or greater relative to number prior to treatment; more preferably, tumor number is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75%. Number of tumors may be measured by any reproducible means of measurement. The number of tumors may be measured by counting tumors visible to the naked eye or at a specified magnification. Preferably, the specified magnification is 2x, 3x, 4x, 5x, 10x, or 50x.

[079] Treating cancer can result in a decrease in number of metastatic lesions in other tissues or organs distant from the primary tumor site. Preferably, after treatment, the number of metastatic lesions is reduced by 5% or greater relative to number prior to treatment; more preferably, the number of metastatic lesions is reduced by 10% or greater; more preferably, reduced by 20% or greater; more preferably, reduced by 30% or greater; more preferably, reduced by 40% or greater; even more preferably, reduced by 50% or greater; and most preferably, reduced by greater than 75%. The number of metastatic lesions may be measured by any reproducible means of measurement. The number of metastatic lesions may be measured by counting metastatic lesions visible to the naked eye or at a specified magnification. Preferably, the specified magnification is 2x, 3x, 4x, 5x, 10x, or 50x.

[080] Treating cancer can result in an increase in average survival time of a population of treated subjects in comparison to a population receiving carrier alone. Preferably, the average survival time is increased by more than 30 days; more preferably, by more than 60 days; more preferably, by more than 90 days; and most preferably, by more than 120 days. An increase in average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with an active compound. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with an active compound.

[081] Treating cancer can result in an increase in average survival time of a population of treated subjects in comparison to a population of untreated subjects. Preferably, the average survival time is increased by more than 30 days; more preferably, by more than 60 days; more preferably, by more than 90 days; and most preferably, by more than 120 days. An increase in

average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with an active compound. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with an active compound.

[082] Treating cancer can result in increase in average survival time of a population of treated subjects in comparison to a population receiving monotherapy with a drug that is not a compound of the present invention, or a pharmaceutically acceptable salt thereof. Preferably, the average survival time is increased by more than 30 days; more preferably, by more than 60 days; more preferably, by more than 90 days; and most preferably, by more than 120 days. An increase in average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with an active compound. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with an active compound.

[083] Treating cancer can result in a decrease in the mortality rate of a population of treated subjects in comparison to a population receiving carrier alone. Treating cancer can result in a decrease in the mortality rate of a population of treated subjects in comparison to an untreated population. Treating cancer can result in a decrease in the mortality rate of a population of treated subjects in comparison to a population receiving monotherapy with a drug that is not a compound of the present invention, or a pharmaceutically acceptable salt thereof. Preferably, the mortality rate is decreased by more than 2%; more preferably, by more than 5%; more preferably, by more than 10%; and most preferably, by more than 25%. A decrease in the mortality rate of a population of treated subjects may be measured by any reproducible means. A decrease in the mortality rate of a population may be measured, for example, by calculating for a population the average number of disease-related deaths per unit time following initiation of treatment with an active compound. A decrease in the mortality rate of a population may also be measured, for example, by calculating for a population the average number of disease-related deaths per unit time following completion of a first round of treatment with an active compound.

[084] Treating cancer can result in a decrease in tumor growth rate. Preferably, after treatment, tumor growth rate is reduced by at least 5% relative to number prior to treatment; more preferably, tumor growth rate is reduced by at least 10%; more preferably, reduced by at least 20%; more preferably, reduced by at least 30%; more preferably, reduced by at least 40%; more preferably, reduced by at least 50%; even more preferably, reduced by at least 50%; and most preferably, reduced by at least 75%. Tumor growth rate may be measured by any reproducible means of measurement. Tumor growth rate can be measured according to a change in tumor diameter per unit time.

[085] Treating cancer can result in a decrease in tumor regrowth. Preferably, after treatment, tumor regrowth is less than 5%; more preferably, tumor regrowth is less than 10%; more preferably, less than 20%; more preferably, less than 30%; more preferably, less than 40%; more preferably, less than 50%; even more preferably, less than 50%; and most preferably, less than 75%. Tumor regrowth may be measured by any reproducible means of measurement. Tumor regrowth is measured, for example, by measuring an increase in the diameter of a tumor after a prior tumor shrinkage that followed treatment. A decrease in tumor regrowth is indicated by failure of tumors to reoccur after treatment has stopped.

[086] Treating or preventing a cell proliferative disorder can result in a reduction in the rate of cellular proliferation. Preferably, after treatment, the rate of cellular proliferation is reduced by at least 5%; more preferably, by at least 10%; more preferably, by at least 20%; more preferably, by at least 30%; more preferably, by at least 40%; more preferably, by at least 50%; even more preferably, by at least 50%; and most preferably, by at least 75%. The rate of cellular proliferation may be measured by any reproducible means of measurement. The rate of cellular proliferation is measured, for example, by measuring the number of dividing cells in a tissue sample per unit time.

[087] Treating or preventing a cell proliferative disorder can result in a reduction in the proportion of proliferating cells. Preferably, after treatment, the proportion of proliferating cells is reduced by at least 5%; more preferably, by at least 10%; more preferably, by at least 20%; more preferably, by at least 30%; more preferably, by at least 40%; more preferably, by at least 50%; even more preferably, by at least 50%; and most preferably, by at least 75%. The proportion of proliferating cells may be measured by any reproducible means of measurement. Preferably, the proportion of proliferating cells is measured, for example, by quantifying the

number of dividing cells relative to the number of nondividing cells in a tissue sample. The proportion of proliferating cells can be equivalent to the mitotic index.

[088] Treating or preventing a cell proliferative disorder can result in a decrease in size of an area or zone of cellular proliferation. Preferably, after treatment, size of an area or zone of cellular proliferation is reduced by at least 5% relative to its size prior to treatment; more preferably, reduced by at least 10%; more preferably, reduced by at least 20%; more preferably, reduced by at least 30%; more preferably, reduced by at least 40%; more preferably, reduced by at least 50%; even more preferably, reduced by at least 50%; and most preferably, reduced by at least 75%. Size of an area or zone of cellular proliferation may be measured by any reproducible means of measurement. The size of an area or zone of cellular proliferation may be measured as a diameter or width of an area or zone of cellular proliferation.

[089] Treating or preventing a cell proliferative disorder can result in a decrease in the number or proportion of cells having an abnormal appearance or morphology. Preferably, after treatment, the number of cells having an abnormal morphology is reduced by at least 5% relative to its size prior to treatment; more preferably, reduced by at least 10%; more preferably, reduced by at least 20%; more preferably, reduced by at least 30%; more preferably, reduced by at least 40%; more preferably, reduced by at least 50%; even more preferably, reduced by at least 50%; and most preferably, reduced by at least 75%. An abnormal cellular appearance or morphology may be measured by any reproducible means of measurement. An abnormal cellular morphology can be measured by microscopy, *e.g.,* using an inverted tissue culture microscope. An abnormal cellular morphology can take the form of nuclear pleiomorphism.

[090] As used herein, the term "selectively" means tending to occur at a higher frequency in one population than in another population. The compared populations can be cell populations. Preferably, a compound of the present invention, or a pharmaceutically acceptable salt thereof, acts selectively on a cancer or precancerous cell but not on a normal cell. Preferably, a compound of the present invention, or a pharmaceutically acceptable salt thereof, acts selectively to modulate one molecular target (*e.g.,* a target protein methyltransferase) but does not significantly modulate another molecular target (*e.g.,* a non-target protein methyltransferase). The invention also provides a method for selectively inhibiting the activity of an enzyme, such as a protein methyltransferase. Preferably, an event occurs selectively in population A relative to population B if it occurs greater than two times more frequently in population A as compared to population B. An event occurs selectively if it occurs greater than five times more frequently in

population A. An event occurs selectively if it occurs greater than ten times more frequently in population A; more preferably, greater than fifty times; even more preferably, greater than 100 times; and most preferably, greater than 1000 times more frequently in population A as compared to population B. For example, cell death would be said to occur selectively in cancer cells if it occurred greater than twice as frequently in cancer cells as compared to normal cells.

[091] A compound of the present invention, or a pharmaceutically acceptable salt thereof, can modulate the activity of a molecular target (*e.g.*, a target protein methyltransferase). Modulating refers to stimulating or inhibiting an activity of a molecular target. Preferably, a compound of the present invention, or a pharmaceutically acceptable salt thereof, modulates the activity of a molecular target if it stimulates or inhibits the activity of the molecular target by at least 2-fold relative to the activity of the molecular target under the same conditions but lacking only the presence of said compound. More preferably, a compound of the present invention, or a pharmaceutically acceptable salt thereof, modulates the activity of a molecular target if it stimulates or inhibits the activity of the molecular target by at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold relative to the activity of the molecular target under the same conditions but lacking only the presence of said compound. The activity of a molecular target may be measured by any reproducible means. The activity of a molecular target may be measured *in vitro* or *in vivo*. For example, the activity of a molecular target may be measured *in vitro* by an enzymatic activity assay or a DNA binding assay, or the activity of a molecular target may be measured *in vivo* by assaying for expression of a reporter gene.

