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(54) **Title**: METHODS FOR TREATING MDS1-EVI1 MEDIATED CANCER

(57) **Abstract**: The present invention relates to a method of treating a cancerous condition mediated by the protein MDS1 -EVI1 (ME). The method includes administering to a patient an amount of an inhibitor of ME protein activity that is effective to cause cell death of cancer cells that are ME-dependent, thereby treating the cancerous condition. The present invention further relates to a method of causing cell death of a cancer cell that requires MDS1-EVI1 (ME) for survival. The method includes introducing an inhibitor of ME activity into a cancer cell that requires ME for survival, whereby said introducing is effective to cause cancer cell death. Novel agents that can inhibit the activity of ME in vitro or in vivo are also disclosed.

METHODS FOR TREATING MDS1-EVI1 MEDIATED CANCER

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/666,223, filed June 29, 2012, and U.S. Provisional Patent Application Serial No. 61/738,137, filed December 17, 2012, both of which are hereby incorporated by reference in their entirety.

[0002] This invention was made with government support under grant number R01CA120313 awarded by the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates to methods and compositions for treating MDS1-EVI1 mediated cancer.

BACKGROUND OF THE INVENTION

[0004] Leukemia is the most common form of cancer among children and adolescents (Arai et al., "Evi-1 Is a Direct Target of MLL Oncoproteins in Hematopoietic Stem Cells," *Blood* (ASH Annual Meeting Abstracts) 112:3807 (Nov 2008)), and approximately 2,000 infants within their first year of life develop life threatening acute leukemia in the United States. The presence of the MLL (Mixed Lineage Leukemia) gene translocation is the most significant independent factor associated with poor outcome and high risk of relapse in infant leukemia (Chen et al., "Malignant Transformation Initiated by Mll-AF9: Gene Dosage and Critical Target Cells," *Cancer Cell* 13:432-440 (2008); Keller and Maniatis, "Identification and Characterization of a Novel Repressor of Betainterferon Gene Expression," *Genes Dev.* 5:868-879 (1991); Turner et al., "Blimp-1, a Novel Zinc Finger-Containing Protein That Can Drive the Maturation of B Lymphocytes Into Immunoglobulin-Secreting Cells," *Cell* 77:297-306 (1994); and Buyse et al., "The Retinoblastoma Protein Binds to RIZ, a Zinc-Finger Protein That Shares an Epitope With the Adenovirus E1A Protein," *Proc Natl Acad Sci. U.S.A.* 92:4467-4471 (1995)). The MLL-AF9 translocation t(9;11)(p22;q23) is the most common, present in 70-80% of infant and nearly 60% of AML cases (Chuikov et al., "Regulation of p53 Activity Through Lysine Methylation," *Nature* 432:353-360 (2004)). Treatment with

chemotherapy regimens achieve infant survival rates of about 25-45%, with high relapse rates pretransplantation being a major contributor of mortality (Nanjundan et al., "Amplification of MDS1/EVI1 and EVI1, Located in the 3q26.2 Amplicon, is Associated with Favorable Patient Prognosis in Ovarian Cancer," *Cancer Res.* 67:3074-3084 (2007); Nitta et al., "Oligomerization of Evi-1 Regulated by the PR Domain Contributes to Recruitment of Corepressor CtBP," *Oncogene* 24:6165-6173 (2005); and Senyuk et al., "The Distal Zinc Finger Domain of AML1/MDS1/EVI1 is an Oligomerization Domain Involved in Induction of Hematopoietic Differentiation Defects in Primary Cells *In Vitro*," *Cancer Res.* 65:7603-7611 (2005)). Thus, a need exists for more effective therapeutic regimens.

[0005] Studies have demonstrated the MLL-AF9 fusion protein activates transcription of the *MECOM* (MDS1-EVI1, Myelodysplastic syndrome 1-Ecotropic virus integration site 1 COMPLEX) locus, a highly conserved proto-oncogene that plays a critical role in normal hematopoiesis (Hoyt et al., "The Evi1 Proto-Oncogene is Required at Midgestation for Neural, Heart, and Paraxial Mesenchyme Development," *Mechanisms of Development* 65:55-70 (1997), Goyama et al., "Evi-1 is a Critical Regulator for Hematopoietic Stem Cells and Transformed Leukemic Cells," *Cell Stem Cell* 3:207-220 (2008)). ME induces oncogenic activity by deregulation of functions such as apoptosis (Dobson et al., "The mll-AF9 Gene Fusion in Mice Controls Myeloproliferation and Specifies Acute Myeloid Leukaemogenesis," *EMBO J.* 18:3564-3574 (1999)) and failure of terminal myeloid maturation (Krivtsov et al., "Transformation From Committed Progenitor to Leukaemia Stem Cell Initiated by MLL-AF9," *Nature* 442(7104):818-22 (2006); and Somervaille et al., "Identification and Characterization of Leukemia Stem Cells in Murine MLL-AF9 Acute Myeloid Leukemia," *Cancer Cell* 10:257-268 (2006)).

[0006] AMLs bearing MLL fusion proteins (MFPs) have a poor prognosis; chemotherapy is inadequate, indicating the need for more effective therapies (Frankel et al., "Therapeutic Trial for Infant Acute Lymphoblastic Leukemia: The Pediatric Oncology Group Experience (POG 8493)," *J. Pediatr. Hematol. Oncol.* 19(1):35-42 (1997); Silverman et al., "Intensified Therapy for Infants With Acute Lymphoblastic Leukemia: Results From the Dana-Farber Cancer Institute Consortium," *Cancer* 80(12):2285-2295 (1997); and Reaman et al., "Treatment Outcome and Prognostic Factors for Infants with Acute Lymphoblastic Leukemia Treated on Two Consecutive

Trials of the Children's Cancer Group," *J. Clin. Oncol.* 17(2):445-455 (1999)). Insights into the molecular pathogenesis of ME-mediated leukemias will greatly facilitate the development of targeted agents to treat these leukemias.

[0007] Therefore, it would be desirable to determine how to target ME to treat these types of leukemias, as well as identify agents that can be used to disrupt ME activity in these and other forms of ME-dependent cancer. The present invention is directed to overcoming these and other deficiencies in the art.

SUMMARY OF THE INVENTION

[0008] A first aspect of the invention relates to a method of treating a cancerous condition mediated by the protein MDS1-EVI1 (ME). The method includes administering to a patient an amount of an inhibitor of ME protein activity that is effective to cause cell death of cancer cells that are ME-dependent, thereby treating the cancerous condition.

[0009] A second aspect of the invention relates to a method of causing cell death of a cancer cell that requires MDS1-EVI1 (ME) for survival. The method includes introducing an inhibitor of ME activity into a cancer cell that requires ME for survival, whereby said introducing is effective to cause cancer cell death.

[0010] A third aspect of the invention relates to an inhibitor of ME protein activity selected from the group consisting of an antibody or antibody fragment that binds specifically to a PR domain of the ME protein, an anti-ME nucleic acid aptamer, a dominant negative ME fragment, or an inhibitory nucleic acid molecule that interferes with ME expression to cause a reduction in ME activity. Pharmaceutical compositions containing one or more of these inhibitors of ME protein activity, and their use in accordance with the first and second aspects of the invention, are also contemplated.

[0011] In a subset of MFP AMLs, specifically those lacking monocytic features (Bindels et al., "EVI1 is Critical for the Pathogenesis of a Subset of MLL-AF9-rearranged AMLs," *Blood* 119(24):5838-5849 (2012), which is hereby incorporated by reference in its entirety), MFPs can bind to and activate transcription of *MECOM* (Bindels et al., EVI1 is Critical for the Pathogenesis of a Subset of MLL-AF9-rearranged AMLs," *Blood* 119(24):5838-5849 (2012); Arai et al., "Evi-1 is a

Transcriptional Target of Mixed-Lineage Leukemia Oncoproteins in Hematopoietic Stem Cells," *Blood* 117(23):6304-6314 (2011); Chen et al., "Malignant Transformation Initiated by Mll-AF9: Gene Dosage and Critical Target Cells," *Cancer Cell* 13(5):432-440 (2008), all of which are hereby incorporated by reference in their entirety), a highly conserved proto-oncogene encoding MDS1-EVI1 (ME), and EVI1 isoforms via distinct transcription sites. Relative to EVI1, ME possesses a "PR" domain with histone methyltransferase activity (Pinheiro et al., "Prdm3 and Prdm16 are H3K9me1 Methyltransferases Required for Mammalian Heterochromatin Integrity," *Cell* 150(5):948-960 (2012), which is hereby incorporated by reference in its entirety). In this invention, using mouse alleles where ME is constitutively (ME^{m1}) or conditionally (ME^{fl4}) lost (Zhang et al., "PR Domain–Containing Mds1-Evi1 is Critical for Long-Term Hematopoietic Stem Cell Function," *Blood* 118(14):3853-3861 (2011), which is hereby incorporated by reference in its entirety), it is revealed that the PR domain is essential in MFP transformation in mouse. These results implicate ME as a novel target for therapeutic intervention.

[0012] A subgroup of leukemogenic MLL fusion proteins (MFP) including MLL-AF9 activates *Mecom* locus, and exhibits extremely poor clinical prognosis. *MECOM* encodes EVI1 and MDS1-EVI1 (ME) proteins via alternative transcription start sites; these differ by the presence of a SET-like PR domain at the N-terminus of ME. SET domains are known to have histone methyltransferase (HMT) activity, and to play important roles in chromatin modification and the regulation of gene expression. The function of the PR domain of ME is unknown. The ability of ME-deficient Lin-/Sca1+/c-Kit+ (LSK) cells to be transformed by different leukemogenic oncogenes were tested. This revealed that while NUP98-HOXA9, E2A-HLF, and MEIS1-HOXA9 were able to transform *ME*-deficient cells, both MLL-AF9 and MLL-ENL (MLL Fusion Proteins, (MFP)) were ineffective, indicating an essential function for ME in the context of MFP-induced acute myeloid leukemia (AML). Experiments with a conditional allele of *ME* show that the gene is important not only for the initiation but also the continued survival of MFP AML cells. Structure-function studies demonstrated that within ME, the PR domain is essential for MFP-induced AML. *In silico* analysis of the PR domain indicates strong similarity to the structure of SET domains with known HMT activity: a series of β-sheet structures with a substrate lysine binding pocket stabilized by a salt bridge between D_{130} and

R_{176}, and with a conserved Y (111) residue at the catalytic site. This *in silico* analysis also revealed important differences, including the absence of the i-SET domain, which is known to provide contacts for binding of substrate histone and methyl donor, S-adenosylmethionine (SAM). In its place were W(149) and Q(115) residues, pointing towards the exterior; the placement of these residues indicates a role in the docking of another protein that serves as the i-SET domain. Finally, there is a change of a Y involved in catalysis to M (residue 191 in ME). To test if the PR domain of ME requires these putative structures for activity, three residues were mutated to A: Y_{111}, R_{176}, and W_{149}. All three mutations resulted in loss of rescue activity, while a control mutation away from the pocket/catalytic site (C158A) had no effect. These data support the contention that (1) ME is essential for MFP-induced AML; (2) this activity depends on the PR domain; and (3) within the PR domain, critical structures include a putative substrate binding pocket, catalytic Y residue, and docking site for an interacting protein. Importantly, these findings strongly indicate that it should be possible to inhibit the function of the PR domain of ME, and that such inhibition should result in the death of MFP-induced leukemias. These studies clearly indicate an essential role of PR-domain protein ME in MFP leukemia, in which case ME represents a novel target for therapeutic intervention for this group of leukemias.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figures 1A-F illustrate that $ME^{m1/m1}$ bone marrow is resistant to MLL-AF9-induced transformation. Figure 1A is a diagram of the ME^{m1} allele, showing exon 1 of *Mds1*, with putative transcription start site and splice donor site, as well as site of *lacZ* insertion and the extent of the DNA of the first intron deleted. Also shown are the locations of MFP binding described by Arai et al., "Evi-1 is a Transcriptional Target of Mixed-Lineage Leukemia Oncoproteins in Hematopoietic Stem Cells," *Blood* 117(23):6304-6314 (2011), which is hereby incorporated by reference in its entirety), as well as the MFP-responsive region identified by this same group by luciferase reporter assays (Arai et al., "Evi-1 is a Transcriptional Target of Mixed-Lineage Leukemia Oncoproteins in Hematopoietic Stem Cells," *Blood* 117(23):6304-6314 (2011), which is hereby incorporated by reference in its entirety). Figure 1B shows quantitation of the number of colonies formed in growth factor-

supplemented methylcellulose at each of four replatings, for wildtype (WT) and *ME*$^{m1/m1}$ bone marrow. Error bars represent standard deviation of platings done in triplicate, 1000 cells/plate. The experiment was repeated multiple times with the same result. Figures 1C, 1D, and 1E show results of *ME* requirement being restricted to transformation by MLL fusion genes. In Figure 1C, quantitation of the number of colonies formed in growth factor-supplemented methylcellulose at each of four replatings for *ME*$^{m1/m1}$ LSK cells transduced with the virus indicated is shown. Error bars are standard deviation; p values determined by Student T test, comparing the first and fourth plating. Figures 1D and 1E show serial replating assay of bone marrow from mice of the genotype indicated, transduced with the leukemogenic oncogene indicated, and treated or not with 4-hydroxy tamoxifen (4-OH-TAM) (1 μM) as indicated. Error bars are not shown, to avoid an overly busy figure, but are within 10% of the value of each bar; p values were calculated by Student T test, comparing first and fourth plating. In Figure 1F, ME but not EVI1 or MDS1 can rescue the transformation deficiency of *ME*$^{m1/m1}$ bone marrow. Serial replating transformation assay of LSK cells isolated from either WT or *ME*$^{m1/m1}$ bone marrow, transduced with MLL-AF9 as well as retroviral expression construct indicated: MIGR1 (empty vector), MDS1, EVI1 or ME. Error bars denote standard deviation. A third replating yielded no colonies for MIGR1 and EVI1-transduced cells.