[092] A compound of the present invention, or a pharmaceutically acceptable salt thereof, does not significantly modulate the activity of a molecular target if the addition of the compound does not stimulate or inhibit the activity of the molecular target by greater than 10% relative to the activity of the molecular target under the same conditions but lacking only the presence of said compound.

[093] As used herein, the term "isozyme selective" means preferential inhibition or stimulation of a first isoform of an enzyme in comparison to a second isoform of an enzyme (*e.g.,* preferential inhibition or stimulation of a protein methyltransferase isozyme alpha in comparison to a protein methyltransferase isozyme beta). Preferably, a compound of the present invention, or a pharmaceutically acceptable salt thereof, demonstrates a minimum of a fourfold differential, preferably a tenfold differential, more preferably a fifty fold differential, in the dosage required to achieve a biological effect. Preferably, a compound of the present invention, or a

pharmaceutically acceptable salt thereof, demonstrates this differential across the range of inhibition, and the differential is exemplified at the IC_{50}, *i.e.,* a 50% inhibition, for a molecular target of interest.

[094] Treating cancer or a cell proliferative disorder can result in cell death, and preferably, cell death results in a decrease of at least 10% in number of cells in a population. More preferably, cell death means a decrease of at least 20%; more preferably, a decrease of at least 30%; more preferably, a decrease of at least 40%; more preferably, a decrease of at least 50%; most preferably, a decrease of at least 75%. Number of cells in a population may be measured by any reproducible means. A number of cells in a population can be measured by fluorescence activated cell sorting (FACS), immunofluorescence microscopy and light microscopy. Methods of measuring cell death are as shown in Li *et al.*, *Proc Natl Acad Sci U S A.* 100(5): 2674-8, 2003. In an aspect, cell death occurs by apoptosis.

[095] Preferably, an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof, is not significantly cytotoxic to normal cells. A therapeutically effective amount of a compound is not significantly cytotoxic to normal cells if administration of the compound in a therapeutically effective amount does not induce cell death in greater than 10% of normal cells. A therapeutically effective amount of a compound does not significantly affect the viability of normal cells if administration of the compound in a therapeutically effective amount does not induce cell death in greater than 10% of normal cells. In an aspect, cell death occurs by apoptosis.

[096] Contacting a cell with a compound of the present invention, or a pharmaceutically acceptable salt thereof, can induce or activate cell death selectively in cancer cells. Administering to a subject in need thereof a compound of the present invention, or a pharmaceutically acceptable salt thereof, can induce or activate cell death selectively in cancer cells. Contacting a cell with a compound of the present invention, or a pharmaceutically acceptable salt thereof, can induce cell death selectively in one or more cells affected by a cell proliferative disorder. Preferably, administering to a subject in need thereof a compound of the present invention, or a pharmaceutically acceptable salt thereof, induces cell death selectively in one or more cells affected by a cell proliferative disorder.

[097] The present invention relates to a method of treating or preventing cancer by administering a compound of the present invention, or a pharmaceutically acceptable salt thereof, to a subject in need thereof, where administration of the compound of the present invention, or a

pharmaceutically acceptable salt thereof, results in one or more of the following: accumulation of cells in G1 and/or S phase of the cell cycle, cytotoxicity via cell death in cancer cells without a significant amount of cell death in normal cells, antitumor activity in animals with a therapeutic index of at least 2, and activation of a cell cycle checkpoint. As used herein, "therapeutic index" is the maximum tolerated dose divided by the efficacious dose.

[098] One skilled in the art may refer to general reference texts for detailed descriptions of known techniques discussed herein or equivalent techniques. These texts include Ausubel *et al., Current Protocols in Molecular Biology*, John Wiley and Sons, Inc. (2005); Sambrook *et al., Molecular Cloning, A Laboratory Manual* (3rd edition), Cold Spring Harbor Press, Cold Spring Harbor, New York (2000); Coligan *et al., Current Protocols in Immunology*, John Wiley & Sons, N.Y.; Enna *et al., Current Protocols in Pharmacology*, John Wiley & Sons, N.Y.; Fingl *et al., The Pharmacological Basis of Therapeutics* (1975), *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, 18th edition (1990). These texts can, of course, also be referred to in making or using an aspect of the invention

[099] The present invention relates to use of the compounds disclosed herein in preparation of a medicament for treating or preventing cancer. Preferably, the cancer is leukemia. More preferably, the cancer is acute myeloid leukemia, acute lymphocytic leukemia or mixed lineage leukemia.

[0100] The present invention also provides pharmaceutical compositions comprising a compound disclosed herein in combination with at least one pharmaceutically acceptable excipient or carrier.

[0101] A "pharmaceutical composition" is a formulation containing the compounds of the present invention in a form suitable for administration to a subject. In one embodiment, the pharmaceutical composition is in bulk or in unit dosage form. The unit dosage form is any of a variety of forms, including, for example, a capsule, an IV bag, a tablet, a single pump on an aerosol inhaler or a vial. The quantity of active ingredient (*e.g.*, a formulation of the disclosed compound or salt, hydrate, solvate or isomer thereof) in a unit dose of composition is an effective amount and is varied according to the particular treatment involved. One skilled in the art will appreciate that it is sometimes necessary to make routine variations to the dosage depending on the age and condition of the patient. The dosage will also depend on the route of administration. A variety of routes are contemplated, including oral, pulmonary, rectal, parenteral, transdermal, subcutaneous, intravenous, intramuscular, intraperitoneal, inhalational, buccal, sublingual,

intrapleural, intrathecal, intranasal, and the like. Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. In one embodiment, the active compound is mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that are required.

[0102] As used herein, the phrase "pharmaceutically acceptable" refers to those compounds, materials, compositions, carriers, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0103] "Pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes excipient that is acceptable for veterinary use as well as human pharmaceutical use. A "pharmaceutically acceptable excipient" as used in the specification and claims includes both one and more than one such excipient.

[0104] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.,* intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), and transmucosal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0105] A compound or pharmaceutical composition of the invention can be administered to a subject in many of the well-known methods currently used for chemotherapeutic treatment. For example, for treatment of cancers, a compound of the invention may be injected directly into tumors, injected into the blood stream or body cavities or taken orally or applied through the skin with patches. The dose chosen should be sufficient to constitute effective treatment but not as

high as to cause unacceptable side effects. The state of the disease condition (*e.g.*, cancer, precancer, and the like) and the health of the patient should preferably be closely monitored during and for a reasonable period after treatment.

[0106] The term "therapeutically effective amount", as used herein, refers to an amount of a pharmaceutical agent to treat, ameliorate, or prevent an identified disease or condition, or to exhibit a detectable therapeutic or inhibitory effect. The effect can be detected by any assay method known in the art. The precise effective amount for a subject will depend upon the subject's body weight, size, and health; the nature and extent of the condition; and the therapeutic selected for administration. Therapeutically effective amounts for a given situation can be determined by routine experimentation that is within the skill and judgment of the clinician. In a preferred aspect, the disease or condition to be treated is cancer. In another aspect, the disease or condition to be treated is a cell proliferative disorder.

[0107] For any compound, the therapeutically effective amount can be estimated initially either in cell culture assays, *e.g.*, of neoplastic cells, or in animal models, usually rats, mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. Therapeutic/prophylactic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}. Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The dosage may vary within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[0108] Dosage and administration are adjusted to provide sufficient levels of the active agent(s) or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug interaction(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

[0109] The pharmaceutical compositions containing active compounds of the present invention may be manufactured in a manner that is generally known, *e.g.*, by means of conventional

mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. Pharmaceutical compositions may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers comprising excipients and/or auxiliaries that facilitate processing of the active compounds into preparations that can be used pharmaceutically. Of course, the appropriate formulation is dependent upon the route of administration chosen.

[0110] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol and sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0111] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0112] Oral compositions generally include an inert diluent or an edible pharmaceutically acceptable carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0113] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser, which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[0114] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0115] The active compounds can be prepared with pharmaceutically acceptable carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can

also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[0116] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved.

[0117] In therapeutic applications, the dosages of the pharmaceutical compositions used in accordance with the invention vary depending on the agent, the age, weight, and clinical condition of the recipient patient, and the experience and judgment of the clinician or practitioner administering the therapy, among other factors affecting the selected dosage. Generally, the dose should be sufficient to result in slowing, and preferably regressing, the growth of the tumors and also preferably causing complete regression of the cancer. Dosages can range from about 0.01 mg/kg per day to about 5000 mg/kg per day. In preferred aspects, dosages can range from about 1 mg/kg per day to about 1000 mg/kg per day. In an aspect, the dose will be in the range of about 0.1 mg/day to about 50 g/day; about 0.1 mg/day to about 25 g/day; about 0.1 mg/day to about 10 g/day; about 0.1 mg to about 3 g/day; or about 0.1 mg to about 1 g/day, in single, divided, or continuous doses (which dose may be adjusted for the patient's weight in kg, body surface area in m^2, and age in years). An effective amount of a pharmaceutical agent is that which provides an objectively identifiable improvement as noted by the clinician or other qualified observer. For example, regression of a tumor in a patient may be measured with reference to the diameter of a tumor. Decrease in the diameter of a tumor indicates regression. Regression is also indicated by failure of tumors to reoccur after treatment has stopped. As used herein, the term "dosage effective manner" refers to amount of an active compound to produce the desired biological effect in a subject or cell.

[0118] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[0119] The compounds of the present invention are capable of further forming salts. All of these forms are also contemplated within the scope of the claimed invention.

[0120] As used herein, "pharmaceutically acceptable salts" refer to derivatives of the compounds of the present invention wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines, alkali or organic salts of acidic residues such as carboxylic acids, and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include, but are not limited to, those derived from inorganic and organic acids selected from 2-acetoxybenzoic, 2-hydroxyethane sulfonic, acetic, ascorbic, benzene sulfonic, benzoic, bicarbonic, carbonic, citric, edetic, ethane disulfonic, 1,2-ethane sulfonic, fumaric, glucoheptonic, gluconic, glutamic, glycolic, glycollyarsanilic, hexylresorcinic, hydrabamic, hydrobromic, hydrochloric, hydroiodic, hydroxymaleic, hydroxynaphthoic, isethionic, lactic, lactobionic, lauryl sulfonic, maleic, malic, mandelic, methane sulfonic, napsylic, nitric, oxalic, pamoic, pantothenic, phenylacetic, phosphoric, polygalacturonic, propionic, salicyclic, stearic, subacetic, succinic, sulfamic, sulfanilic, sulfuric, tannic, tartaric, toluene sulfonic, and the commonly occurring amine acids, *e.g.*, glycine, alanine, phenylalanine, arginine, etc.

[0121] Other examples of pharmaceutically acceptable salts include hexanoic acid, cyclopentane propionic acid, pyruvic acid, malonic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo-[2.2.2]-oct-2-ene-1-carboxylic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, muconic acid, and the like. The present invention also encompasses salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, *e.g.*, an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like.

[0122] It should be understood that all references to pharmaceutically acceptable salts include solvent addition forms (solvates) or crystal forms (polymorphs) as defined herein, of the same salt.

[0123] The compounds, or pharmaceutically acceptable salts thereof, are administered orally, nasally, transdermally, pulmonary, inhalationally, buccally, sublingually, intraperintoneally, subcutaneously, intramuscularly, intravenously, rectally, intrapleurally, intrathecally and

parenterally. In one embodiment, the compound is administered orally. One skilled in the art will recognize the advantages of certain routes of administration.

[0124] The dosage regimen utilizing the compounds is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the condition.

[0125] Techniques for formulation and administration of the disclosed compounds of the invention can be found in *Remington: the Science and Practice of Pharmacy*, 19th edition, Mack Publishing Co., Easton, PA (1995). In an embodiment, the compounds described herein, and the pharmaceutically acceptable salts thereof, are used in pharmaceutical preparations in combination with a pharmaceutically acceptable carrier or diluent. Suitable pharmaceutically acceptable carriers include inert solid fillers or diluents and sterile aqueous or organic solutions. The compounds will be present in such pharmaceutical compositions in amounts sufficient to provide the desired dosage amount in the range described herein.

[0126] All percentages and ratios used herein, unless otherwise indicated, are by weight. Other features and advantages of the present invention are apparent from the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

[0127] In the synthetic schemes described herein, compounds may be drawn with one particular configuration for simplicity. Such particular configurations are not to be construed as limiting the invention to one or another isomer, tautomer, regioisomer or stereoisomer, nor does it exclude mixtures of isomers, tautomers, regioisomers or stereoisomers.

[0128] All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety

of embodiments and that the foregoing description and examples below are for purposes of illustration and not limitation of the claims that follow.

[0129] **Example 1 Cell Culture**

[0130] Human leukemia cell line EOL-1 (Catalog # ACC-386) was purchased from DSMZ and were grown in Roswell Park Memorial Institute medium (RPMI) with 10% Fetal Bovine Serum (FBS). Cells were kept in log growth as outlined in the technical data sheet provided by the vendor.

[0131] Exponentially growing EOL-1 cells were plated in 96-well plates at a density of 3×10^4 viable cells/well. Each treatment was seeded in triplicate with a final well volume of 150 μLs. Cells were incubated with increasing concentrations of DOT1L inhibitor up to50 μM. Viable cell number was determined every 3 – 4 days for 11 days using the Guava Viacount assay (Millipore # 4000-0040) and analyzed on a Guava EasyCyte Plus instrument according to the manufacturer's protocol. On the days of cell counts, growth media and inhibitor were replenished and cells maintained in log phase culture by reseeding at a density of 5×10^4 viable cells/well. Total cell number was expressed as split-adjusted viable cells per well. For each cell inhibitor IC50 values were determined from concentration-dependence curves at day 11. All calculations were done using GraphPad Prism, version 5.00 for Windows, GraphPad Software, San Diego California USA (graphpad.com).

[0132] **Example 2 Histone Extraction of Cell Pellets**

[0133] Frozen pellets were allowed to thaw briefly on ice and then lysed by a 5 minute incubation on ice with 250 μl nuclear extraction buffer (10 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 25 mM KCl, 1% Triton X-100, 8.6% Sucrose, plus a Roche protease inhibitor tablet 1836153001). Nuclei were collected by centrifugation at 600 g for 5 minutes at 4°C and washed once in Tris/EDTA buffer (pH 7.4). Supernatant was removed and histones extracted for one hour with 60 μl 0.4 N cold sulfuric acid. Extracts were clarified by centrifugation at 10,000 g for 10 minutes at 4°C and transferred to a fresh microcentrifuge tube containing 600 μl ice cold acetone. Histones were precipitated at -20° C for 2 hours, pelleted by centrifugation at 10,000 g for 10 minutes and resuspended in 60 μl distilled water (DI water). Total protein of the acid extracts was assessed using a bicinchoninic acid (BCA) protein quantification assay with a bovine serum albumin (BSA) standard (Pierce Biotechnology).

[0134] **Example 3 H3K79me2 Immunoblot**

[0135] For immunoblot analysis of the H3K79me2 inhibition by EPZ-5676, exponentially

growing EOL-1 cells were seeded at 2×10^5 cells/mL and incubated in the presence of increasing concentrations of EPZ-5676 for 4 days. Following incubation, cells ($2\text{-}3\times10^6$) were harvested and histones extracted as described. Histones (400 ng) were fractionated on a 10-20% Tris HCl gels (Bio-Rad) with Tris-Glycine SDS running buffer (Invitrogen) under denaturing conditions and transferred to a nitrocellulose filter. The filter was incubated for 1 hour in blocking buffer (Odyssey blocking buffer, Li-cor, 927-40000) at RT and then incubated overnight at 4°C in blocking buffer containing a antibody specific for H3K79me2 (1:5000 dilution, abcam ab3594). Filters were washed 3 times for 5 minutes with wash buffer (PBST) and incubated with infrared tagged secondary antibody (Alexa Flour 680 goat anti-rabbit IgG (1:20,000), Invitrogen A-21076) at RT for 1 hour. Filters were washed in PBST and reprobed for 1 hour at RT with the appropriate total histone antibody control (mouse anti-histone H3 (1:20,000), CST 3638, or mouse anti-histone H4 (1:10,000), CST 2935). Filters were washed again in PBST and incubated with infrared tagged secondary antibody (IRDye 800Cw donkey-anti-mouse IgG (1:20,000), Li-Cor 926-32212) at RT for 1 hour. After a final wash in PBST, filters were scanned using the Odyssey infared imager (Li-cor). Signal intensities specific for each methyl-specific antibody was quantified using Odyssey software and normalized to that of the appropriate total histone control signal on the same filter by dividing the methyl-specific antibody signal intensity by the total histone control signal intensity.

[0136] **Example 4 Quantitative Real-Time PCR**

[0137] Exponentially growing EOL-1 cells were plated in a 12 well plate at 2×10^5 cells/mL. Cells were incubated in the presence of increasing concentrations of EPZ-5676 up to 10 μM. On day 4, cells were maintained in log phase culture by reseeding at 5×10^5 cells/mL and compound was replenished. At day 6 cells were washed twice with PBS and pelleted by centrifugation at 200 X g. Cell pellets were lysed in 300 μL RLT buffer (Qiagen) and total RNA was isolated using the RNeasy total RNA isolation kit (Qiagen 74106). Total RNA (1 μg) was reverse transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems 4368813). RNA isolation and cDNA synthesis were carried out according to the manufacturer's protocol. Predesigned labeled primer and probe sets for *HOXA9* (Hs00365956), *MEIS1* (Hs00180020) and *FLT3* (Hs00975659) were purchased from Applied Biosystems. Quantitative real-time PCR (qPCR) reactions contained 50 ng cDNA, 1X labeled primer and probe set, and 1X Taqman universal PCR master mix (Applied Biosystems 4304437). Samples were run on a 7900 HT Fast

Real Time PCR machine (Applied Biosystems 4351405) with cycling conditions of 2 min 50°C, 10 min 95°C, 40 cycles at 15 sec 95°C and 1 min 60°C. Target gene cycle numbers were normalized to the house keeping gene β2-microglobulin (Applied Biosystems 4333766) to get a ΔCT value. Percent of DMSO control was calculated with the equation $(2^{-\Delta\Delta CT})*100$ where the ΔΔCT is the difference between normalized target gene and DMSO control (ΔCT sample – ΔCT control = ΔΔCT).