[0014] Figures 2A-H illustrate that deletion of *ME* results in failure of MLL-AF9 leukemic cells to transplant into syngeneic sublethally irradiated recipient mice. Figure 2A is a diagram that depicts experimental procedures. Figures 2B, 2C, and 2D as shown are complete blood count data from weeks 0 to 4 post-transplant for recipients of *ME*$^{fl4/m1}$ cells, with and without pre-treatment with 4-OH TAM; extent of normal values is indicated by greyed zone. Mice receiving cells with no 4-OH TAM pre-treatment became frankly leukemic, anemic, and thrombocytopenic over the four weeks of monitoring. At week four, most recipients of the untreated cells were moribund, and the mice were necropsied. Error bars depict standard deviation. Statistical significance (Student T test) was observed at week four for leukocytes and platelets ($p<0.05$). Figures 2E, 2F, 2G, and 2H show that deletion of *ME* results in failure of leukemic cells to significantly infiltrate organs of irradiated recipients. Mice receiving 4-OH TAM-pretreated MLL-AF9 leukemia cells maintained normal spleen weights (Figs. 2E and 2F), as well as livers and bone marrow essentially

devoid of infiltrating leukemia. Non-pretreated cells infiltrated spleen, liver, and bone marrow. In Figure 2E, gross photographs of spleens from mice injected with un-treated and 4-OH TAM pre-treated cells are shown. Figure 2F shows scattergrams of spleen weights of the four experimental groups, as indicated; the number of spleens in each cohort is in parentheses. The average is demarcated by horizontal bar; and p value determined by Student T test. Figures 2G and 2H show photomicrographs of liver (Figure 2G) and bone marrow (Figure 2H), showing extensive infiltration by leukemic cells. Photographs are at 200 X magnification with H&E staining.

[0015] Figures 3A-C show that the PR domain conforms in structure to enzymatically active SET domains, albeit with important differences. Figure 3A is a partial alignment of PR domain of murine ME (SEQ ID NO: 15) with the SET domains of human Suvar39H1 (SEQ ID NO: 16) and *P. sativa* RubiscoLSMT (SEQ ID NO: 17), with demarcation of the regions of beta sheet 1 through 12 and location of the iSET domain. The numbering above refers to ME, below to RuLSMT. Figures 3B-C illustrate the ribbon structure of the lysine binding pocket from RubiscoLSMT and ME, respectively, with key residues underlined. The diagonal line denotes actual (Fig. 3B) and potential (Fig. 3C) interaction between residues as discussed in the examples. Beta sheets 6, 7, 9, and 12 are indicated; β6 is perpendicular to the viewing plane.

[0016] Figures 4A-E illustrate that Y_{111}, W_{149}, and R_{176} of PR domain of ME are required for biologic activity. Figure 4A shows the results of a replating assay performed with lineage-negative cells of $ME^{m1/m1}$ mice. Shown is a photograph of the third plating, in duplicate. Figure 4B is a backbone depiction of *in silico* structure of PR protein, showing locations of the amino acids mutated. Figure 4C shows a higher magnification view of "substrate pocket" of PR domain, showing location of the W149, protruding from the pocket, as well as the Y111 and R176. Figure 4D is an alignment showing the conservation of Y111 among enzymatically active SET domains, including MDS-EVI1 (amino acids 90-123 of SEQ ID NO: 1), HS Suvar39H1 (amino acids 12-44 of SEQ ID NO: 16), EZH2 (SEQ ID NO: 18), EZH1 (SEQ ID NO: 19), E(Z) (SEQ ID NO: 20), and MLL (SEQ ID NO: 21). Figure 4E is a Western blot of transfected HEK293T cells showing expression of mutant or wildtype ME, at the expected molecular weight.

[0017] Figure 5 illustrates the evolutionary relationship of PR/SET proteins. (Figure is adapted from Wu et al., "Structural Biology of Human H3K9 Methyltransferases," *PLoS One* 1:e8570 (2010), which is hereby incorporated by reference in its entirety.)

[0018] Figures 6A-B illustrate the structure of the Mecom genomic locus (Fig. 6A) and the structure of the Mecom proteins (Fig. 6B). In Figure 6A, the genomic structure of the Mecom locus is illustrated, showing starts of transcription at Mds1 exon 1 (Mds1ex1) and Evi1, as well as mRNA splicing pattern. Scale is in basepairs. Below is an enlargement of the Evi1 portion of the locus, showing Evi1 exons 1-15, as well as locations of the genetic lesions that have been reported: floxed exon 3 (Zhang et al., "PR-Domain–Containing Mds1-Evi1 is Critical for Long-Term Hematopoietic Stem Cell Function," *Blood* 118:3853 (2011), which is hereby incorporated by reference in its entirety); floxed exon 4 (Goyama et al., "Evi-1 is a Critical Regulator for Hematopoietic Stem Cells and Transformed Leukemic Cells," *Cell Stem Cell* 3:207 (2008), which is hereby incorporated by reference in its entirety); and Neo gene insertion into exon 7 (Hoyt et al., "The Evi1 Proto-Oncogene is Required at Midgestation for Neural, Heart, and Paraxial Mesenchyme Development," *Mechanisms of Development* 65:55 (1997), which is hereby incorporated by reference in its entirety). Also indicated are the presence of initiator methionines in exons 3 and 4. In Figure 6B, the structure of the proteins derived from the Mecom locus are shown, with the apparent molecular weights indicated to the left, as well as key structural elements, including the PR domain, the zinc fingers (ZF), the C-terminal binding protein (CtBP).

[0019] Figure 7 illustrates one example of a chimeric therapeutic agent that includes an AML-specific aptamer KH1C12 and either an ME-inhibiting aptamer or ME-inhibiting RNAi molecule. The therapeutic agent is targeted to AML cells recognized by the KH1C12 aptamer.

[0020] Figure 8 illustrates an approach for targeted delivery of a therapeutic agent with a conjugated aptamer molecule. One or more AML-specific KH1C12 aptamers and one or more ME-inhibiting aptamers or ME-inhibiting RNAi molecules form the functional components of the conjugate. The therapeutic agent is targeted to an AML cell recognized by the KH1C12 aptamer.

[0021] Figure 9 shows a targeted approach for delivery of a therapeutic agent having a conjugate that includes a polycation-aptamer or RNAi vector linked via phenyl(di)boronic acid-salicylhydroxamic acid assembly to an antibody that is specific for a cancer cell surface marker (e.g., CD-33, CD-19, or CD-20). The therapeutic agent is targeted to cells that express the cancer cell surface marker.

DETAILED DESCRIPTION OF THE INVENTION

[0022] One aspect of the invention relates to a method of treating a cancerous condition mediated by the protein MDS1-EVI1 ("ME"). The method includes administering to a patient an amount of an inhibitor of ME protein activity that is effective to cause cell death of cancer cells that are ME-dependent, thereby treating the cancerous condition.

[0023] A related aspect of the invention relates to a method of causing death of a cancer cell that requires ME for survival. This method includes introducing an inhibitor of ME activity into a cancer cell that requires ME for survival under conditions effective to cause cancer cell death.

[0024] Messenger RNA transcripts initiating at *Mds1* can splice from exon 2 of *Mds1* into exon 2 of *Evi1* to encode a larger ME protein. Relative to EVI1 proper, the ME protein possesses a 190 amino acid N-terminal extension encoded by exon 2 of *Mds1* and exon 2 of *Evi1*. The latter is all open reading frame, in frame with the coding of exon 3 of *Evi1*, but cannot be translated in the context of an *Evi1* transcript due to the lack of an initiator methionine (the first of which resides in exon 3 of *Evi1*). However, within the context of *Mds1-Evi1* mRNA transcripts, exon 2 is translated, and, together with exon 2 of *Mds1*, encodes a 110 amino acid domain with homology to a domain present in other proteins, specifically positive regulatory domain 1 binding factor 1 (PRD1-BF1) (Keller and Maniatis, "Identification and Characterization of a Novel Repressor of Betainterferon Gene Expression," *Genes Dev.* 5:868-879 (1991), which is hereby incorporated by reference in its entirety) (also known as B-lymphocyte-induced maturation protein 1 (Blimp-1) (Turner et al., "Blimp-1, a Novel Zinc Finger-Containing Protein That can Drive the Maturation of B Lymphocytes into Immunoglobulin-Secreting Cells," *Cell* 77:297-306 (1994), which is hereby incorporated by reference in its entirety) and Rb-interacting zinc

finger protein (RIZ) (Buyse et al., "The Retinoblastoma Protein Binds to RIZ, a Zinc-Finger Protein That Shares an Epitope With the Adenovirus E1A Protein," *Proc. Natl. Acad. Sci. USA* 92:4467-4471 (1995), which is hereby incorporated by reference in its entirety). This domain, termed the PRD1-BF1-RIZ (PR) domain is evolutionarily distantly related to the SET domain (Su(var)3-9, Enhancer of zeste (E(z)), and Trithorax (trx).

[0025] A number of types of cancer are associated with ME expression and are believed to be ME-dependent, i.e., requiring ME expression and activity for either cancer cell development, cancer cell survival, or both. Exemplary types of cancer or precancerous conditions that are associated with ME expression include, without limitation, cancer cells that are leukemic or dysplastic such as acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), myelodysplastic syndrome (MDS), chronic myelogenous leukemia (CML), and epithelial cancers where the 3q26.2 aberration is present (ovary, breast, head and neck, cervix, and lung). In certain AML or ALL cancers, the cancerous condition is associated with the presence of the MLL-AF9 translocation t(9;11)(p22;q23) or MLL-ENL translocation t(11;19)(q23;p13.3); these translocations encode MLL fusion proteins, and some of these have been found to activate transcription of *Mecom* and MLL fusion protein induced leukemias have been found to depend on an intact *Mecom* gene (Goyama et al., "Evi-1 is a Critical Regulator for Hematopoietic Stem Cells and Transformed Leukemic Cells," *Cell Stem Cell* 3:207-220 (2008), which is hereby incorporated by reference in its entirety).

[0026] The human ME amino acid sequence, variant c, is described at Genbank Accession NP_004982.2 and comprises the amino acid sequence set forth below (SEQ ID NO: 1):

MRSKGRARKL ATNNECVYGN YPEIPLEEMP DADGVASTPS LNIQEPCSPA TSSEAFTPKE
GSPYKAPIYI PDDIPIPAEF ELRESNMPGA GLGIWTKRKI EVGEKFGP**Y**V GEQRSNLKDP
SYGWEILDEF YNVKFCIDAS QPDVGS**W**LKY IRFAGCYDQH NLVACQINDQ IFY**R**VVADIA
PGEELLLFMK SEDYPHETMA PDIHEERQYR CEDCDQLFES KAELADHQKF PCSTPHSAFS
MVEEDFQQKL ESENDLQEIH TIQECKECDQ VFPDLQSLEK HMLSHTEERE YKCDQCPKAF
NWKSNLIRHQ MSHDSGKHYE CENCAKVFTD PSNLQRHIRS QHVGARAHAC PECGKTFATS
SGLKQHKHIH SSVKPFICEV CHKSYTQFSN LCRHKRMHAD CRTQIKCKDC GQMFSTTSSL
NKHRRFCEGK NHFAAGGFFG QGISLPGTPA MDKTSMVNMS HANPGLADYF GANRHPAGLT
FPTAPGFSFS FPGLFPSGLY HRPPLIPASS PVKGLSSTEQ TNKSQSPLMT HPQILPATQD
ILKALSKHPS VGDNKPVELQ PERSSEERPF EKISDQSESS DLDDVSTPSG SDLETTSGSD
LESDIESDKE KFKENGKMFK DKVSPLQNLA SINNKKEYSN HSIFSPSLEE QTAVSGAVND
SIKAIASIAE KYFGSTGLVG LQDKKVGALP YPSMFPLPFF PAFSQSMYPF PDRDLRSLPL
KMEPQSPGEV KKLQKGSSES PFDLTTKRKD EKPLTPVPSK PPVTPATSQD QPLDLSMGSR
SRASGTKLTE PRKNHVFGGK KGSNVESRPA SDGSLQHARP TPFFMDPIYR VEKRKLTDPL
EALKEKYLRP SPGFLFHPQF QLPDQRTWMS AIENMAEKLE SFSALKPEAS ELLQSVPSMF
NFRAPPNALP ENLLRKGKER YTCRYCGKIF PRSANLTRHL RTHTGEQPYR CKYCDRSFSI

SSNLQRHVRN IHNKEKPFKC HLCDRCFGQQ TNLDRHLKKH ENGNMSGTAT SSPHSELEST
GAILDDKEDA YFTEIRNFIG NSNHGSQSPR NVEERMNGSH FKDEKALVTS QNSDLLDDEE
VEDEVLLDEE DEDNDITGKT GKEPVTSNLH EGNPEDDYEE TSALEMSCKT SPVRYKEEEY
KSGLSALDHI RHFTDSLKMR KMEDNQYSEA ELSSFSTSHV PEELKQPLHR KSKSQAYAMM
LSLSDKESLH STSHSSSNVW HSMARAAAES SAIQSISHV

The PR domain is underlined in the sequence shown above, and residues Y_{109}, R_{174}, and W_{147} critical to PR domain (and ME protein) function are shown in bold typeface. The nucleotide sequence encoding this ME protein is provided below and reported at Genbank Accession NM_004991.3 as follows (SEQ ID NO: 2):