[0138] **Example 5 Rat EOL-1 xenograft studies**

[0139] All studies performed were conducted after review by the appropriate animal care and use committee at Charles River Discovery Research Services (North Carolina). EOL-1 ($1 X 10^7$) cells resuspended in 100% Matrigel (BD Biosciences) were implanted subcutaneously into female athymic nude rats (*rnu/rnu*, Harlan). Tumors were measured by calipers and rats were randomized according to tumor size into treatment groups before the initiation of dosing with EPZ-5676. EPZ-5676 was delivered by IV infusion into the femoral vein continuously for 21 days in 5% hydroxypropyl-β-cyclodextrin (HPBCD) in saline. The dosing rate was fixed at 7 mL/animal/day in all groups, and dosing concentrations were adjusted for the last recorded weights of individual animals. For efficacy studies 10 animals were randomized into each treatment group when tumor volumes reached approximately 500 mm^3. Rats were weighed and tumors measured with calipers twice weekly until the end of the study. Each test animal was euthanized when its neoplasm reached the predetermined endpoint volume or on the last day of the study, whichever came first. Optimal tumor volume endpoints were chosen based the growth characteristics of the EOL-1 xenograft model in pilot studies. Significance of treatment compared to control was measured using the repeated measures ANOVA with Dunnet post test versus the vehicle treated group. For pharmacokinetic studies, blood samples (0.2 mL) were drawn from the saphaneous vein, flash frozen and subsequently analysed for EPZ-5676 levels by HPLC/MS/MS. For the pharmacodynamic study, 5 animals were randomized in each treatment group when tumors volumes reached approximately 1000 mm^3 and then animals were dosed by continuous IV infusion for 7 days. On day 7 animals were euthanized and tumors, peripheral blood mononuclear cells and bone marrow cells were isolated and flash frozen. A detailed description of tissue isolation and processing for PBMC whole cell lysates, tumor and bone marrow histones and tumor RNA can be found below.

[0140] **Whole cell lysate preparation from rat PBMCs**

[0141] Rats were euthanized at the end of infusion by terminal cardiac puncture under CO_2

anesthesia and sampled for full blood volume. PBMCs were isolated from whole blood by centrifugation over Ficoll-Paque Plus (GE Healthcare) and treatment with ACK lysis buffer (Gibco). PBMC pellets were flash frozen and stored at –80 °C. PBMC whole cell lysates were prepared by thawing pellets and adding 50 µL Tris-Glycine SDS protein sample buffer (Invitrogen, LC2676) containing 1% 2-Mercaptoethanol. Lysate was mixed by pipet and incubated at 95°C for 2 minutes.

[0142] **Histone isolation from tumor xenografts**

[0143] Rats were euthanized as described above and tumors were harvested, snap frozen in liquid nitrogen, pulverized using a mortar and pestle and stored at -80°C. 30 mg of tumor powder was lysed in 500 µL nuclear extraction buffer (10 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 25 mM KCl, 1% Triton X-100, 8.6% Sucrose, plus a Roche protease inhibitor tablet 1836145). After 5 minutes in extraction buffer the samples were homogenized using a handheld homogenizer (Cole Parmer, R-04727-11). Nuclei were collected by centrifugation at 600 × g for 5 minutes in a 4°C rotor and washed once in ice cold PBS. Supernatant was removed and histones extracted for one hour following addition of 0.4 N cold sulfuric acid at a ratio of 1 µL per 0.6 mg powder. Extracts were clarified by centrifugation at 10,000 × g for 10 minutes at 4°C and transferred to a fresh microcentrifuge tube containing ice cold acetone at a 10 X volume of the sulfuric acid. Histones were precipitated at -20°C for 2 hours, pelleted by centrifugation at 10,000 × g for 10 minutes at 4°C and resuspended in water with a volume equal to that used in the extraction step. Histone protein concentrations were measured using a bicinchoninic acid protein quantification assay with a BSA standard (Pierce Biotechnology).

[0144] **Histone isolation from rat bone marrow tissue**

[0145] Rats were euthanized as described above and, bone marrow cells were flushed from rat tibia, femur and hip bones using ice cold PBS. Cell pellets were flash frozen and stored at –80 °C. Histones were extracted from cell pellets following lysis in 500 µls of nuclear extraction buffer (10 mM Tris-HCl, 10 mM MgCl2, 25 mM KCl, 1% Triton X-100, 8.6% Sucrose, plus a Roche protease inhibitor tablet 1836145). Nuclei were collected by centrifugation at 600 g for 5 minutes at 4° C and washed once in TE buffer (pH 7.4). Supernatant was removed and histones extracted for one hour with 0.4 N cold sulfuric acid. Extracts were clarified by centrifugation at 10000 g for 10 minutes at 4°C and transferred to a fresh microcentrifuge tube containing 10 x volume of ice cold acetone. Histones were precipitated at -20° C for 2 hours, pelleted by centrifugation at 10000 g for 10 minutes and resuspended in 75 µls water. Histones were

quantified using the BCA protein assay (Pierce 23225).

[0146] **RNA isolation from tumor xenografts**

[0147] Rats were euthanized and tumors were collected and powdered as described above. 10 mg of powdered tumor was lysed in 600 μL RLTlysis buffer (Qiagen) and homogenized in a TissueLyser (Qiagen, 85210). Supernatant was collected and total RNA was isolated using the RNeasy Total RNA isolation kit (Qiagen 74106) according to manufacturer's instructions.

[0148] **Example 6**

[0149] An exemplary method according to the disclosure:

[0150] Step 1: obtaining a biological sample from a subject. Any biological sample derived from the subject can be used in the assay. For example, cells, tissues samples, body fluids (including, but not limited to, mucus, blood, plasma, serum, urine, saliva, and semen), tumor cells, and tumor tissues. Preferably, the sample is selected from bone marrow, peripheral blood cells, blood, plasma and serum. Samples can be provided by the subject under treatment or testing. Alternatively samples can be obtained by the physician according to routine practice in the art.

[0151] Step 2: isolating a nucleic acid sample (DNA or mRNA) from the biological sample obtained from a subject according to any known method in the art.

[0152] Step 3: carrying out PCR, real time PCR, nested PCR or multiplex ligation dependent probe amplification assay according to the standard protocol available in the art utilizing the isolated nucleic acid as the template and one or more primers (SEQ ID NO: 1-60) described herein.

[0153] Step 4: determining the molecular weight (size) of the amplified nucleic acid by, for example, running an electrophoresis gel. If the molecular weight of at least some of the amplified nucleic acid is larger than the molecular weight of an amplified nucleic acid of the corresponding wild type *MLL* gene, the tested subject has chromosomal rearrangement MLL-PTD and could be treated with a DOT1L inhibitor *(e.g.*, Compound A2).

[0154] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

[0155] The invention can be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and

all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

CLAIMS

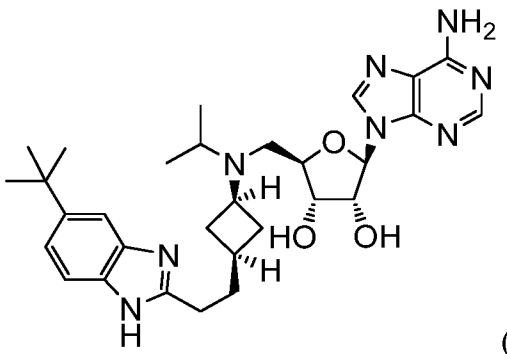
What is claimed is:

1. A method comprising steps of:
   (i) providing a nucleic acid sample from a biological sample obtained from a subject;
   (ii) detecting the presence of a chromosomal rearrangement, wherein the chromosomal rearrangement is partial tandem duplication of MLL (MLL-PTD);
   (iii) identifying the subject as a candidate for treatment; and
   (iv) selecting a therapy that includes the administration of a therapeutically effective amount of a DOT1L inhibitor to the subject identified in step (iii).

2. A method comprising steps of:
   (i) providing a nucleic acid sample from a biological sample obtained from a subject;
   (ii) contacting the nucleic acid sample with at least one primer that specifically hybridizes to a nucleic acid sequence of the *MLL* gene SEQ ID NO:61 and/or SEQ ID NO:62, a complement or a fragment thereof;
   (iii) amplifying the nucleic acid sequence of the *MLL* gene, a complement or a fragment thereof;
   (iv) detecting the presence of the chromosomal rearrangement MLL-PTD by detecting the presence of an amplified nucleic acid with molecular weight larger than the molecular weight of an amplified nucleic acid of the corresponding wild type *MLL* gene;
   (v) identifying the subject as a candidate for treatment; and
   (vi) selecting a therapy that includes the administration of a therapeutically effective amount of a DOT1L inhibitor to the subject identified in step (v).

3. The method of claim 1 or claim 2, further comprising administering a therapeutically effective amount of a DOT1L inhibitor to the subject.