atgagatccaaaggcagggcaaggaaactggccacaaataatgagtgtgtatatggcaactaccctgaa
atacctttggaagaaatgccagatgcagatggagtagccagcactccctccctcaatattcaagagcca
tgctctcctgccacatccagtgaagcattcactccaaaggagggttctccttacaaagcccccatctac
atccctgatgatatccccattcctgctgagtttgaacttcgagagtcaaatatgcctggggcaggacta
ggaatatggaccaaaaggaagatcgaagtaggtgaaaagtttgggccttatgtgggagagcagaggtca
aacctgaaagaccccagttatggatgggagatcttagacgaattttacaatgtgaagttctgcatagat
gccagtcaaccagatgttggaagctggctcaagtacattagattcgctggctgttatgatcagcacaac
cttgttgcatgccagataaatgatcagatattctatagagtagttgcagacattgcgccgggagaggag
cttctgctgttcatgaagagcgaagactatccccatgaaactatggcgccggatatccacgaagaacgg
caatatcgctgcgaagactgtgaccagctctttgaatctaaggctgaactagcagatcaccaaaagttt
ccatgcagtactcctcactcagcattttcaatggttgaagaggactttcagcaaaaactcgaaagcgag
aatgatctccaagagatacacacgatccaggagtgtaaggaatgtgaccaagtttttcctgatttgcaa
agcctggagaaacacatgctgtcacatactgaagagagggaatacaagtgtgatcagtgtcccaaggca
tttaactggaagtccaatttaattcgccaccagatgtcacatgacagtggaaagcactatgaatgtgaa
aactgtgccaaggttttcacggaccctagcaaccttcagcggcacattcgctctcagcatgtcggtgcc
cgggcccatgcatgcccggagtgtggcaaaacgtttgccacttcgtcgggcctcaaacaacacaagcac
atccacagcagtgtgaagccctttatctgtgaggtctgccataaatcctatactcagttttcaaacctt
tgccgtcataagcgcatgcatgctgattgcagaacccaaatcaagtgcaaagactgtggacaaatgttc
agcactacgtcttccttaaataaacacaggaggttttgtgagggcaagaaccattttgcggcaggtgga
tttttggccaaggcatttcacttcctggaaccccagctatggataaaacgtccatggttaatatgagt
catgccaacccgggccttgctgactattttggcgccaataggcatcctgctggtcttacctttccaaca
gctcctggatttcttttagcttccctggtctgtttccttccggcttgtaccacaggcctcctttgata
cctgctagttctcctgttaaaggactatcaagtactgaacagacaaacaaaagtcaaagtcccctcatg
acacatcctcagatactgccagctacacaggatattttgaaggcactatctaaacacccatctgtaggg
gacaataagccagtggagctccagcccgagaggtcctctgaagagaggccctttgagaaaatcagtgac
cagtcagagagtagtgaccttgatgatgtcagtacaccaagtggcagtgacctggaaacaacctcgggc
tctgatctggaaagtgacattgaaagtgataaagagaaatttaaagaaaatggtaaaatgttcaaagac
aaagtaagccctcttcagaatctggcttcaataaataataagaaagaatacagcaatcattccatttc
tcaccatctttagaggagcagactgcggtgtcaggagctgtgaatgattctataaaggctattgcttct
attgctgaaaaatactttggttcaacaggactggtggggctgcaagacaaaaaagttggagctttacct
tacccttccatgtttcccctcccatttttccagcattctctcaatcaatgtacccatttcctgataga
gacttgagatcgttacctttgaaaatggaaccccaatcaccaggtgaagtaaagaaactgcagaagggc
agctctgagtcccccttttgatctcaccactaagcgaaaggatgagaagcccttgactccagtcccctcc
aagcctccagtgacacctgccacaagccaagaccagcccctggatctaagtatgggcagtaggagtaga
gccagtgggacaaagctgactgagcctcgaaaaaccacgtgtttgggggaaaaaaaggaagcaacgtc
gaatcaagacctgcttcagatggttccttgcagcatgcaagacccactcctttctttatggaccctatt
tacagagtagagaaaagaaaactaactgacccacttgaagctttaaaagagaaatacttgaggccttct
ccaggattcttgtttcacccacaattccaactgcctgatcagagaacttggatgtcagctattgaaaac
atggcagaaaagctagagagcttcagtgccctgaaacctgaggccagtgagctcttacagtcagtgccc
tctatgttcaacttcagggcgcctcccaatgccctgccagagaaccttctgcggaagggaaaggagcgc
tatacctgcagatactgtggcaagattttttccaaggtctgcaaacctaacacggcacttgagaacccac
acaggagagcagccttacagatgcaaatactgtgacagatcatttagcatatcttctaacttgcaaagg
catgttcgcaacatccacaataaagagaagccatttaagtgtcacttatgtgataggtgttttggtcaa
caaaccaatttagacagacacctaaagaaacatgagaatgggaacatgtccggtacagcaacatcgtcg
cctcattctgaactggaaagtacaggtgcgattctggatgacaaagaagatgcttacttcacagaaatt

cgaaatttcattgggaacagcaaccatggcagccaatctcccaggaatgtggaggagagaatgaatggc
agtcattttaaagatgaaaaggctttggtgaccagtcaaaattcagacttgctggatgatgaagaagtt
gaagatgaggtgttgttagatgaggaggatgaagacaatgatattactggaaaaacaggaaaggaacca
gtgacaagtaatttacatgaaggaaaccctgaggatgactatgaagaaaccagtgccctggagatgagt
tgcaagacatccccagtgaggtataaagaggaagaatataaaagtggactttctgctctagatcatata
aggcacttcacagatagcctcaaaatgaggaaaatggaagataatcaatattctgaagctgagctgtct
tcttttagtacttcccatgtgccagaggaacttaagcagccgttacacagaaagtccaaatcgcaggca
tatgctatgatgctgtcactgtctgacaaggagtccctccattctacatcccacagttcttccaacgtg
tggcacagtatggccagggctgcggcggaatccagtgctatccagtccataagccacgtatga

[0027] An exemplary mouse ME amino acid sequence is described partially by Swissprot accession number G3UWT0 and comprises the amino acid sequence set forth below (SEQ ID NO: 3):

MRSKGRARKL ATSNECAYGN YPEIPLEEMP DADADGITSV PSLHIQEPCS PATSSESFTP
KEGSPYKAPI YIPDDIPIPD EFELRESTMP GAGLGIWTKR KIEIGEKFGP **Y**MGEQRSDLK
DSSYGWEILD EFCNVKFCID ASQPDVGS**W**L KYIRFAGCYD QHNLVACQIN DQIFY**R**VVAD
IAPGEELLLF MKSEEDPHEP MAPDIHEERQ HRCEDCDQLF ESKAELADHQ KFPCSTPHSA
FSMVEEDLQQ NLESESDLRE IHGNQDCKEC DRVFPDLQSL EKHMLSHTEE REYKCDQCPK
AFNWKSNLIR HQMSHDSGKH YECENCAKVF TDPSNLQRHI RSQHVGARAH ACPECGKTFA
TSSGLKQHKH IHSSVKPFIC EVCHKSYTQF SNLCRHKRMH ADCRTQIKCK DCGQMFSTTS
SLNKHRRFCE GKNHFAAGGF FGQGISLPGT PAMDKTSMVN MSHANPGLAD YFGTNRHPAG
LTFPTAPGFS FSFPGLFPSG LYHRPPLIPA SPPVKGLSST EQSNKCQSPL LTHPQILPAT
QDILKALSKH PPVGDNKPVE LLPERSSEER PLEKISDQSE SSDLDDVSTP SGSDLETTSG
SDLESDLESD KEKCKENGKM FKDKVSPLQN LASITNKKEH NNHSVFSASV EEQSAVSGAV
NDSIKAIASI AEKYFGSTGL VGLQDKKVGA LPYPSMFPLP FFPAFSQSMY PFPDRDLRSL
PLKMEPQSPS EVKKLQKGSS ESPFDLTTKR KDEKPLTSGP SKPSGTPATS QDQPLDLSMG
SRGRASGTKL TEPRKNHVFG EKKGSNMDTR PSSDGSLQHA RPTPFFMDPI YRVEKRKLTD
PLEALKEKYL RPSPGFLFHP QMSAIENMAE KLESFSALKP EASELLQSVP SMFSFRAPPN
TLPENLLRKG KERYTCRYCG KIFPRSANLT RHLRTHTGEQ PYRCKYCDRS FSISSNLQRH
VRNIHNKEKP FKCHLCDRCF GQQTNLDRHL KKH**E**NGNMSG TATSSPHSEL ESAGAILDDK
EDAYFTEIRN FIGNSNHGSQ SPRNMEERMN GSHFKDKKAL ATSQNSDLLD DEEVEDEVLL
DEEDEDNDIP GKPRKELGVT RLDEEIPEDD YEEAGALEMS CKASPVRYKE EDYKSGLSAL
DHIRHFTDSL KMREMEENQY TDAELSSISS SHVPEELKQT LHRKSKSQAY AMMLSLSDKD
SLHPTSHSSS NVWHSMARAA AESSAIQSIS HV

The PR domain is underlined in the sequence shown above. This protein sequence is further analyzed in the Examples section, specifically in association with Figures 4A-E. The nucleotide sequence encoding this ME protein is provided below and reported at Genbank Accession CN697723.1 plus M21829. CN697723 contains a partial sequence that bridges between MDS1 and EVI1 (M21829), and the remainder of the open reading frame is afforded by the remainder of EVI1 (SEQ ID NO: 4):

ATGAGATCCAAAGGCAGGGCAAGGAAACTGGCCACAAGTAATGAGTGTGCCTATGGCAACTATCCTGAAATACCTT
TGGAAGAAATGCCAGATGCTGATGCAGATGGGATAACCAGTGTTCCCTCCCTCCACATTCAAGAGCCATGCTCTCC
TGCGACGTCCAGTGAGTCATTTACTCCTAAGGAGGGCTCGCCATACAAAGCTCCCATCTACATCCCTGATGACATC
CCTATTCCTGATGAGTTTGAGCTTCGAGAGTCAACTATGCCTGGAGCAGGACTTGGAATATGGACCAAAAGGAAGA
TTGAAATAGGCGAAAAGTTTGGGCCATACATGGGAGAGCAGAGATCAGACCTGAAAGATTCCAGCTATGGATGGG**A**
GATCTTAGATGAGTTTTGCAATGTGAAGTTCTGCATAGATGCCAGTCAACCAGATGTAGGAAGCTGGCTCAAGTAC
ATCAGATTCGCTGGCTGCTATGATCAGCACAACCTTGTTGCATGCCAGATAAATGATCAGATATTCTACCGAGTAG
TCGCAGACATTGCGCCTGGGGAAGAGCTCTTGCTGTTCATGAAGAGTGAAGAGGACCCGCACGAACCCATGGCGCC
TGACATCCACGAAGAACGGCAGCACCGCTGTGAGGACTGTGACCAGCTCTTTGAATCCAAGGCAGAGCTAGCCGAT

CACCAGAAGTTCCCATGCAGCACACCTCACTCGGCCTTCTCCATGGTGGAGGAGGACTTGCAACAAAACCTGGAGA
GTGAGAGCGATCTCCGAGAGATCCATGGCAACCAGGACTGTAAGGAATGTGACCGAGTTTTCCCCGATCTGCAAAG
CTTGGAGAAGCACATGCTGTCACATACTGAGGAGAGGGAATACAAGTGTGATCAGTGTCCCAAGGCATTTAACTGG
AAGTCCAATTTAATTCGCCACCAGATGTCACATGACAGTGGAAAGCACTATGAGTGTGAAAACTGTGCCAAGGTTT
TCACGGACCCTAGCAACCTTCAGCGACACATTCGATCTCAGCATGTTGGTGCCCGGGCTCATGCTTGCCCCGAGTG
TGGTAAAACATTTGCCACTTCGTCAGGCCTCAAACAGCACAAGCACATCCACAGCAGTGTGAAGCCCTTTATCTGT
GAGGTCTGCCATAAATCCTATACTCAGTTTTCAAACCTTTGTCGTCATAAGCGCATGCATGCTGATTGCAGAACCC
AAATCAAGTGCAAAGACTGTGGACAAATGTTCAGCACTACGTCTTCCTTAAATAAACACAGGAGGTTTTGTGAGGG
CAAGAACCATTTTGCGGCAGGTGGATTTTTTGGCCAAGGCATTTCACTTCCTGGAACCCCAGCTATGGATAAAACG
TCCATGGTTAATATGAGTCATGCCAACCCGGGCCTGGCTGACTATTTTGGCACCAATAGGCATCCTGCTGGTCTTA
CCTTTCCAACAGCTCCTGGATTTTCCTTTAGCTTTCCTGGTCTGTTTCCTTCGGGCTTATACCACAGGCCTCCTTT
GATACCCGCTAGTCCTCCTGTTAAAGGATTATCAAGTACCGAACAGTCAAACAAATGTCAAAGTCCCCTCTTGACA
CATCCTCAGATACTGCCAGCTACACAGGATATTTTGAAGGCACTATCTAAACACCCACCTGTAGGGGACAATAAGC
CAGTGGAACTGCTGCCCGAGAGGTCCTCTGAAGAGAGGCCCCTTGAGAAAATCAGTGACCAGTCAGAGAGTAGTGA
CCTTGATGATGTCAGTACACCAAGTGGCAGTGACCTGGAAACAACCTCGGGCTCTGATCTGGAAAGTGACCTTGAA
AGTGATAAAGAGAAATGTAAAGAAAATGGTAAAATGTTCAAAGACAAAGTAAGCCCTCTGCAGAATCTGGCTTCGA
TAACTAATAAGAAAGAACACAACAATCATTCCGTTTTCTCAGCATCTGTAGAGGAGCAAAGTGCCGTGTCAGGAGC
TGTGAATGATTCTATAAAGGCTATTGCGTCTATTGCTGAAAAATACTTTGGTTCTACAGGACTGGTGGGGCTGCAA
GACAAAAAAGTTGGAGCGTTACCTTACCCTTCCATGTTTCCCCTCCCGTTTTTTCCAGCATTCTCTCAATCAATGT
ACCCATTTCCTGATAGAGACTTGAGGTCGTTACCTTTGAAAATGGAGCCCCAATCACCAAGTGAAGTTAAGAAACT
GCAGAAGGGAAGCTCTGAGTCCCCTTTTGACCTCACCACTAAGAGAAAGGATGAGAAGCCCTTGACTTCAGGCCCC
TCGAAGCCTTCAGGAACACCAGCCACAAGCCAAGACCAGCCCCTGGATCTAAGTATGGGCAGTAGGGGTAGAGCCA
GTGGGACAAAGTTGACTGAGCCTCGAAAAAACCATGTGTTTGGGGAAAAGAAAGGAAGCAACATGGATACTAGGCC
ATCTTCAGATGGCTCCTTGCAGCATGCCAGACCCACTCCCTTCTTCATGGACCCCATTTATAGAGTAGAGAAAAGA
AAGTTAACTGACCCGCTTGAAGCTTTGAAAGAAAAATACTTGAGACCTTCTCCAGGATTCTTGTTTCACCCGCAAA
TGTCAGCAATTGAGAACATGGCAGAAAAGCTGGAAAGCTTCAGCGCCCTCAAACCTGAGGCCAGCGAGCTCCTGCA
GTCCGTGCCCTCCATGTTCAGCTTCCGAGCTCCTCCCAACACCCTGCCAGAGAACCTGCTGCGGAAGGGGAAAGAG
CGCTACACCTGCAGGTACTGTGGCAAGATATTTCCAAGGTCTGCGAACCTAACACGGCACTTGAGAACCCACACAG
GAGAGCAACCTTACAGATGCAAATACTGTGATAGATCATTCAGCATTTCTTCCAACCTGCAGCGACATGTGCGCAA
CATCCACAACAAGGAGAAGCCATTTAAGTGTCATTTATGTGACAGATGTTTTGGTCAACAAACCAATCTTGACAGA
CACCTGAAGAAACATGAGAACGGCAACATGTCTGGGACGGCAACGTCCTCGCCTCACTCAGAGCTAGAAAGCGCAG
GCGCAATCCTGGATGACAAAGAAGATGCGTACTTTACAGAGATCCGCAATTTCATCGGGAACAGCAACCATGGTAG
CCAGTCTCCTCGGAACATGGAAGAGAGGATGAATGGCAGTCACTTCAAGGATAAAAAGGCTTTGGCAACCAGCCAA
AATTCAGATTTATTGGATGATGAAGAAGTAGAAGATGAGGTGTTGTTGGATGAGGAGGATGAAGACAATGATATTC
CTGGAAAGCCCAGAAAGGAGCTAGGGGTGACTCGTTTAGACGAGGAGATCCCGGAGGATGACTACGAAGAAGCTGG
TGCCCTGGAGATGAGCTGTAAGGCGTCCCCGGTGAGGTATAAAGAGGAAGACTATAAATCTGGCCTTTCTGCTCTA
GATCACATAAGGCACTTCACAGATAGCCTCAAAATGAGGGAAATGGAAGAGAATCAATACACTGACGCTGAGCTGT
CCTCCATTAGTTCTTCTCATGTGCCAGAGGAGCTTAAACAGACGTTACACAGAAAGTCCAAATCACAGGCATATGC
TATGATGTTGTCACTGTCTGACAAGGATTCCCTCCATCCCACCTCCCACAGTTCCTCCAACGTGTGGCACAGCATG
GCAAGGGCTGCAGCAGAATCCAGTGCCATCCAGTCCATAAGCCATGTATGA