4. The method of any preceding claim, wherein the DOT1L inhibitor is Compound A2 having the formula



(A2) or a pharmaceutically acceptable salt thereof.

5. The method of claim 1, wherein the detecting step comprises contacting the nucleic acid sample with at least one primer that specifically hybridizes to a nucleic acid sequence of the *MLL* gene SEQ ID NO:61 and/or SEQ ID NO:62, a complement or a fragment thereof.

6. The method of claim 5, wherein the detecting step further comprises amplifying at least a portion of the nucleic acid sequence of the *MLL* gene, a complement or a fragment thereof.

7. The method of claim 6, wherein the amplification is carried out by polymerase chain reaction (PCR), nested polymerase chain reaction (PCR), real-time PCR or multiplex ligation dependent probe amplification.

8. The method of claim 1, wherein the detecting step is carried out by polymerase chain reaction (PCR), nested PCR, real-time PCR or multiplex ligation dependent probe amplification.

9. The method of any preceding claim, wherein the primer is any one of SEQ ID NOs: 1-60.

10. The method of any preceding claim, wherein the subject has a hematological cancer.

11. The method of any preceding claim, wherein the subject had at least one prior therapy to treat a hematological cancer.

12. The method of claim 11, wherein the hematological cancer is refractory to the prior therapy.

13. The method of claim 11, wherein the hematological cancer shows recurrence following remission.

14. The method of claim 10, wherein the subject received and failed all known effective therapies for the hematological cancer.

15. The method of claim 10, wherein the hematological cancer is selected from the group consisting of acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, a myeloproliferative disorder, and chronic myelogenous leukemia.

16. The method of claim 10, wherein the hematological cancer is leukemia.

17. The method of claim 16, wherein the subject has a leukemia characterized by MLL gene rearrangement.

18. The method of claim 17, wherein the MLL gene rearrangement is a partial tandem duplication of the MLL gene.

19. The method of claim 1, wherein the subject is simultaneously being treated with another therapy to treat acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, a myeloproliferative disorder, or chronic myelogenous leukemia.

20. The method of claim 19, wherein the another therapy is standard of care for the treatment of acute myeloid leukemia.

21. The method of claim 19, wherein the another therapy is standard of care for the treatment of acute lymphoblastic leukemia.

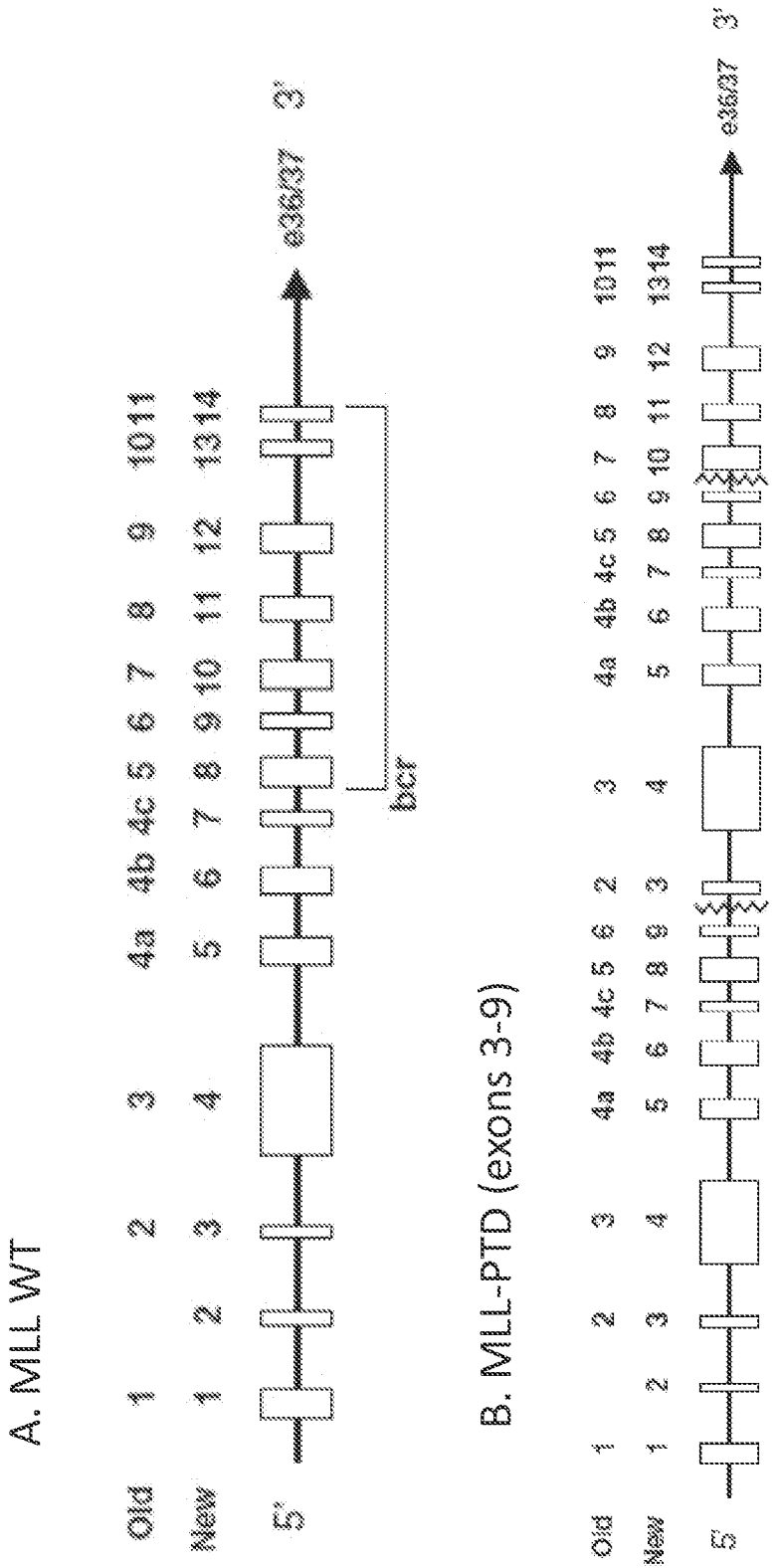

A. MLL WT
Old 1 2 3 4a 4b 4c 5 6 7 8 9 10 11
New 1 2 3 4 5 6 7 8 9 10 11 12 13 14
5' e36/37 3'
bcr
B. MLL-PTD (exons 3-9)
Old 1 2 3 4a 4b 4c 5 6 7 8 9 10 11
New 1 2 3 4 5 6 7 8 9 10 11 12 13 14
5' e36/37 3'