[0028] A ClustalW alignment of the human and mouse ME PR domains (amino acids 84-193 of SEQ ID NOs: 1, 3) is provided below:

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                                   111 115             130
MousePR  LRESTMPGAGLGIWTKRKIEIGEKFGPYMGEQRSDLKDSSYGWEILDEFCNVKFCIDASQ
HumanPR  LRESNMPGAGLGIWTKRKIEVGEKFGPYVGEQRSNLKDPSYGWEILDEFYNVKFCIDASQ
         ****.***************:*******:*****:***.********** **********

             149                         176            191
MousePR  PDVGSWLKYIRFAGCYDQHNLVACQINDQIFYRVVADIAPGEELLLFMKS
HumanPR  PDVGSWLKYIRFAGCYDQHNLVACQINDQIFYRVVADIAPGEELLLFMKS
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where the symbol (:) indicates that the amino acids variations share strongly similar properties, scoring >0.5 in the Gonnet PAM 250 matrix; the symbol (.) indicates that the amino acid variations share weakly similar properties, scoring ≤ 0.5 in the Gonnet PAM 250 matrix; and a space identifies a non-conserved amino acid variation. Based on the

above alignment, the human and mouse sequences share PR domains that are about 95% identical.

[0029] Based on the foregoing alignment, it is contemplated that the present invention can be practiced with ME proteins that share at least 60% identity, more preferably at least about 70% identity, most preferably at least about 80%, 85%, 90%, or 95% identity with the human or mouse PR domains, where residues critical to PR domain function, such as Y_{111}, R_{176}, and W_{149} in the mouse ME and Y_{109}, R_{174}, and W_{147} in the human ME, remain unaltered. It is further contemplated that the present invention can be practiced with ME proteins that share the consensus sequence below (SEQ ID NO: 5):

LRESXMPGAG LGIWTKRKIE XGEKFGP**Y**XG EQRSXLKDXS YGWEILDEFX NVKFCIDASQ
PDVGS**W**LKYI RFAGCYDQHN LVACQINDQI FY**R**VVADIAP GEELLLFMKS

where X can be any amino acid.

[0030] The SET domain is present in a variety of nuclear regulatory proteins and in many of these has been shown to have histone methyltransferase (HMT) activity, which is the ability to transfer a methyl group from S-adenosyl methionine (SAM) to lysine residues of specific target proteins. The most commonly investigated and tested target protein for this enzymatic activity is histone, though other proteins may also be targets (e.g., p53; Chuikov et al., "Regulation of p53 Activity Through Lysine Methylation," *Nature* 432:353-360 (2004), which is hereby incorporated by reference in its entirety). The potential of the PR domain of ME to harbor histone methyltransferase activity has been addressed, and while one report suggests that it does not in the absence of any other binding partner (Nanjundan et al., "Amplification of MDS1/EVI1 and EVI1, Located in the 3q26.2 Amplicon, is Associated with Favorable Patient Prognosis in Ovarian Cancer," *Cancer Res.* 67:3074-3084 (2007), which is hereby incorporated by reference in its entirety), a more recent report confirms that baculovirus-expressed, recombinant ME does possess histone methyltransferase activity (Pinheiro et al., "Prdm3 and Prdm16 are H3K9me1 Methyltransferases Required for Mammalian Heterochromatin Integrity." *Cell* 150: 948-960 (2012), which is hereby incorporated by reference in its entirety). Another report suggests the PR domain of ME may function in oligomerization (Nitta et al., "Oligomerization of Evi-1 Regulated by the PR Domain Contributes to Recruitment

of Corepressor CtBP," *Oncogene* 24:6165-6173 (2005), which is hereby incorporated by reference in its entirety); however, this is controversial (Senyuk et al., "The distal Zinc Finger Domain of AML1/MDS1/EVI1 is an Oligomerization Domain Involved in Induction of Hematopoietic Differentiation Defects in Primary Cells *In Vitro*," *Cancer Res*. 65:7603-7611 (2005), which is hereby incorporated by reference in its entirety). Instead, the accompanying Examples explain why it is believed that ME does, indeed, possesses methyltransferase activity, either alone or in the presence of a binding partner.

[0031] The methods of the present invention utilize an inhibitor of ME protein activity. These inhibitors can interact directly or indirectly with the ME protein to completely or partially inhibit ME protein function within the targeted cancer cells. As demonstrated in the accompanying Examples, ME protein function is critical to the survival of certain types of cancer cells (i.e., ME-dependent cancer cells). Alternatively, the inhibitors can interfere with ME protein expression so as to diminish or abolish the level of ME protein present in the targeted cancer cells.

[0032] In accordance with one embodiment, the inhibitor of ME protein activity is an antibody or antibody fragment or antibody mimic that binds specifically to a PR domain of the ME protein. Because of their specificity, monoclonal antibodies are particularly desirable; however, mono-specific polyclonal antibody populations can also be used. Antibodies are preferably directed to the binding of the PR domain at or near residues critical to PR domain function, such as Y_{111}, R_{176}, and W_{149} in the mouse ME and Y_{109}, R_{174}, and W_{147} in the human ME. Binding to the PR domain at or near these locations should interfere with ME activity and inhibit its function in the cancer cells.

[0033] Methods for monoclonal antibody production may be achieved using the techniques described herein or other well-known in the art (MONOCLONAL ANTIBODIES – PRODUCTION, ENGINEERING AND CLINICAL APPLICATIONS (Mary A. Ritter and Heather M. Ladyman eds., 1995), which is hereby incorporated by reference in its entirety). Generally, the process involves obtaining immune cells (lymphocytes) from the spleen of a mammal which has been previously immunized with the antigen of interest (i.e., the ME protein or specific peptide fragments thereof, such as the PR domain, alone or fused with a suitable immunogenic conjugate). Briefly, the antigen of interest is administered subcutaneously to New Zealand white

rabbits which have first been bled to obtain pre-immune serum. The antigen can be injected at a total volume of 100 µl per site at six different sites, and each injection will contain synthetic surfactant adjuvant pluronic polyols, or pulverized acrylamide gel containing the purified antigen. The rabbits are then bled two weeks after the first injection and periodically boosted with the same antigen three times every six weeks. A sample of serum is then collected 10 days after each boost. Ultimately, the rabbits are euthanized prior to harvesting lymphocytes from the spleen.

[0034] The antibody-secreting lymphocytes are then fused with myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is achieved by standard and well-known techniques, for example, by using polyethylene glycol (PEG) or other fusing agents (Milstein and Kohler, "Derivation of Specific Antibody-Producing Tissue Culture and Tumor Lines by Cell Fusion," *Eur. J. Immunol.* 6:511 (1976), which is hereby incorporated by reference in its entirety). The immortal cell line, which is preferably murine, but may also be derived from cells of other mammalian species, is selected to be deficient in enzymes necessary for the utilization of certain nutrients, to be capable of rapid growth, and have good fusion capability. The resulting fused cells, or hybridomas, are cultured, and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, and grown either *in vivo* or *in vitro* to produce large quantities of antibody.

[0035] In addition to whole antibodies, the present invention encompasses binding portions of such antibodies. Such binding portions include the monovalent Fab fragments, Fv fragments (e.g., single-chain antibody, scFv), and single variable V_H and V_L domains, and the bivalent $F(ab')_2$ fragments, Bis-scFv, diabodies, triabodies, minibodies, etc. These antibody fragments can be made by conventional procedures, such as proteolytic fragmentation procedures, as described in James Goding, MONOCLONAL ANTIBODIES:PRINCIPLES AND PRACTICE 98-118 (Academic Press, 1983) and Ed Harlow and David Lane, ANTIBODIES: A LABORATORY MANUAL (Cold Spring Harbor Laboratory, 1988); Houston et al., "Protein Engineering of Antibody Binding Sites: Recovery of Specific Activity in an Anti-Digoxin Single-Chain Fv Analogue Produced in *Escherichia coli,*" *Proc. Natl. Acad. Sci. USA*

85:5879-5883 (1988); Bird et al, "Single-Chain Antigen-Binding Proteins," *Science* 242:423-426 (1988), all of which are hereby incorporated by reference in their entirety, or other methods known in the art.

[0036] It may further be desirable, especially in the case of antibody fragments, to modify the antibody to increase its serum half-life. This can be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment by mutation of the appropriate region in the antibody fragment or by incorporating the epitope binding site into a peptide tag that is then fused to the antibody fragment at either end or in the middle (e.g., by DNA or peptide synthesis).

[0037] Antibody mimics are also suitable for use in accordance with the present invention. A number of antibody mimics are known in the art including, without limitation, those known as monobodies, which are derived from the tenth human fibronectin type III domain (^{10}Fn3) (Koide et al., "The Fibronectin Type III Domain as a Scaffold for Novel Binding Proteins," *J. Mol. Biol.* 284:1141-1151 (1998); Koide et al., "Probing Protein Conformational Changes in Living Cells by Using Designer Binding Proteins: Application to the Estrogen Receptor," *Proc. Natl. Acad. Sci. USA* 99:1253-1258 (2002), each of which is hereby incorporated by reference in its entirety); and those known as affibodies, which are derived from the stable alpha-helical bacterial receptor domain Z of staphylococcal protein A (Nord et al., "Binding Proteins Selected from Combinatorial Libraries of an alpha-helical Bacterial Receptor Domain," *Nature Biotechnol.* 15(8):772-777 (1997), which is hereby incorporated by reference in its entirety).

[0038] In preparing these antibody mimics the CDR sequences of the V_H and/or V_L chains (from monoclonal antibodies raised again ME or its PR domain) can be grafted into the variable loop regions of these antibody mimics. The grafting can involve a deletion of at least two amino acid residues up to substantially all but one amino acid residue appearing in a particular loop region along with the substitution of the CDR sequence. Insertions can be, for example, an insertion of the CDR domain at one or more locations of a particular loop region. The deletions, insertions, and replacements on the polypeptides can be achieved using recombinant techniques beginning with a known nucleotide sequence.

[0039] In accordance with another embodiment, the inhibitor of ME protein activity is an anti-ME nucleic acid aptamer that binds specifically to a PR domain of the ME protein.

[0040] Anti-ME nucleic acid aptamers can be formed of DNA or RNA, and are characterized by specificity for the ME PR domain. Aptamers are single-stranded, partially single-stranded, partially double-stranded, or double-stranded nucleotide sequences, advantageously a replicatable nucleotide sequence, capable of specifically recognizing a selected non-oligonucleotide molecule or group of molecules by a mechanism other than Watson-Crick base pairing or triplex formation. Aptamers include, without limitation, defined sequence segments and sequences comprising nucleotides, ribonucleotides, deoxyribonucleotides, nucleotide analogs, modified nucleotides and nucleotides comprising backbone modifications, branchpoints and nonnucleotide residues, groups or bridges.

[0041] Nucleic acid aptamers include multivalent aptamers and bivalent aptamers. Methods of making bivalent and multivalent aptamers and their expression in multi-cellular organisms are described in U.S. Pat. No. 6,458,559 to Shi et al., which is hereby incorporated by reference in its entirety. A method for modular design and construction of multivalent nucleic acid aptamers, their expression, and methods of use are described in U.S. Patent Publication No. 2005/0282190 to Shi et al, which is hereby incorporated by reference in its entirety.

[0042] Identifying suitable nucleic acid aptamers that inhibit the activity of ME, as described above, basically involves selecting aptamers that bind ME, specifically its PR domain, with sufficiently high affinity (e.g., Kd=20-50 nM) and specificity from a pool of nucleic acids containing a random region of varying or predetermined length (Shi et al., "A Specific RNA Hairpin Loop Structure Binds the RNA Recognition Motifs of the Drosophila SR Protein B52," *Mol. Cell Biol.* 17:1649-1657 (1997); Shi, "Perturbing Protein Function with RNA Aptamers" (thesis, Cornell University) microformed on (University Microfilms, Inc. 1997), each of which is hereby incorporated by reference in their entirety). For example, identifying suitable nucleic acid aptamers can be carried out using an established *in vitro* selection and amplification scheme known as SELEX using the PR domain of ME as the target for aptamer selection. The SELEX scheme is described in detail in U.S. Pat. No. 5,270,163 to Gold et al.; Ellington and Szostak, "*In Vitro* Selection of RNA

Molecules that Bind Specific Ligands," *Nature* 346:818-822 (1990); and Tuerk & Gold, "Systematic Evolution of Ligands by Exponential Enrichment: RNA Ligands to Bacteriophage T4 DNA Polymerase," *Science* 249:505-510 (1990), each of which is hereby incorporated by reference in its entirety. The SELEX procedure can be modified so that an entire pool of aptamers with binding affinity can be identified by selectively partitioning the pool of aptamers. This procedure is described in U.S. Patent Application Publication No. 2004/0053310 to Shi et al., which is hereby incorporated by reference in its entirety.

[0043] Once selected for their binding affinity, aptamers that bind to and inhibit activity of ME can be identified for use in the present invention based on their ability to inhibit cancer cell survival *in vitro* or *in vivo* as demonstrated in the accompanying Examples for shRNA.

[0044] In accordance with another embodiment, the inhibitor of ME protein activity is a dominant negative ME polypeptide that interferes with ME protein interaction with its putative binding partner. One example of such a ME polypeptide is the PR domain, identified above, which contains the putative binding pocket that interacts with the interacting partner of ME. Any dominant negative polypeptides that inhibit ME activity can also be screened *in vitro* and *in vivo* to assess their ability to inhibit cancer cell survival as described in the accompanying Examples for shRNA.

[0045] In accordance with a further embodiment, the inhibitor of ME protein activity can be a small molecule ME inhibitor that disrupts ME protein interaction with its putative binding partner. One approach for measuring such disruption is via a transcriptional readout. Any small molecules that inhibit ME activity can also be screened *in vitro* and *in vivo* to assess their ability to inhibit cancer cell survival as described in the accompanying Examples for shRNA.

[0046] A further embodiment of the inhibitor of ME protein activity is an inhibitory nucleic acid (RNAi) molecule that interferes with ME expression to cause a reduction in ME expression levels and, hence, overall levels of ME activity.

[0047] An important feature of RNAi affected by siRNA is the double stranded nature of the RNA and the absence of large overhanging pieces of single stranded RNA, although dsRNA with small overhangs and with intervening loops of RNA has been shown to effect suppression of a target gene. It will be understood that in this specification the terms siRNA and RNAi are interchangeable. Furthermore, as

is well-known in this field RNAi technology may be effected by siRNA, miRNA or shRNA or other RNAi inducing agents. Although siRNA will be referred to in general in the specification. It will be understood that any other RNA inducing agent may be used, including shRNA, miRNA or an RNAi-inducing vector whose presence within a cell results in production of an siRNA or shRNA targeted to a target *MECOM* or ME-encoding transcript.

[0048] RNA interference is a multistep process and is generally activated by double-stranded RNA (dsRNA) that is homologous in sequence to the targeted *MECOM* transcript. Introduction of long dsRNA into the cancer cells expressing *MECOM* leads to the sequence-specific degradation of those *MECOM* gene transcripts. The long dsRNA molecules are metabolized to small (e.g., 21 -23 nucleotide (nt)) interfering RNAs (siRNAs) by the action of an endogenous ribonuclease known as Dicer. The siRNA molecules bind to a protein complex, termed RNA-induced silencing complex (RISC), which contains a helicase activity and an endonuclease activity. The helicase activity unwinds the two strands of RNA molecules, allowing the antisense strand to bind to the targeted ME-encoding RNA molecule. The endonuclease activity hydrolyzes the ME-encoding RNA at the site where the antisense strand is bound. Therefore, RNAi is an antisense mechanism of action, as a single stranded (ssRNA) RNA molecule binds to the target ME-encoding RNA molecule and recruits a ribonuclease that degrades the ME-encoding RNA.

[0049] Preferably, siRNA, miRNA or shRNA targeting ME-encoding RNA are used.

[0050] Exemplary RNAi for ME (and Evi1) include, without limitation, an shRNA molecule encoded by the sequences:

5’-ATCCGAGCGCCTGACATCCACGAAGAACTTCAAGAGAGTTCTTCGTGG ATGTCAGGCGCtttttACGCGTG-3’ (SEQ ID NO: 6), corresponding to bp 511-532 of GenBank sequence M21829, and designated as sh11, which has the following structure:

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GATCCGAGCGCCTGACATCCACGAAGAACTTCA
          ||||||||||||||||||||||      A
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GTGCGCATTTTTCGCGGACTGTAGGTGCTTCTTGAGAG
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(SEQ ID NO: 6);

and

5'-GATCCGAGTTGTTGGATGAGGAGGATGAATTCAAGAGATTCATCCTC CTCATCCAACAACtttttACGCGTG-3' (SEQ ID NO: 7), corresponding to bp 3139-3167 of M21829 and designated as sh54, which has the following structure:

```
        GATCCGAGTTGTTGGATGAGGAGGATGAATTCA
                    |||||||||||||||||||||||    A
GTGCGCATTTTTCAACAACCTACTCCTCCTACTTAGAG
```
(SEQ ID NO: 7).

These sequences were selected using software at the RNAi OligoRetriever Database.

[0051] Exemplary shRNA specific for the murine ME PR domain include, without limitation:

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CCTATTCCTGATGAGTTTGAGCTTCA
|||||||||||||||||||||||    A
GGATAAGGACTACTCAAACTCGAGAG
```
(SEQ ID NO: 8)

This sequence corresponds to basepairs 229-250 of the composite (CN697723-M21829) sequence listed above, and was obtained from GeneLink, Inc.

[0052] Exemplary RNAi specific for the ME PR domain region include, without limitation: RNAi targeting nt 494-514 of the above identified human ME-encoding sequence (target = GCCAGATAAATGATCAGATAT) (SEQ ID NO: 9), which was identified using the Invitrogen RNAi Designer website; RNAi targeting nt 403-421 of the above-identified human ME-encoding sequence (uuCUGCAUAGAUGCCAGUCAAcc) (SEQ ID NO: 10), which was identified by the Kribbs siRNA design website; and RNAi targeting nt 266-284 of the above-identified human ME-encoding sequence (ggGCAGGACUAGGAAUAUGGAcc) (SEQ ID NO: 11), which was identified by the Kribbs siRNA design website.

[0053] In certain embodiments, delivery of the inhibitor of ME protein activity can be achieved systemically using a non-targeted delivery system. Non-targeted delivery of RNAi specific for the ME PR domain region is feasible, because blocking ME function in normal cells is not detrimental. This is confirmed via MDS1 KO homozygous mice, which are perfectly viable. For example, delivery agents for the inhibitor of ME protein activity may include those selected from the following

non-limiting group of cationic polymers, modified cationic polymers, peptide molecular transporters, lipids, liposomes and/or non-cationic polymers. Viral vector delivery systems may also be used for RNAi inducing agents. For example, an alternative delivery route includes the direct delivery of RNAi inducing agents (including siRNA, shRNA and miRNA) and even anti-sense RNA (asRNA) in gene constructs followed by the transformation of cells within bone marrow compartment with the resulting recombinant DNA molecules. This results in the transcription of the gene constructs encoding the RNAi inducing agent, such as siRNA, shRNA and miRNA, or even asRNA and provides for the transient and stable expression of the RNAi inducing agent in those transformed cells of the bone marrow compartment.

[0054] In other embodiments, a targeted delivery system or complex can be used to deliver the inhibitor of ME protein activity primarily or exclusively to the targeted cancerous cells. There are a number of targeted delivery vehicles that use peptide/receptor-, antibody-, or aptamer-mediated delivery of molecular complexes or particles to the cancer cells of interest.

[0055] One example of a peptide/receptor targeting system is described by Jin et al. ("Targeted Delivery of Antisense Oligodeoxynucleotide by Transferrin Conjugated pH-sensitive Lipopolyplex Nanoparticles: A Novel Oligonucleotide-based Therapeutic Strategy in Acute Myeloid Leukemia," *Mol. Pharm.* 7(1):196-206 (2010), which is hereby incorporated by reference in its entirety). Jin et al. describes the effective use of transferrin conjugated pH-sensitive lipopolyplex nanoparticles (LPs) that incorporate a therapeutic nucleic acid molecule and can release the same at acidic endosomal pH to facilitate the cytoplasmic delivery of the therapeutic nucleic acid molecule after endocytosis.

[0056] Several examples describe the use of monoclonal anti-CD-33 antibody-mediated delivery of therapeutic complexes to AML cells. One example, reported by Simard et al. ("*In vivo* Evaluation of pH-sensitive Polymer-based Immunoliposomes Targeting the CD33 Antigen ," *Mol. Pharm.* 7(4):1098-1107 (2010), which is hereby incorporated by reference in its entirety), involves pH-sensitive immunoliposomes obtained by anchoring a copolymer of dioctadecyl, N-isopropylacrylamide and methacrylic acid in bilayers of PEGylated liposomes and coupling the whole anti-CD33 monoclonal antibody (mAb) or its Fab' fragments, which was then used to deliver a payload of 1-beta-d-arabinofuranosylcytosine (ara-C) to human myeloid

leukemia cells in a mouse model. Another example, reported by Rothdiener et al., ("Targeted Delivery of SiRNA to CD33-positive Tumor Cells with Liposomal Carrier Systems," *J. Control Release* 144(2):251-258 (2010), which is hereby incorporated by reference in its entirety), involves siRNA-loaded immunoliposomes (IL) and immunolipoplexes (ILP) containing free or polyethylene imine (PEI)-complexed into PEGylated liposomes endowed with an anti-CD33 single-chain Fv fragment (scFv). One well known anti-CD33 mAb is gemtuzumab.

[0057] Several examples describe the use of monoclonal anti-CD-19 antibody-mediated delivery of therapeutic complexes to ALL cells. One example, reported by Harata et al., ("CD19-targeting Liposomes Containing Imatinib Efficiently Kill Philadelphia Chromosome-positive Acute Lymphoblastic Leukemia Cells," *Blood* 104(5):1442-1449 (2004), which is hereby incorporated by reference in its entirety), involves immunoliposome carrying anti-CD19 antibody (CD19-liposomes) for the delivery of the BCR-ABL tyrosine kinase inhibitor Imatinib to Philadelphia chromosome-positive acute lymphoblastic leukemia at near 100% internalization efficiency.

[0058] As an alternative to antibody-based targeting of the ME-expressing cancer cells, aptamers can also be used. One exemplary aptamer that targets myeloid leukemic cells is KH1C12 [5'-
dAdTdCdCdAdGdAdGdTdGdAdCdGdCdAdGdCdAdTdGdCdCdC
dTdAdGdTdTdAdCdTdAdCdTdAdCdTdCdTdTdTdTdTdAdGdCdAdAdAdCdGdCd
CdCdTdCdGdCdTdTdTdGdGdAdCdAdCdGdGdTdGdGdCdTdTdAdGdT-3'] (SEQ ID NO: 12). This aptamer can be joined to an aptamer or RNAi molecule that inhibits ME expression or activity, as described. Before joining two functional RNA molecules, it is often beneficial to first predict the secondary structures of the chimeric nucleic acid molecule to ensure that their combination is unlikely to disrupt their secondary structures. Secondary structure predictions can be performed using a variety of software including, without limitation, RNA Structure Program (Dr. David Mathews, University of Rochester) and MFold (Dr. Michael Zuker, The RNA Institute, SUNY at Albany), among others. If the secondary structure predictions suggest no problems, then the chimeric nucleic acid molecules can be generated. Double-stranded DNA templates can be prepared by cloning their PCR products into a cloning vector and using the clones as templates for PCR with the appropriate

primers (e.g., 5' primer for one aptamer portion and 3' primer for the other aptamer portion). These same primers can be used to generate the chimeric DNA template for transcription, and *in vitro* transcription can be carried out using standard procedures to obtain the RNA chimeras, which can then be gel purified prior to use.

[0059] One embodiment of the chimeric therapeutic agent (10) shown in Figure 7 includes the AML-specific aptamer KH1C12 (12) and either an ME-inhibiting aptamer or ME-inhibiting RNAi molecule, generally denoted at (14), which is targeted to AML cells recognized by the KH1C12 aptamer. Upon binding of the KH1C12 aptamer to the AML cell, the cancer cell will take up the molecule and the ME-inhibiting component (14) will interfere with ME expression or activity. As demonstrated in the accompanying Examples, disruption of ME expression or activity will diminish both proliferation and survival of the targeted cancer cells.

[0060] In another approach for targeted delivery of the therapeutic agent, illustrated in Figure 8, a conjugated aptamer molecule (20) is provided. In this embodiment, one or more AML-specific KH1C12 aptamers (26) and one or more ME-inhibiting aptamer or ME-inhibiting RNAi molecule, generally denoted at (28), form the functional components of the conjugate (20). (In Figure 8, two of each are shown.) All four of these molecules are biotinylated (24), and the conjugate is formed upon incubation of the biotinylated aptamers/RNAi molecules with streptavidin (22). Biotinylation of the aptamers at their 3′ ends is known not to interfere with the activity of these RNA molecules (*see* Chu et al., "Aptamer Mediated siRNA Delivery," *Nuc.l Acids Res.* 34(10):e73 (2006), which is hereby incorporated by reference in its entirety). Conjugate (20) is targeted to AML cells recognized by the KH1C12 aptamer. Upon binding of the KH1C12 aptamer to the AML cell, the cancer cell will take up the molecule and the ME-inhibiting component (28) will interfere with ME expression or activity. As demonstrated in the accompanying Examples, disruption of ME expression or activity will diminish both proliferation and survival of the targeted cancer cells.

[0061] In a further approach for targeted delivery of the therapeutic agent, illustrated in Figure 9, a conjugate (30) includes a polycation-aptamer or RNAi vector (32) linked via phenyl(di)boronic acid-salicylhydroxamic acid assembly to an antibody (34) that is specific for a cancer cell surface marker (e.g., CD-33, CD-19, CD-20 as described above). In this embodiment, the phenyl(di)boronic acid is first

coupled to the antibody via a PEG linker using the methodology of Moffatt et al., "Successful *In Vivo* Tumor Targeting of Prostate-specific Membrane Antigen with a Highly Efficient J591/PEI/DNA Molecular Conjugate," *Gene Therapy* 13:761-772 (2006), which is hereby incorporated by reference in its entirety. The salicylhydroxamic acid is coupled to polyethyleneimine (PEI), a polycation, using the procedures of Moffatt et al. ("Successful *In Vivo* Tumor Targeting of Prostate-specific Membrane Antigen with a Highly Efficient J591/PEI/DNA Molecular Conjugate," *Gene Therapy* 13:761-772 (2006), which is hereby incorporated by reference in its entirety), and thereafter the aptamer/RNAi molecule can be introduced to the SHA-PEI solution to form the self-assembled conjugate (30).

[0062] One embodiment of this type of therapeutic agent includes a CD-33-specific monoclonal antibody and one or more ME-specific RNAi molecules in the PEI matrix, which are conjugated together via PDB-SHA bridge. This conjugate is targeted to CD-33-positive AML cells. Upon binding of the antibody to the CD-33-positive AML cells, the cancer cell will take up the conjugate and the ME-specific RNAi will interfere with ME expression and activity. As demonstrated in the accompanying Examples, disruption of ME expression or activity will diminish both proliferation and survival of the targeted cancer cells.

[0063] Another embodiment of this type of therapeutic agent includes a CD-19-specific monoclonal antibody and one or more ME-specific RNAi molecules in the PEI matrix, which are conjugated together via PDB-SHA bridge. This conjugate is targeted to CD-19-positive AML cells. Upon binding of the antibody to the CD-19-positive AML cells, the cancer cell will take up the conjugate and the ME-specific RNAi will interfere with ME expression and activity. As demonstrated in the accompanying Examples, disruption of ME expression or activity will diminish both proliferation and survival of the targeted cancer cells.

[0064] Another embodiment of this type of therapeutic agent includes a CD-20-specific monoclonal antibody and one or more ME-specific RNAi molecules in the PEI matrix, which are conjugated together via PDB-SHA bridge. This conjugate is targeted to CD-20-positive AML cells. Upon binding of the antibody to the CD-20-positive AML cells, the cancer cell will take up the conjugate and the ME-specific RNAi will interfere with ME expression and activity. As demonstrated in the

accompanying Examples, disruption of ME expression or activity will diminish both proliferation and survival of the targeted cancer cells.

[0065] Polymeric nanoparticles can be targeted to cell-surface marked using aptamers designed using the SELEX procedure (Farokhzad et al., "Targeted Nanoparticle-aptamer Bioconjugates for Cancer Chemotherapy *in vivo*," *Proc. Natl. Acad. Sci. USA* 103(16):6315-6320 (2006), which is hereby incorporated by reference in its entirety). Nanoparticles and microparticles may comprise a concentrated core of drug that is surrounded by a polymeric shell (nanocapsules) or as a solid or a liquid dispersed throughout a polymer matrix (nanospheres). General methods of preparing nanoparticles and microparticles are described by Soppimath et al., "Biodegradable Polymeric Nanoparticles as Drug Delivery Devices," *J. Control Release* 70(1-2):1-20 (2001), which is hereby incorporated by reference in its entirety. Other polymeric delivery vehicles that may be used include block copolymer micelles that comprise a drug containing a hydrophobic core surrounded by a hydrophilic shell; they are generally utilized as carriers for hydrophobic drugs and can be prepared as found in Allen et al., "Colloids and Surfaces," *Biointerfaces* 16(1-4):3-27 (1999), which is hereby incorporated by reference in its entirety. Polymer-lipid hybrid systems consist of a polymer nanoparticle surrounded by a lipid monolayer. The polymer particle serves as a cargo space for the incorporation of hydrophobic drugs while the lipid monolayer provides a stabilizing interference between the hydrophobic core and the external aqueous environment. Polymers such as polycaprolactone and poly(D,L-lactide) may be used while the lipid monolayer is typically composed of a mixture of lipids. Suitable methods of preparation are similar to those referenced above for polymer nanoparticles. Derivatized single chain polymers are polymers adapted for covalent linkage of a biologically active agent to form a polymer-drug conjugate. Numerous polymers have been proposed for synthesis of polymer-drug conjugates including polyaminoacids, polysaccharides such as dextrin or dextran, and synthetic polymers such as N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer. Suitable methods of preparation are detailed in Veronese et al, "Bioconjugation in Pharmaceutical Chemistry," *IL Farmaco* 54(8):497-516 (1999), which is hereby incorporated by reference in its entirety.

[0066] The various therapeutic agents of the present invention can be administered systemically (e.g., orally, parenterally, subcutaneously, intravenously,

intramuscularly, intraperitoneally, by intranasal instillation, by implantation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, transdermally, by application to mucous membranes, or by introduction into one or more lymph nodes), and/or directly into a bone marrow compartment.

[0067] For the use in accordance with the invention, the appropriate dosage of the therapeutic agent, e.g. inhibitor of ME activity, will, of course, vary depending upon, for example, the particular agent to be employed, the host, the mode of administration and the severity of the cancerous condition being treated, and the effects desired. Satisfactory results can be obtained at dosages from about 0.1 mg to about 1000 mg, preferably from 1 to 100 mg, more preferably 20-50 mg. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight, or within the range of 1-10 mg/kg body weight. Administration may be in a single dose or in several doses over a period of time as long as may be indicated in relation to the time the disease is clinically evident or prophylactically to suppress further clinical relapse, for example a dose from about 5 up to about 100 mg, may be administered once a month, until control or amelioration of the disease is achieved. A preferred dosage regimen comprises administration of 20-50 mg of the therapeutic inhibitor of ME activity every two weeks or once a month. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months.

[0068] For oral therapeutic administration (e.g., sublingual delivery), the therapeutic agents may be incorporated with excipients and used in the form of tablets, capsules, elixirs, suspensions, syrups, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compound in these compositions may, of course, be varied and may conveniently be between about 2% to about 60% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained.

[0069] The tablets, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch, or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a

lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose, or saccharin. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a fatty oil.

[0070] Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar, or both. A syrup may contain, in addition to active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye, and flavoring such as cherry or orange flavor.

[0071] The therapeutic agent may also be administered parenterally. Solutions or suspensions of these therapeutic agents can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols such as, propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0072] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability 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, saline, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

[0073] The therapeutic agents of the present invention may also be administered directly to the airways in the form of an aerosol or via a lung surfactant formulation. For use as aerosols, the inhibitors of the present invention in solution or suspension may be packaged in a pressurized aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The materials of the present invention also may be

administered in a non-pressurized form such as in a nebulizer or atomizer. A number of commercially available lung surfactant formulations exist, including synthetic surfactant formulations and exogenous formulations.

[0074] Persons of skill in the art are readily able to test and assess optimal dosage schedules based on the balance of efficacy and any undesirable side effects. The optimal dosage of each type of ME inhibitor will vary, of course, and the minimal effective dose will be administered for therapeutic regimen.

[0075] Sustained release formulations include implantable devices that include a slow-dissolving polymeric matrix and the therapeutic agent of the invention retained within the polymeric matrix. The matrix can be designed to deliver substantially the entire payload of the vehicle over a predetermined period of time, such as about one to two weeks or about one to three months.

[0076] As indicated above, the therapeutic agents of the present invention can be administered in using a delivery vehicle for passive or targeted delivery to cancer cells that express the ME protein for its continued survival. As indicated, any suitable passive or targeted delivery vehicle can be employed, including liposomes, polymeric nanoparticles, chimeric proteins, polyethylene glycol conjugates, and oligoarginine.

[0077] The therapeutic agents of the present invention can also be used alone or in combination with one or more additional therapies, including without limitation, bone marrow transplant, chemotherapies, radiation therapies, immunotherapies, and combinations thereof with or without adjuvants that enhance the efficacy of those therapies, such as CXCR4 antagonists of the type described in O'Callaghan et al., "Targeting CXCR4 with Cell-penetrating Pepducins in Lymphoma and Lymphocytic Leukemia," *Blood* 119(7):1717-1725 (2012); Parameswaran et al., "Treatment of Acute Lymphoblastic Leukemia with an rGel/BLyS Fusion Toxin," *Leukemia* doi: 10.1038/leu.2012.54 (February 2012), each of which is hereby incorporated by reference in its entirety.