FIG. 1

Compound	K_i (nM)	Prolif. IC_{50} (μM)
EPZ004777	0.3	0.012
EPZ-5676	< 0.08	0.002

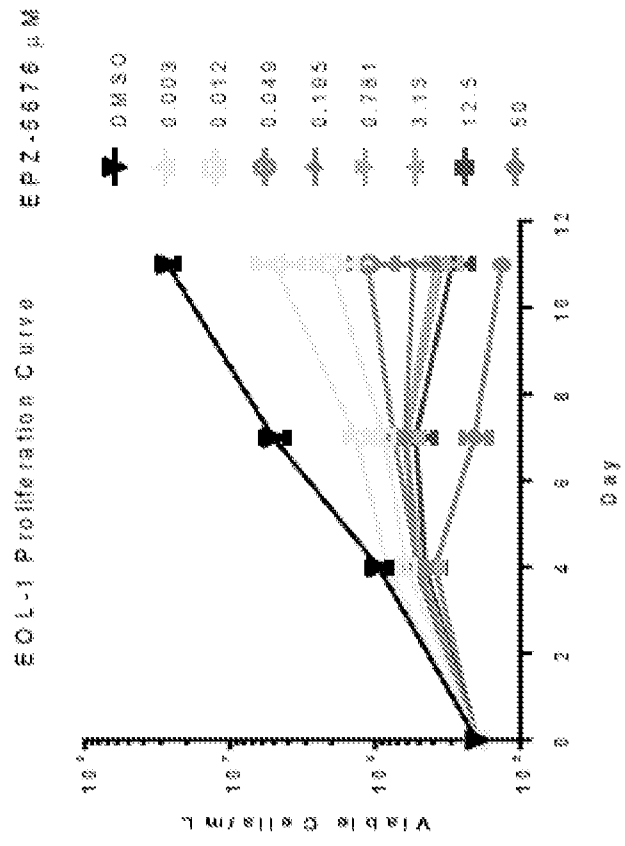


FIG. 2

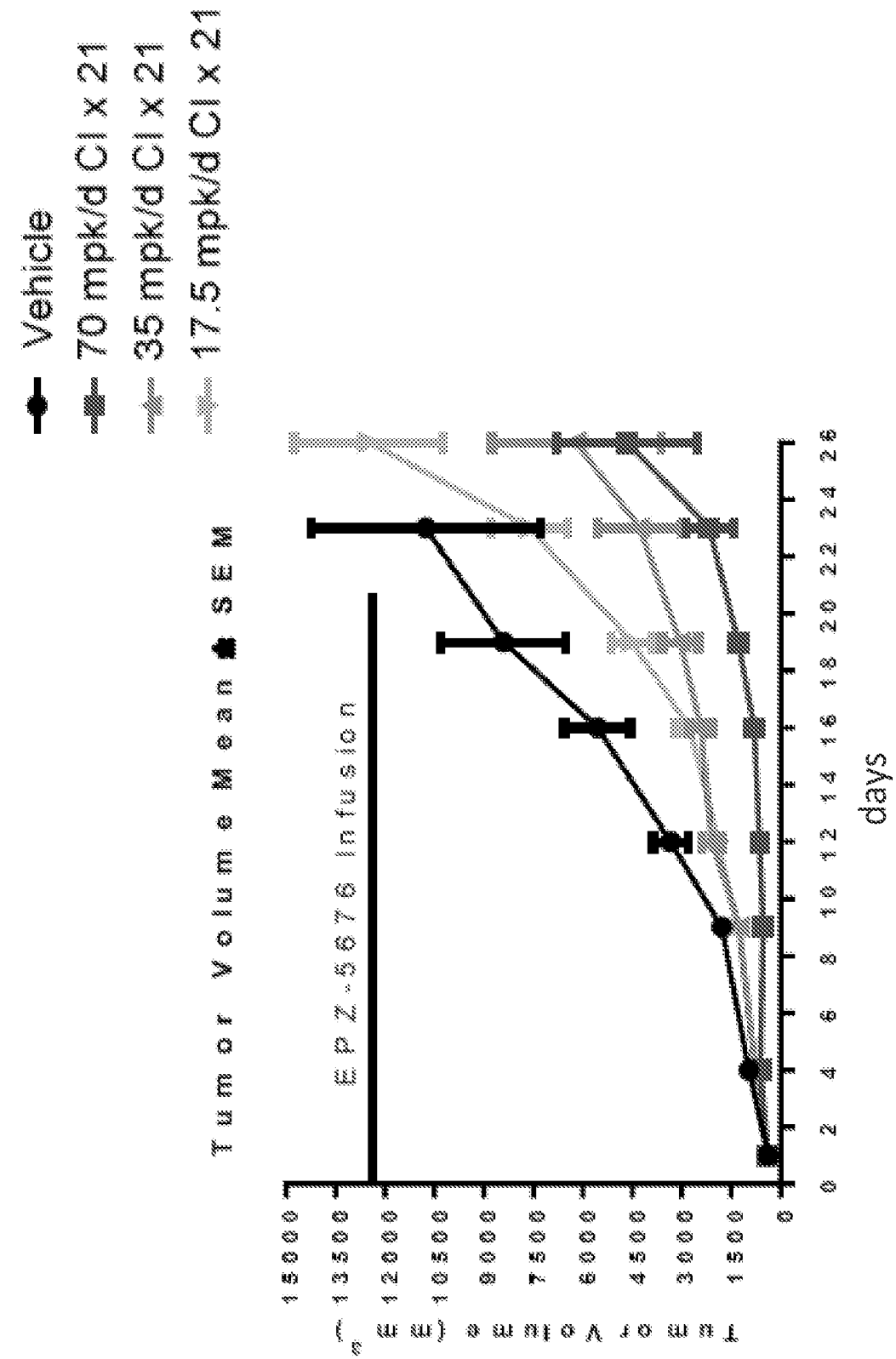

Vehicle
70 mpk/d CI x 21
35 mpk/d CI x 21
17.5 mpk/d CI x 21
Tumor Volume Mean ± SEM
EPZ-5676 Infusion
Tumor Volume (mm³)
15000
13500
12000
10500
9000
7500
6000
4500
3000
1500
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
days

FIG. 3

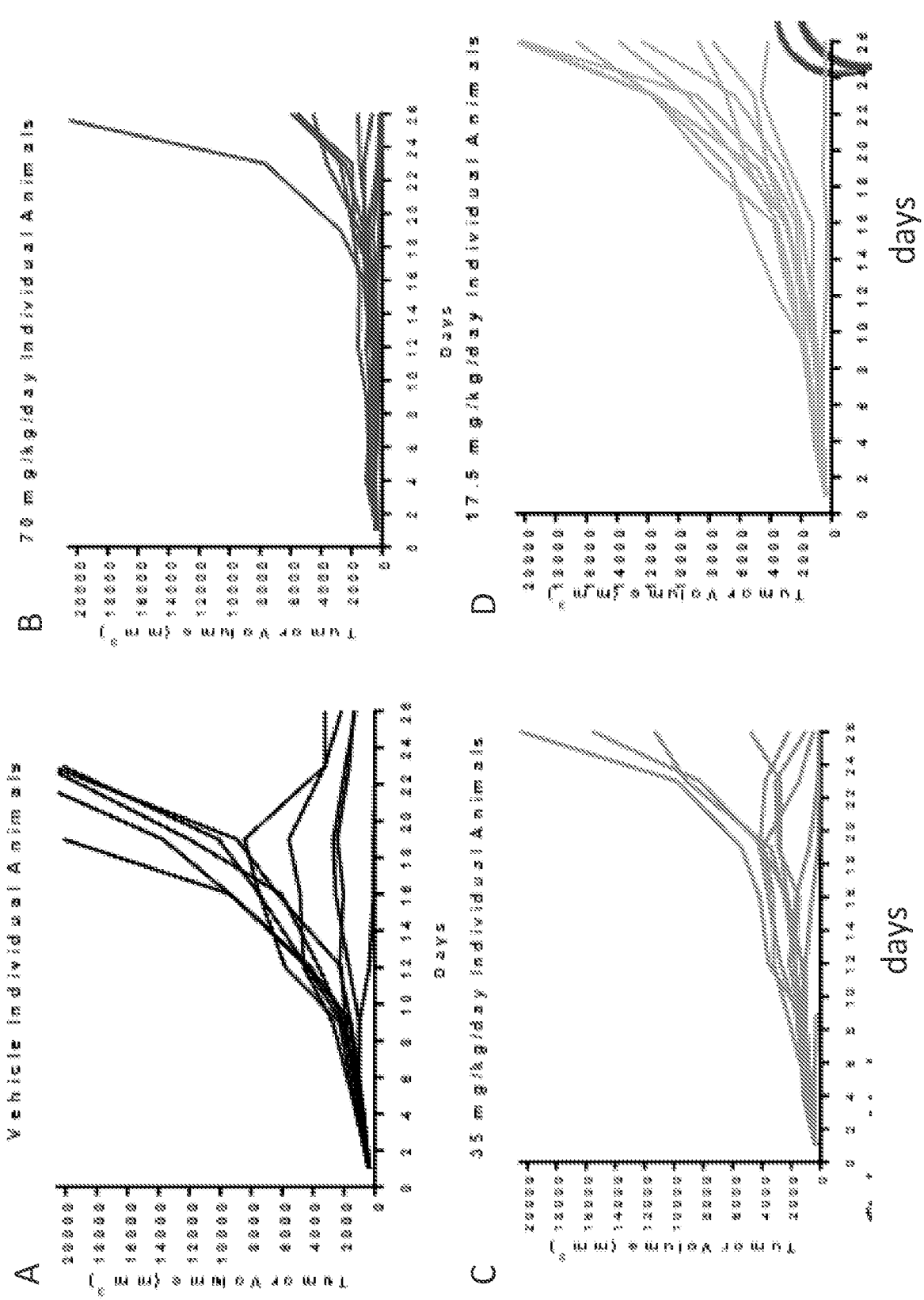

A
Vehicle Individual Animals
B
70 mg/kg/day Individual Animals
C
35 mg/kg/day Individual Animals
D
17.5 mg/kg/day Individual Animals
Tumor Volume (mm3)
Days
days