[0078] Exemplary conventional and experimental therapies for AML include, without limitation, cytarabine and anthracycline chemotherapy regimen, gemtuzumab ozogamicin, stem cell transplant, clofarabine, farnesyl transferase inhibitors, decitabine, MDR1 inhibitors, arsenic trioxide, all-trans retinoic acid.

[0079] Exemplary conventional and experimental therapies for ALL include, without limitation, one or more of the following: radiation therapy on affected bone

areas; combinations of prednisone or dexamethasone, vincristine, asparaginase, and daunorubicin to induce remission; combinations of vincristine, cyclophosphamide, cytarabine, etoposide, thioguanine, or mercaptopurine during intensification, or for CNS protection intrathecal methotrexate or cytarabine, alone or combined, and either with or without cranio-spinal irradiation; intrathecal administration of hydrocortisone, methotrexate, and cytarabine for CNS relapse; and the scheduled separate administration of mercaptopurine, methotrexate, vincristine and corticosteroids for maintenance therapy.

EXAMPLES

[0080] The Examples set forth below are for illustrative purposes only and are not intended to limit, in any way, the scope of the present invention.

Materials and Methods for Examples 1-5

[0081] *Mice* – ME^{ml} and ME^{fl4} ($Evi1^{fl3}$ previously) alleles (Zhang et al., “PR Domain–Containing Mds1-Evi1 is Critical for Long-Term Hematopoietic Stem Cell Function,” *Blood* 118(14):3853-3861 (2011), which is hereby incorporated by reference in its entirety) and *Esr-Cre* (Hayashi et al., “Efficient Recombination in Diverse Tissues by a Tamoxifen-Inducible Form of Cre: A Tool for Temporally Regulated Gene Activation/Inactivation in the Mouse,” *Dev. Biol.* 244:305-318 (2002), which is hereby incorporated by reference in its entirety) have been described. All procedures were approved by an animal care and use committee.

[0082] *Retroviral constructs* – The MLL-AF9 expression construct (Barabé et al., “Modeling the Initiation and Progression of Human Acute Leukemia in Mice,” *Science* 316:600-604 (2007), which is hereby incorporated by reference in its entirety) was moved to mCherry. pMIGR1-ME (p1183) was made by inserting MDS1 (Zhang et al., “PR Domain–Containing Mds1-Evi1 is Critical for Long-Term Hematopoietic Stem Cell Function *Blood*. 118(14):3853-3861 (2011), which is hereby incorporated by reference in its entirety) into BglII-cut p854 (Zhang et al., “Targeting a DNA Binding Motif of the EVI1 Protein by a Pyrrole-Imidazole Polyamide,” *Biochemistry* 50(48):10431-10441 (2011), which is hereby incorporated by reference in its entirety) pMIGR1-MDS (p1211) was made by PCR

(5'atgaattcgcatgagatccaaaggcagggc3'/5'atgaattcttcacctggtctcccatccatagctg3') (SEQ ID NOS: 13 and 14, respectively) and cloning.

[0083] *Serial replating assay* – Lineage-negative/Sca-1$^+$/c-kit$^+$ (LSK) cells were isolated (t=0hr), infected (Zhang et al., "PR Domain–Containing Mds1-Evi1 is Critical for Long-Term Hematopoietic Stem Cell Function," *Blood* 118(14):3853-3861 (2011), which is hereby incorporated by reference in its entirety) with retrovirus (t=15hr), sorted (t=63hr), and plated in M3434 (StemCell). For Figure 1F, add-back infections were performed following MLL-AF9 sort (t=64h), sorted for GFP (t=90h) and plated in M3434 (Lavau et al., "Immortalization and Leukemic Transformation of a Myelomonocytic Precursor by Retrovirally Transduced HRX-ENL," *EMBO J.* 16:4226–4237 (1997), which is hereby incorporated by reference in its entirety).

[0084] *In vivo leukemogenesis assay* – Primary leukemias were harvested, explanted to culture, +/- 4-OH TAM; 10^6 spleen cells/mouse were transplanted into sublethally irradiated secondary recipient mice via tail vein injection. Leukemia development in transplanted mice was monitored by assessing total blood counts (CBC) every week.

Example 1 – Knock-in of *lacZ* into *Mds1* Exon 1

[0085] To assess the role of the *Mds1* gene in hematopoiesis, a targeted disruption of the *Mds1* was created by inserting a promoterless *lacZ* gene into the first coding exon in the mouse designated $Mds1^{tm1ap}$ (herein termed ME^{m1}; Barabé et al., "Modeling the Initiation and Progression of Human Acute Leukemia in Mice," *Science* 316:600-604 (2007), which is hereby incorporated by reference in its entirety). The structure of the ME^{m1} allele is such that the expression of *lacZ* is under the control of the *Mds1* promoter, and the splice donor is deleted. The β-galactosidase protein produced contains a nuclear localization signal from SV40 virus; staining for the enzyme was found to be concentrated to the nucleus. Matings between heterozygous mice generated live born homozygous $ME^{m1/m1}$ mice close to the expected Mendelian frequency (Barabé et al., "Modeling the Initiation and Progression of Human Acute Leukemia in Mice," *Science* 316:600-604 (2007), which is hereby incorporated by reference in its entirety). $ME^{m1/m1}$ mice appeared normal at birth, but grew more slowly than wildtype and all developed a progressive kyphosis (Zhang et al., "Targeting a DNA Binding Motif of the EVI1 Protein by a Pyrrole-

Imidazole Polyamide," *Biochemistry* 50(48):10431-10441 (2011), which is hereby incorporated by reference in its entirety). To assess allelism with *Evi1*, matings of *Mds1*$^{m1/+}$ and *Evi1*$^{+/-}$ mice (Lavau et al., "Immortalization and Leukemic Transformation of a Myelomonocytic Precursor by Retrovirally Transduced HRX-ENL," *EMBO J.* 16:4226–4237 (1997), which is hereby incorporated by reference in its entirety) were performed which revealed that no mice carrying both mutations were identified ($p<0.0001$). These data indicate an essential role for the combination of both *ME* and *Evi1* transcripts during development.

[0086] It is known that *Evi1* is one of the highest upregulated genes in hematopoietic stem and progenitor cells of preleukemic MLL-AF9 knockin mice (Silverman et al., "Intensified Therapy for Infants With Acute Lymphoblastic Leukemia: Results From the Dana-Farber Cancer Institute Consortium," *Cancer* 80(12):2285-2295 (1997), which is hereby incorporated by reference in its entirety); however this study did not distinguish *Evi1* from *ME* mRNA transcripts. While conditional knockout studies deleting exon 4 of *Evi1* (Figure 5) have shown that MLL-AF9 transformation is dependent on an intact *Mecom* gene (Lavau et al., "Immortalization and Leukemic Transformation of a Myelomonocytic Precursor by Retrovirally Transduced HRX-ENL," *EMBO J.* 16:4226-4237 (1997), which is hereby incorporated by reference in its entirety), this exon is required for both *ME* and *Evi1* transcripts (Figures 6A-B); as such, it was not possible to discern which transcript was responsible for the resistance to transformation by MLL-AF9. Herein, ME is identified as being a critical downstream component in MFP-induced transformation. Further, it is shown that within ME, it is the PR domain that is critical. The mutagenesis and *in silico* structural analysis of this domain strongly suggests that its function in MFP-induced transformation depends on a substrate binding pocket, and thus, is likely druggable.

Example 2 – Knockout of *Mds1* Isoforms Abrogates the Ability of MLL-AF9 to Transform Bone Marrow Progenitors; Add-Back Assay Shows Primary Role for PR Domain of ME Isoform

[0087] To test the role of *ME* in MLL-AF9 leukemogenesis, bone marrow cells from *ME*$^{m1/m1}$ mice were infected which bear a *lacZ* insertion in exon 1 of *Mds1* (Figure 1A) and lack MDS1 and ME, but express normal levels of EVI1, and serial replating assay was performed (Lavau et al., "Immortalization and Leukemic

Transformation of a Myelomonocytic Precursor by Retrovirally Transduced HRX-ENL," *EMBO J.* 16:4226-4237 (1997); Dobson et al., "The mll-AF9 Gene Fusion in Mice Controls Myeloproliferation and Specifies Acute Myeloid Leukaemogenesis," *Embo J.* 18(13):3564-3574 (1999); Krivtsov et al., "Transformation From Committed Progenitor to Leukaemia Stem Cell Initiated by MLL-AF9," *Nature* 442(7104):818-822 (2006); and Somervaille et al., "Identification and Characterization of Leukemia Stem Cells in Murine MLL-AF9 Acute Myeloid Leukemia," *Cancer Cell* 10:257-268 (2006), which are hereby incorporated by reference in their entirety). While MLL-AF9 induced wildtype cells to form colonies at each cycle, it was unable to transform $ME^{m1/m1}$ cells (Figure 1B). These findings indicate a dependency of MLL-AF9 transformation on functional *Mds1* and/or *ME*.

[0088] Whether the transformation block was specific for MLL-AF9 was then tested. $ME^{m1/m1}$ cells were transduced with MLL-AF9, Nup98-HoxA9, MLL-ENL, E2A-Hlf, or Meis1-HoxA9, and were assayed for transformation. This revealed that cells lacking *ME* were resistant to transformation only by MLL-AF9 or MLL-ENL (Figure 1C). Thus, the block to transformation is oncogene-specific. To further test this, $ME^{fl4/m1}$/*Esr-Cre* (Figure 1D) and $ME^{fl4/+}$/*Esr-Cre* (Figure 1E) LSK cells were transduced with the same oncogenes, split into two treatment groups (vehicle or 4-OH-TAM) and assayed for transformation. All vehicle-treated cell samples, regardless of genotype, were fully capable of being transformed with all oncogenes (Figures 1D-E), while 4-OH-TAM-treated $ME^{fl4/m1}$/*Esr-Cre* cells displayed the same selective resistance to transformation; 4-OH TAM-treated $ME^{fl4/+}$/*Esr-Cre* cells were susceptible to transformation by all oncogenes. Together, these data indicate a selective requirement for a functional *ME* allele for transformation by MFP oncogenes.

[0089] To test which isoform of *Mecom* is required for MLL-AF9 transformation, LSK cells were retrovirally transduced from $ME^{m1/m1}$ mice with MIGR1, or with constructs for MDS1, EVI1, or ME, and then infected with MLL-AF9 retrovirus; transduced cells were assayed for transformation. While neither MDS1 nor EVI1 were able to rescue the deficiency, ME was so able (Figure 1F), confirming that in the context of the $ME^{m1/m1}$ genotype, ME is the essential isoform that is lacking. Since the major difference between ME and EVI1 is the PR domain, it

is clear that this domain is critical for ME activity in the setting of MLL-AF9-induced leukemogenesis.

Example 3 – Knockout of *ME* Results in Suppression of MLL-AF9-Leukemia Development in Transplanted Mice

[0090] To test if *ME* is required for transformed MLL-AF9 AML cells to survive *in vivo*, transplantable leukemias were established and then the gene was deleted and assayed for leukemogenesis by transplantation. $ME^{fl4/m1}$/*Esr-Cre* or $ME^{fl4/+}$/*Esr-Cre* LSK cells were transduced with MLL-AF9 and transplanted into irradiated recipients. After 3 months, primary AMLs developed, and leukemic spleen cells were harvested, induced with 4-OH-TAM to delete *ME* (yielding genotypes $ME^{ko4/m1}$ and $ME^{ko4/+}$, respectively), and injected into irradiated recipients. Weekly CBCs noted an increasing leukocyte count and persistently low hematocrit and platelet count in mice receiving $ME^{fl4/m1}$/*Esr-Cre* cells not treated with 4-OH-TAM (Figures 2A-C), indicating infiltration of leukemic cells into the blood and bone marrow; in mice receiving 4-OH-TAM-treated $ME^{fl4/m1}$/*Esr-Cre* cells, a normal leukocyte level was maintained, and platelets and hematocrit recovered to normal levels. The control group mice receiving $ME^{fl4/+}$/*Esr-Cre* cells with and without 4-OH-TAM treatment all develop leukemia as expected.

[0091] Four weeks post-transplant, mice receiving $ME^{fl4/+}$/*Esr-Cre* cells with and without 4-OH-TAM treatment, and mice receiving $ME^{fl4/m1}$/*Esr-Cre* cells untreated with 4-OH-TAM became moribund; in contrast, the cohort receiving $ME^{fl4/m1}$/*Esr-Cre* cells treated with 4-OH-TAM remained healthy. Necropsy confirmed that the moribund mice had widely disseminated disease, with enlarged spleens (Figures 2D-E), and, on histopathology, leukemic cell infiltration of bone marrow (Figure 2F), liver (Figure 2H) and spleen. In contrast, mice receiving 4-OH-TAM-treated $ME^{fl4/m1}$/*Esr-Cre* leukemic cells remained healthy and had normal-sized spleens at necropsy (Figures 2C-D), with no or minimal AML infiltration into organs, as assessed by histopathology (Figures 2E-H).

[0092] Bindels et al. showed overexpression of EVI1 in a subset of MLL fusion protein leukemias; those expressing EVI1 were phenotypically distinct in that they rarely showed monoblastic phenotype (Bindels et al., "EVI1 is Critical for the Pathogenesis of a Subset of MLL-AF9-rearranged AMLs," *Blood* 119(24):5838-5849

(2012), which is hereby incorporated by reference in its entirety). Thus, it appears that not all cases of MFP-induced leukemias express the *MECOM* locus; and that there is a distinct phenotype (monoblastic) when *MECOM* is not activated. It is likely that *MECOM* non-expressing MFP-induced leukemias arise via transformation of a cell that is beyond the HSC/CMP stage, at which the *MECOM* locus is normally silenced (Krivtsov et al., "Cell of Origin Determines Clinically Relevant Subtypes of *MLL*-Rearranged AML," *Leukemia* 27:1-9 (2013), which is hereby incorporated by reference in its entirety). Arai et al. established that MFPs can upregulate transcription of both EVI1 and ME (Arai et al., "Evi-1 is a Transcriptional Target of Mixed-Lineage Leukemia Oncoproteins in Hematopoietic Stem Cells," *Blood* 117(23):6304-6314 (2011), which is hereby incorporated by reference in its entirety). While these results reveal an essential role for ME, they do not preclude that EVI1 isoforms are also essential. Nor do they exclude the possibility that it is the ratio of ME to EVI1 that is critical.

[0093] Currently there are no clinically available targeted therapies for MLL fusion protein leukemias. Published reports identify alternative targeted therapies for these leukemias: inhibitors of glycerol synthase kinase (GSK) (Wang et al., "Glycogen Synthase Kinase 3 in MLL Leukemia Maintenance and Targeted Therapy," *Nature* 455:1205-1209 (2008), which is hereby incorporated by reference in its entirety), DOT1L (Daigle et al., "Selective Killing of Mixed Lineage Leukemia Cells by a Potent Small-Molecule DOT1L Inhibitor," *Cancer Cell* 20(1):53-65 (2011), which is hereby incorporated by reference in its entirety), and the MFP-MENIN interaction (Grembecka et al., "Menin-MLL Inhibitors Reverse Oncogenic Activity of MLL Fusion Proteins in Leukemia," *Nat. Chem. Biol.* 8(3):277-284 (2012), which is hereby incorporated by reference in its entirety). However, none of these has yet made it to the clinic, and furthermore, each is likely toxic, based on interpolation from genetic studies (Jones et al., "The Histone H3K79 Methyltransferase Dot1L is Essential for Mammalian Development and Heterochromatin Structure," *PLoS Genet.* 4(9):e1000190 (2008); Feng et al., "Early Mammalian Erythropoiesis Requires the Dot1L Methyltransferase," *Blood* 116(22):4483-4491 (2010); and Chen et al., "The Tumor Suppressor Menin Regulates Hematopoiesis and Myeloid Transformation by Influencing Hox Gene Expression," *Proc. Natl. Acad. Sci. USA* 103:1018-1023 (2006), all of which are hereby

incorporated by reference in their entirety). Thus, despite these reports, there is still a need for additional avenues for therapeutic intervention. The present invention reveals a novel discovery that ME is necessary for MFP transformation.

[0094] The fact that ME but not EVI1 can rescue the mutant phenotype centers attention on the PR domain as being responsible for ME function in the setting of MFP leukemogenesis. Recent studies have shown that this domain harbors H3K9 monomethyltransferase activity, which was expected based on the finding of HMT activity in closely related proteins (Wu et al., "Structural Biology of Human H3K9 Methyltransferases," *PLoS One* 1(1):e8570 (2010), which is hereby incorporated by reference in its entirety). This may open an avenue for novel therapeutic intervention in the treatment of MFP leukemias. The fact that ME is a non-essential gene for cellular and organismal survival (Zhang et al., "PR Domain–Containing Mds1-Evi1 is Critical for Long-Term Hematopoietic Stem Cell Function," *Blood* 118(14):3853-3861 (2011), which is hereby incorporated by reference in its entirety) provides the expectation that therapies that inhibit its function are likely to be nontoxic and well-tolerated.

Example 4 – *In silico* Analysis of the Structure of the "PR" Domain of MDS1-EVI1

[0095] MDS1-EVI1 has a SET-like PR domain at the N-terminus. Amino acid alignment (Figure 3A) shows considerable identity and similarity with Suvar39H1 and RuLSMT, two HMTs with known enzymatic activity (Trievel et al., "Structure and Catalytic Mechanism of a SET Domain Protein Methyltransferase," *Cell* 111:91-103 (2002), which is hereby incorporated by reference in its entirety). Previous studies (Nanjundan et al., "Amplification of MDS1/EVI1 and EVI1, Located in the 3q26.2 Amplicon, is Associated With Favorable Patient Prognosis in Ovarian Cancer," *Cancer Res.* 67:3074-3084 (2007), which is hereby incorporated by reference) failed to detect HMT activity when the PR domain of ME was purified from *E. coli* or when expressed in COS7 cells and pulled down with an anti-epitope tag. To further address the possibility of this PR domain possessing HMT activity, *in silico* structural analysis was performed by "threading" the primary amino acid sequence of the PR domain of MDS1-EVI1 onto the coordinates of a crystallized SET protein (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit

methyltransferase (Rubisco-LSMT)) (Trievel et al., “Structure and Catalytic Mechanism of a SET Domain Protein m=Methyltransferase,” *Cell* 111:91-103 (2002), which is hereby incorporated by reference in its entirety) using ONO software. This revealed a remarkably close fit with some interesting and important differences between the two domains (Figures 3B and 3C).

[0096] The essential features of Rubisco-LSMT structure are NH_2-SET (N-SET) and COOH-SET (C-SET) domains with an unconserved intervening-SET (i-SET) domain in between (Figure 3A). The N-SET portion contains β sheets 1 through 5; C-SET contains sheets 6-12. A catalytic pocket which binds the target lysine is caged by four of the β sheets (β6, β7, β9, and β12; Figure 3B). The pocket is stabilized by inter-sheet interactions, particularly stacking of planar phenylalanine residues F_{224} and F_{269} (diagonal line, Figure 3B), and several hydrophobic interactions involving W_{104} and V_{233} (Figure 3B). The binding of SAM is dependent on interactions between SAM and the i-SET domain. A key residue for the enzymatic activity is a tyrosine residue (Y_{287}) shown at the top of the pocket in Figure 3B.

[0097] This pocket clearly exists in the PR domain of MDS1-EVI1 and is also caged by four β sheets. The stabilizing interactions are different – the stacking F residues are replaced with a pair of + and – charged-amino acids: designated D_{224} (aa 130 in MDS1-EVI1) and R_{269} (aa 176 in ME) in Figure 3C. The hydrophobic residues W_{104} and V_{233} have been replaced with charged or polar residues: R (aa 116 in mouse ME) and N (aa 143 in mouse ME), respectively. These changes make the pocket more hydrophilic overall, and larger in size. All of these amino acids are conserved between human and mouse ME, and are therefore believed to be highly conserved among mammalian ME.