FIG. 4

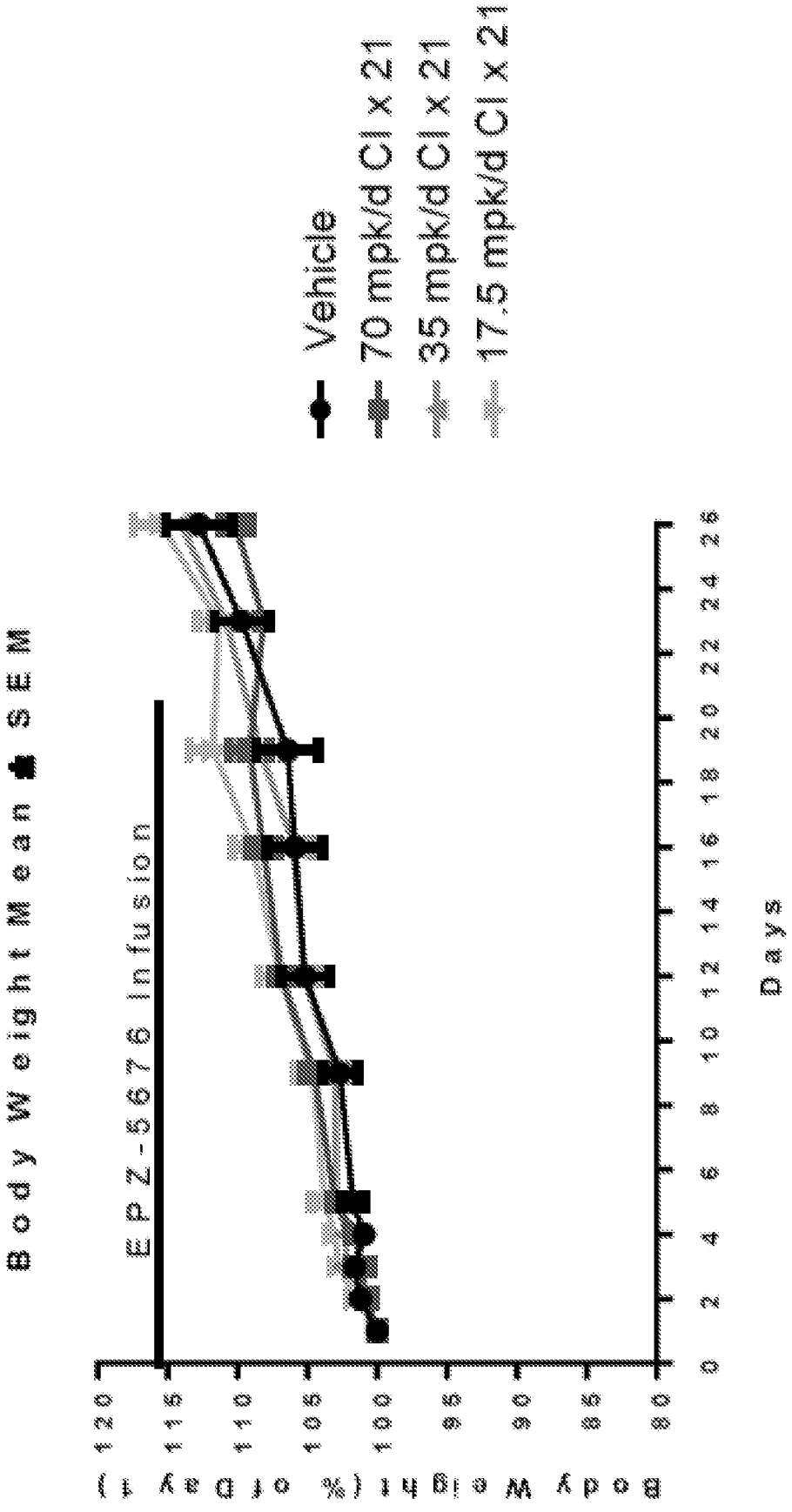

Vehicle
70 mpk/d Cl x 21
35 mpk/d Cl x 21
17.5 mpk/d Cl x 21
Body Weight Mean ± SEM
EPZ-5676 Infusion
Body weight (% of day 1)
120
115
110
105
100
95
90
85
80
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Days

FIG. 5

Mean ±SD EPZ-5676 ng/ml Plasma Levels

	Day 1	Day 8	Day 15	Day 22
70 mg/kg/day	1058 ± 219	970 ± 141	396 ± 127	836 ± 489
35 mg/kg/day	1249 ± 1705	582 ± 315	185 ± 57	348 ± 168
17.5 mg/kg/day	1479 ± 1595	245 ± 75	477 ± 1005	533 ± 762

Median EPZ-5676 ng/ml Plasma Levels

	Day 1	Day 8	Day 15	Day 22
70 mg/kg/day	1,077	1,032	404	757
35 mg/kg/day	520	397	190	399
17.5 mg/kg/day	449	211	168	332

FIG. 6

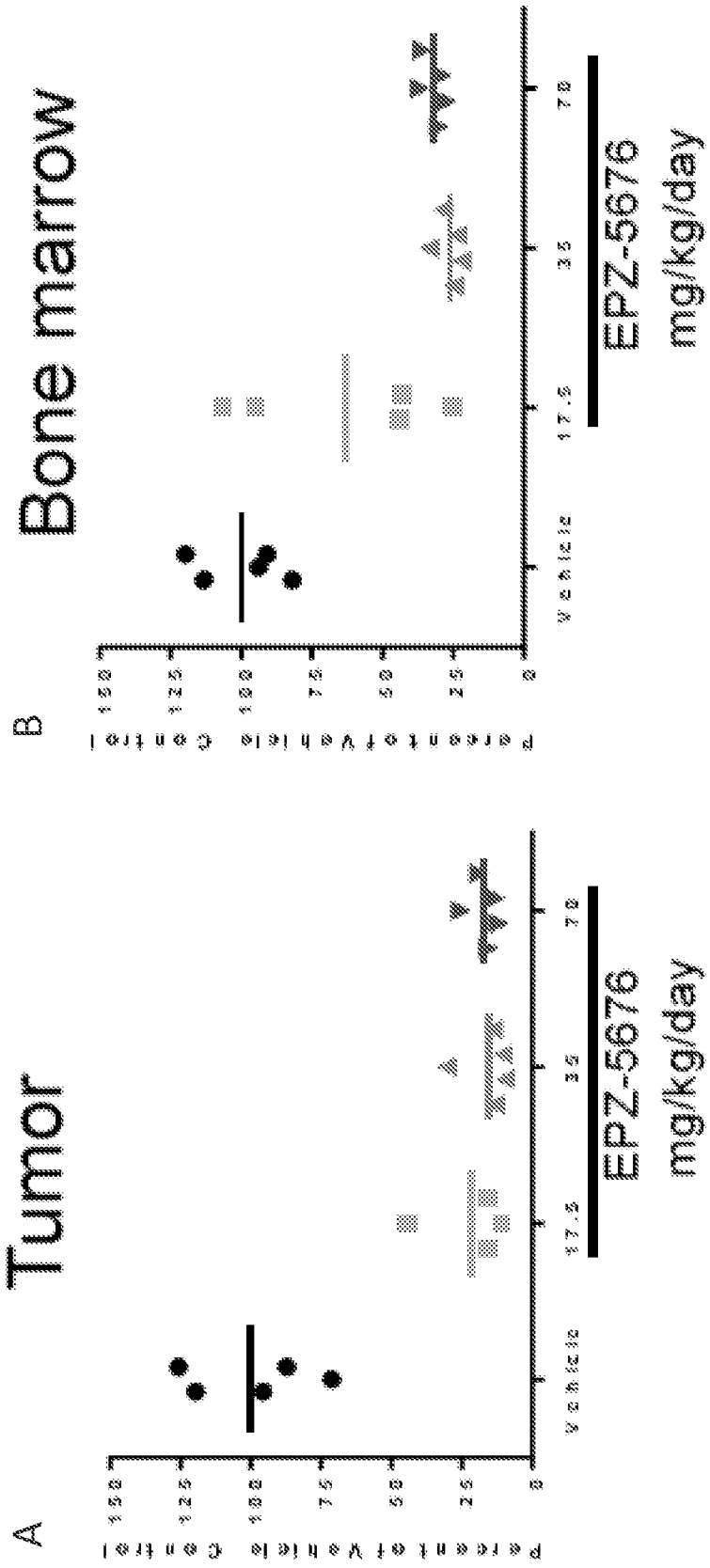

A
Tumor
Percent of Vehicle Control
150
125
100
75
50
25
0
Weeks
17.5
35
70
EPZ-5676
mg/kg/day
B
Bone marrow
Percent of Vehicle Control
150
125
100
75
50
25
0
Weeks
17.5
35
70
EPZ-5676
mg/kg/day

FIG. 7

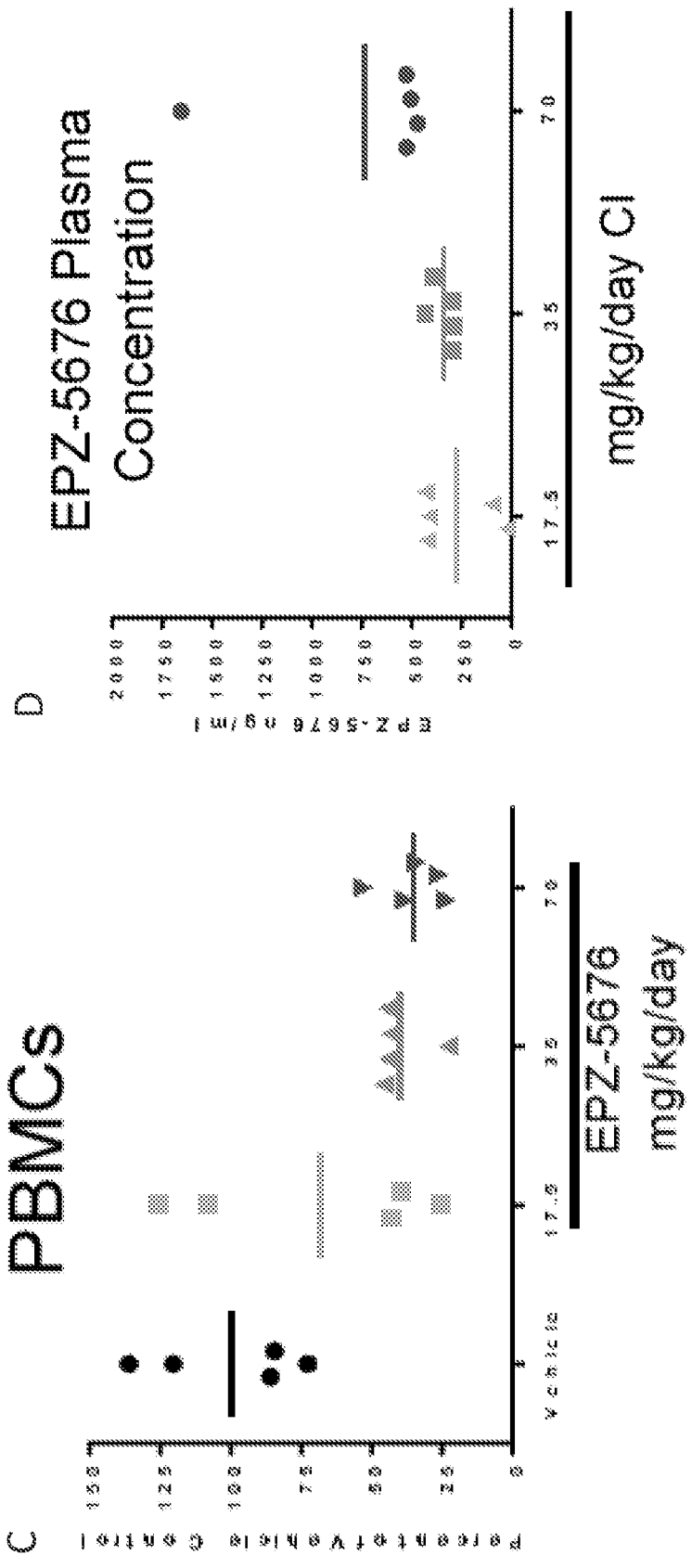

C
PBMCs
Vehicle
17.5
35
70
EPZ-5676
mg/kg/day
D
EPZ-5676 Plasma
Concentration
mg/kg/day CI

FIG. 7

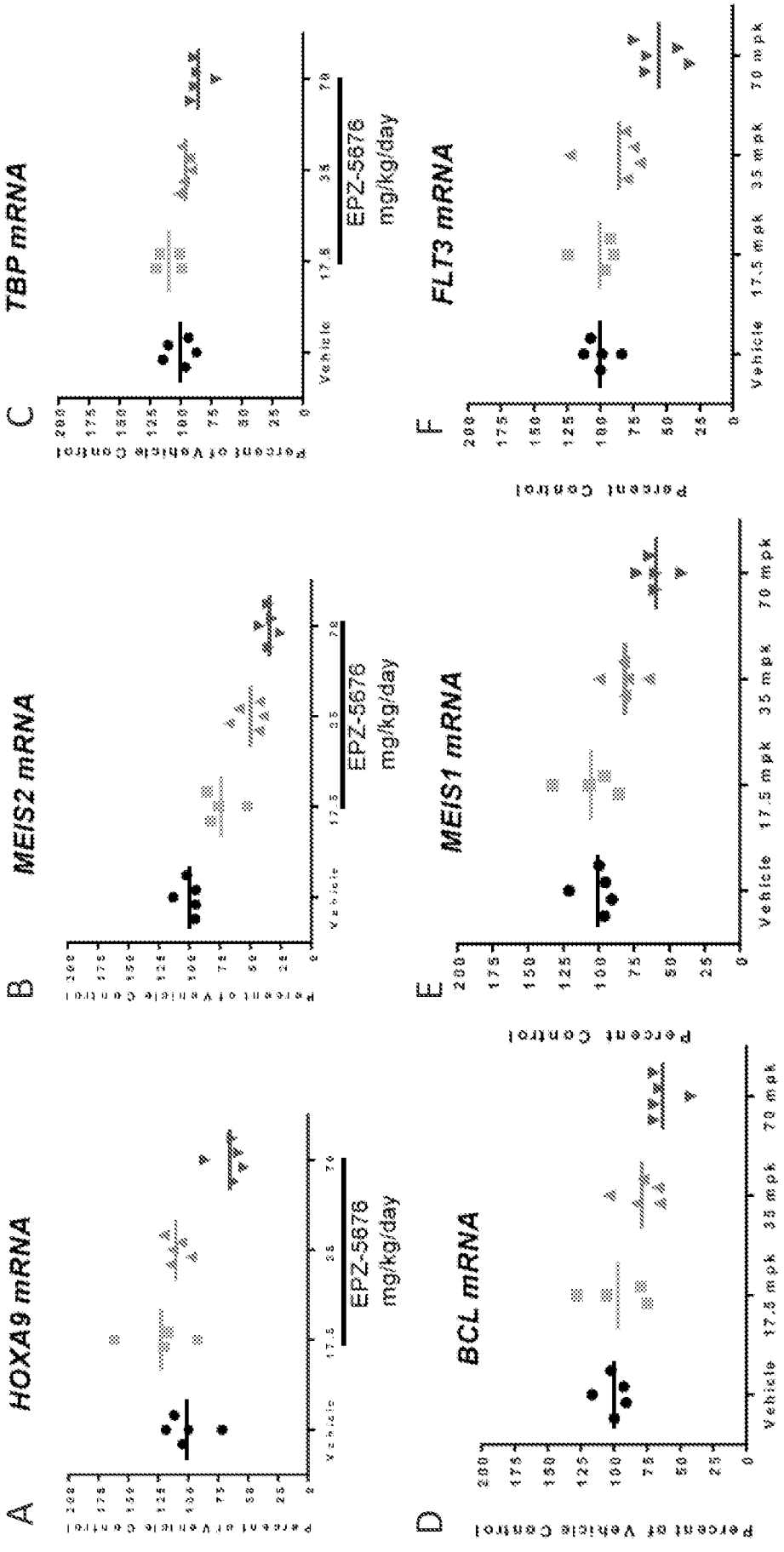
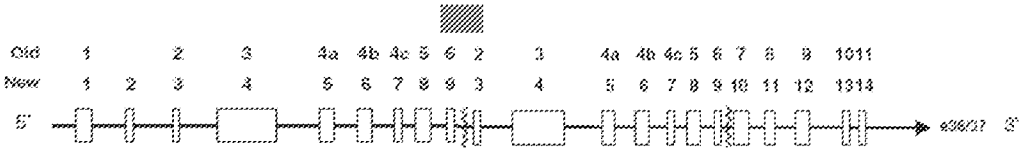

A
HOXA9 mRNA
Percent of Vehicle Control
Vehicle
17.5
35
70
EPZ-5676
mg/kg/day
B
MEIS2 mRNA
Percent of Vehicle Control
Vehicle
17.5
35
70
EPZ-5676
mg/kg/day
C
TBP mRNA
Percent of Vehicle Control
Vehicle
17.5
35
70
EPZ-5676
mg/kg/day
D
BCL mRNA
Percent of Vehicle Control
Vehicle
17.5 mpk
35 mpk
70 mpk
E
MEIS1 mRNA
Percent Control
Vehicle
17.5 mpk
35 mpk
70 mpk
F
FLT3 mRNA
Percent Control
Vehicle
17.5 mpk
35 mpk
70 mpk

FIG. 8

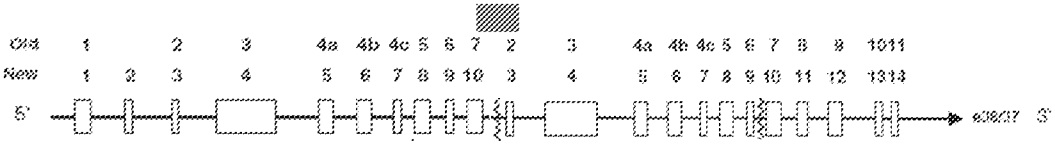
A. Primer set 1 (MLL-PTD 9-3)

MLL-PTD Breakpoint Frequency *



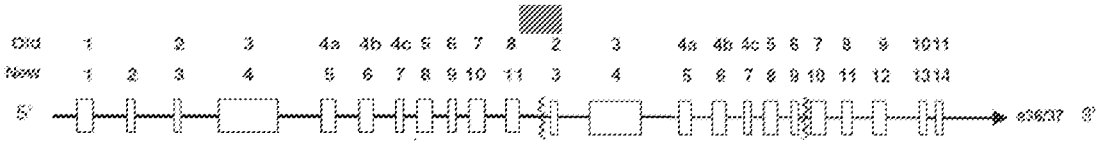
69/133 (52%)

B. Primer set 2 (MLL-PTD 10-3)



27/133 (20%)

C. Primer set 3 (MLL-PTD 11-3)



28/133 (21%)

Less Frequent MLL-PTD Breakpoints

Exons 12-3 (3/133, 2%); Exons 9-5 (2/133, 1.5%); Exons 8-3 (1/133, <1%); Exon 8+ (3/133, 2%)

RT-PCR amplicon
approximately 100 bp

* Fusions identified in literature

FIG. 9