[0098] A second important difference is that the i-SET domain, on which SAM depends for binding, does not exist in ME. This is true in both human and mouse ME. This indicates that the role of the i-SET domain, if such exists for ME, is likely taken up by an interacting protein or another region of the ME protein. Consistent with this, on the face of the protein where i-SET would be, there are two protruding residues, W_{149} and Q_{115}, stabilized by a perfect hydrogen bond, which may provide a docking site for an interacting protein. All of these amino acids are

conserved between human and mouse ME, and are therefore believed to be highly conserved among mammalian ME.

[0099] A third important difference is that the Y_{287} residue thought to be the critical residue for HMT catalytic activity is changed to a methionine residue (M_{191} of ME). This very likely indicates lack of intrinsic HMT activity, even when ME is within its holocomplex.

[0100] In sum, the preceding structural and functional analyses demonstrate a highly related 3-dimensional structure with a larger binding pocket, absence of a full complement of interaction sites for SAM, and an altered residue for the enzymatic activity. Based on these properties, it is believed that these alterations result in an HMT enzyme that requires interaction with another protein for enzymatic activity. Importantly, with this putative structure, specific predictions can be made as to what point mutations will result in loss of activity.

Example 5 – Add-Back Assay Demonstrates Essential Role for Amino Acids at Putative Catalytic Site of MEPRD

[0101] Using the add-back assay in which the rescue of the MLL-AF9 transformation deficiency seen in $ME^{m1/m1}$ mice is scored, the importance of several key amino acids were tested within the MEPRD, with the goal of discerning whether the domain in fact has catalytic activity. Within the binding pocket, the conserved tyrosine (Y_{111}) was mutated to alanine: this amino acid is thought to be at the catalytic site, and to be important for specifying whether HMTs function as monomethylases or di-/tri-methylases. R_{176} was also mutated, which is predicted to stabilize the substrate lysine binding pocket through a salt bridge with D_{130}. In addition, W_{149} was mutated, which is hypothesized to function in allowing interaction with a binding partner protein that takes on the role of the absent i-SET domain. C_{158}, was also mutated, which, it is predicted, is on the opposite side of the domain from the catalytic site, and therefore should not be essential for biologic activity.

[0102] Figures 4A-E reveal that while wildtype ME protein is able to rescue susceptibility to MLL-AF9-induced transformation as expected, the mutation at the active site ($Y_{111}A$) was devoid of activity, indicating the importance of this residue. Mutation at R_{176} also resulted in the loss of activity, indicating that, if it does play a role in maintaining the pocket structure through interaction with D_{130}, the pocket

plays a critical role in ME biologic function. Thus, even though the presence of M_{190} may indicate MEPRD lacks HMT enzymatic activity, this data is consistent with MEPRD binding a lysine substrate with Y111 playing a role in maintaining its orientation within the pocket. The W149 residue, which the present invention postulates serves as a docking site for an interacting protein, also proved to be essential, as the W149A mutant was devoid of activity. Together, this evidence supports the notion that another protein interacts and plays a critical role in activity. C158A functioned as wildtype, as predicted.

[0103] In sum, the analysis of the present invention confirms a structure highly related to enzymatically active SET domains with a larger binding pocket, absence of a full complement of interaction sites for SAM, and an altered residue at the catalytic site. It is therefore likely that this PR domain requires interaction with another protein for activity; this is supported by the loss of activity with the $W_{149}A$ mutant. This analysis also indicates that the PR domain of ME may have target(s) other than histone, or perhaps binding residues other than unmethylated lysine.

Prophetic Example 6 – Construction and Use of Aptamer Conjugate for Targeted Delivery to AML cells in Animal Models

[0104] An aptamer conjugate will be prepared using a 3′ biotinylated AML-specific KH1C12 specific RNA aptamer of SEQ ID NO: 13 and either a 3′ biotinylated ME-inhibiting aptamer or a 3′ biotinylated ME-inhibiting RNAi molecule of one of SEQ ID NOS: 10-12. The ME-inhibiting aptamer and ME-inhibiting RNAi molecule may optionally be formed using modified nucleotides to enhance their half life. Biotinylation of these aptamers at their 3′ ends is known not to interfere with the activity of RNA aptamers (*see* Chu *et al.*, "Aptamer Mediated siRNA Delivery," *Nucl Acids Res.* 34(10):e73 (2006), which is hereby incorporated by reference in its entirety).

[0105] The aptamers and RNAi will be synthesized by *in vitro* transcription from a double-stranded DNA template bearing a T7 RNA polymerase promoter. The aptamers will be purified using polyacrylamide gel electrophoresis, followed by overnight elution in water and ethanol precipitation.

[0106] Biotinylation of the purified aptamers and RNAi will be carried out using the procedures of Chu *et al.*, "Aptamer Mediated siRNA Delivery," *Nucl Acids*

Res. 34(10):e73 (2006), which is hereby incorporated by reference in its entirety. Briefly, purified aptamer (150 nM) will be oxidized in 100 mM NaOAc (pH 5.0), 100 mM $NaIO_4$ (90 min, RT, dark), and the oxidized aptamers will be recovered via ethanol precipitation. The oxidized RNA will be reacted with 200 pmol of freshly prepared biotin-hydrazide in 500 µL 100 mM NaOAc (pH 5.0) (3h, RT), and the hydrazide removed. The biotinylated RNA will be gel-purified.

[0107] Using 100 mM KOAc, 30 mM HEPES–KOH (pH 7.4) and 2 mM MgOAc buffer, 200 pmol of each biotinylated aptamer (1:1 ratio) will be introduced with 100 pmol of streptavidin. The complex will be allowed to equilibrate for a minimum of 10 minutes and then be stored on ice until its use.

[0108] AML-specific aptamer KH1C12 conjugates will be added directly to cell culture media (500 µl) at a final concentration of either 10 nM, 50 nM, or 100 nM conjugate. AML positive cell lines will be introduced at cell densities of 10^4-10^6, and cells will be assessed at 24, 48, and 72 hours after the addition of the conjugate for cell survival.

[0109] With positive *in vitro* results, these same cell lines and conjugates will be used in a xenograft model of human AML as described in the preceding Examples or Zuber et al., “Mouse Models of Human AML Accurately Predict Chemotherapy Response,” *Genes Dev.* 23:877-889 (2009), which is hereby incorporated by reference in its entirety (*see also* Wunderlich et al., “AML Cells Are Differentially Sensitive to Chemotherapy Treatment in a Human Xenograft Model,” *Blood* 121(12):e90-7 (2013), which is hereby incorporated by reference in its entirety). Mice bearing AML positive xenografts will be used. Seven days following implantation animals in several test groups will be administered 500 µg/kg, 2.5 mg/kg, or 12.5 mg/kg of the conjugate via i.p. injection every three days. Animals will be maintained for 20 weeks following start of treatment. Tumor weights will be assessed every three days beginning the day treatment is started. Assessment of treatment versus control will be measured by percent test/control (%T/C) tumor weights calculated on each day that tumors are measured, tumor growth delay, and/or tumor regression.

Prophetic Example 7 – Construction and Use of Conjugate for Targeting Expression Vector Delivery to AML Cells

[0110] A plasmid vector encoding an RNAi molecule of SEQ ID NOS: 6-11 will be used to prepare a polycation conjugated vectors. This will be carried out using the procedure of Moffatt et al., "Successful *in vivo* Tumor Targeting of Prostate-specific Membrane Antigen with a Highly Efficient J591/PEI/DNA Molecular Conjugate," *Gene Therapy* 13:761-772 (2006), which is hereby incorporated by reference in its entirety, except that the plasmid vector will replace the β-gal plasmid of Moffatt and the prostate-specific mAb J591 will be replaced by an antibody that is specific for a AML cell surface marker (e.g., CD-33, CD-19, CD-20).

[0111] The addition of polyethylene glycol (PEG) (Sigma, Mr=3000) as a spacer between phenyl(di)boronic acid (PDBA) and the cancer cell surface marker will be carried out in a stepwise manner by first generating PDBA–PEG. About 300 mg of ω-amino-α-carboxyl PEG will be suspended in 0.1 M $NaHCO_3$ to a final concentration of 2 mg/ml. A 10 mM stock of PDBA–x–N-hydroxysuccinimide (NHS) will be made in N,N-dimethylformyl amine. A molar ratio of 5:1 of PDBA to PEG will be produced by adding 4.4 ml of PDBA–x–NHS solution to the PEG solution. The sample will be dialyzed using a 1000-Mw membrane for 48 h at 41°C against 20 mM HEPES buffer in the cold to yield PDBA–PEG. For PDBA–PEG–cancer cell surface marker formation, the cancer cell surface marker will be warmed in 37°C for 5 minutes to activate the antibody, and then 300 mg of the antibody will be reacted with 0.1 M $NaHCO_3$ in a final volume of 250 ml. Following this, 1.3 ml of 0.1 M DTT solution will be added for 10 minutes in a 37°C water bath to reduce disulfide bond formation. The DTT will be removed with Ultrafree-MC filter unit. In all, 300 mg of the 5:1 PDBA:PEG ratio will be introduced in a solution of 0.1M 2-[N-morpholino]ethane-sulfonic acid, pH 6.0, and 0.5 M NaCl, and the final volume will be brought up to 300 ml with distilled water. To activate the PEG for coupling to the cancer cell surface marker, 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide and NHS will be added to final concentrations of 2 and 5 mM, respectively, allowed to react at room temperature for 15 minutes, and the reaction terminated with β-mercaptoethanol at a final concentration of 20 mM. Finally, 150 mg of the cancer cell surface marker solution (1 mg/ml) will be combined with the PDBA–PEG solution

and allowed to proceed for 2 hours at room temperature, after which dialysis will be performed for 48 hours with 6000–8000 Mw cutoff membrane.

[0112] A working solution of 10 mM salicylhydroxamic acid-polyethylenimine (SHA–PEI) (pH 7.4) will be prepared in endotoxin-free water (BioWhittaker) and stored at 41°C. 4.5 ml of SHA–PEI will be added to 5.5 ml of 20mM HEPES buffer, and then plasmid (6 mg) will be pipetted into polystyrene tubes and the solution brought up to 60 ml with HEPES buffer. Following this, 10 ml of the SHA–PEI solution will be added directly to the plasmid DNA solution and the solution allowed to incubate at room temperature for 5 minutes. After this, appropriate amounts of PDBA–PEG–cancer cell surface marker will be added and the incubation continued for an additional 5 minutes, which will result in the formation of the cancer cell surface maker/PEG/PEI/DNA(RNAi) vector.

[0113] This conjugate will be screened *in vitro* using the cell lines described in the preceding Examples, and also screened for *in vivo* activity using the xenograft mouse model of human AML described in the preceding Examples (Zuber et al., "Mouse Models of Human AML Accurately Predict Chemotherapy Response," *Genes Dev.* 23:877-889 (2009) and Wunderlich et al., "AML Cells Are Differentially Sensitive to Chemotherapy Treatment in a Human Xenograft Model," *Blood* 121(12):e90-7 (2013), both of which are hereby incorporated by reference in their entirety). Tetracycline will be administered to induce RNAi expression by the plasmid vector.

[0114] Mice bearing AML positive xenografts will be used. Seven days following implantation animals in several test groups will be administered 500 μg/kg, 2.5 mg/kg, or 12.5 mg/kg of the conjugate via i.p. injection every three days. Animals will be maintained for 20 weeks following start of treatment. Tumor weights will be assessed every three days beginning the day treatment is started. Assessment of treatment versus control will be measured by percent test/control (%T/C) tumor weights calculated on each day that tumors are measured, tumor growth delay, and/or tumor regression.

[0115] All of the features described herein (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined with any of the above aspects in any combination, except

combinations where at least some of such features and/or steps are mutually exclusive.

[0116] Having thus described the basic concept of the invention, it will be rather apparent to those skilled in the art that the foregoing detailed disclosure is intended to be presented by way of example only, and is not limiting. Various alterations, improvements, and modifications will occur and are intended to those skilled in the art, though not expressly stated herein. Additionally, the recited order of processing elements or sequences, or the use of numbers, letters, or other designations therefore, is not intended to limit the claimed processes to any order except as may be specified in the claims. These alterations, improvements, and modifications are intended to be suggested hereby, and are within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims and equivalents thereto.

WHAT IS CLAIMED:

1. A method of treating a cancerous condition mediated by the protein MDS1-EVI1 ("ME"), the method comprising:

administering to a patient an amount of an inhibitor of ME protein activity that is effective to cause cell death of cancer cells that are ME-dependent, thereby treating the cancerous condition.

2. The method according to claim 1, wherein the inhibitor of ME protein activity is an antibody or antibody fragment that binds specifically to a PR domain of the ME protein, an anti-ME nucleic acid aptamer, a dominant negative ME fragment, or a small molecule ME inhibitor.

3. The method according to claim 2, wherein the antibody or antibody fragment or the anti-ME aptamer binds specifically to at least a portion of the amino acid sequence of AGLGIWTKRKIEVGEKFGPYVGEQRSNLKDPSYG (amino acids 90-123 of SEQ ID NO: 1).

4. The method according to claim 1, wherein the inhibitor of ME activity is an inhibitory nucleic acid molecule that interferes with ME expression to cause a reduction in ME activity.

5. The method according to claim 4, wherein the inhibitory nucleic acid molecule is encoded by an expression vector.

6. The method according to claim 4, wherein the inhibitory nucleic acid molecule comprises an inhibitory RNA ("RNAi") molecule.

7. The method according to claim 6, wherein the RNAi molecule targets nt 511-532 of GenBank sequence M21829, nt 3139-3167 of GenBank sequence M21829, nt 494-514 of the human ME-encoding sequence, nt 403-421 of the human ME-encoding sequence (uuCUGCAUAGAUGCCAGUCAAcc) (SEQ ID NO: 10), or nt 266-284 of the human ME-encoding sequence (ggGCAGGACUAGGAAUAUGGAcc) (SEQ ID NO: 11).

8. The method according to claim 1, wherein the cancerous condition is selected from the group of acute myeloid leukemia (AML), acute

lymphoid leukemia (ALL), myelodysplastic syndrome, chronic myelogenous leukemia, and epithelial cancers where the 3q26.2 aberration is present (ovary, breast, head and neck, cervix, and lung).

9. The method according to claim 1, where the cancerous condition is characterized by the MLL-AF9 translocation t(9;11)(p22;q23) or MLL-ENL translocation t(11;19)(q23;p13.3).

10. The method according to claim 6, wherein the inhibitory nucleic acid molecule further comprises a nucleic acid aptamer targeting an myeloid leukemic cell, myelodysplastic cell, or an epithelial ovary, breast, head and neck, cervix, or lung cancer cell.

11. The method according to claim 10, wherein the aptamer is KH1C12 (SEQ ID NO: 12), which targets a myeloid leukemic cell.

12. The method according to claim 1, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by implantation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, transdermally, by application to mucous membranes, or by introduction into one or more lymph nodes.

13. The method according to claim 1 further comprising administering an additional therapeutic agent selected from the group of a chemotherapeutic, radiation therapy, immunotherapy, and combinations thereof.

14. A method of causing cell death of a cancer cell that requires MDS1-EVI1 ("ME") for survival, the method comprising:

introducing an inhibitor of ME activity into a cancer cell the requires ME for survival under conditions effective to cause cancer cell death.

15. The method according to claim 14, wherein the inhibitor of ME protein activity is an antibody or antibody fragment that binds specifically to a PR domain of the ME protein, an anti-ME nucleic acid aptamer, a dominant negative ME fragment, or a small molecule ME inhibitor.

16. The method according to claim 15, wherein the antibody or antibody fragment or the anti-ME aptamer binds specifically to at least a portion of the amino acid sequence of AGLGIWTKRKIEVGEKFGPYVGEQRSNLKDPSYG (amino acids 90-123 of SEQ ID NO: 1).

17. The method according to claim 14, wherein the inhibitor of ME activity is an inhibitory nucleic acid molecule that interferes with ME expression to cause a reduction in ME activity.

18. The method according to claim 17, wherein the inhibitory nucleic acid molecule is encoded by an expression vector, the expression vector being introduced into the cancer cell under conditions effective to express the inhibitory nucleic acid molecule.

19. The method according to claim 17, wherein the inhibitory nucleic acid molecule comprises an inhibitory RNA ("RNAi") molecule.

20. The method according to claim 19, wherein the RNAi molecule targets nt 511-532 of GenBank sequence M21829, nt 3139-3167 of GenBank sequence M21829, nt 494-514 of the human ME-encoding sequence, nt 403-421 of the human ME-encoding sequence (uuCUGCAUAGAUGCCAGUCAAcc) (SEQ ID NO: 10), or nt 266-284 of the human ME-encoding sequence (ggGCAGGACUAGGAAUAUGGAcc) (SEQ ID NO: 11).

21. The method according to claim 14, wherein the cancer cell is a leukemic or dysplastic myeloid cell, or an epithelial cancer cell where the 3q26.2 aberration is present.

22. The method according to claim 14, where the cancer cell is characterized by the MLL-AF9 translocation t(9;11)(p22;q23) or MLL-ENL translocation t(11;19)(q23;p13.3).

23. The method according to claim 19, wherein the inhibitory nucleic acid molecule further comprises a nucleic acid aptamer targeting an myeloid leukemic cell, myelodysplastic cell, or an epithelial ovary, breast, head and neck, cervix, or lung cancer cell.

24. The method according to claim 23, wherein the aptamer is KH1C12 (SEQ ID NO: 12), which targets a myeloid leukemic cell.

25. The method according to claim 14 further comprising exposing the cell to an additional therapeutic agent selected from the group of a chemotherapeutic, radiation, an immunotherapeutic, and combinations thereof.

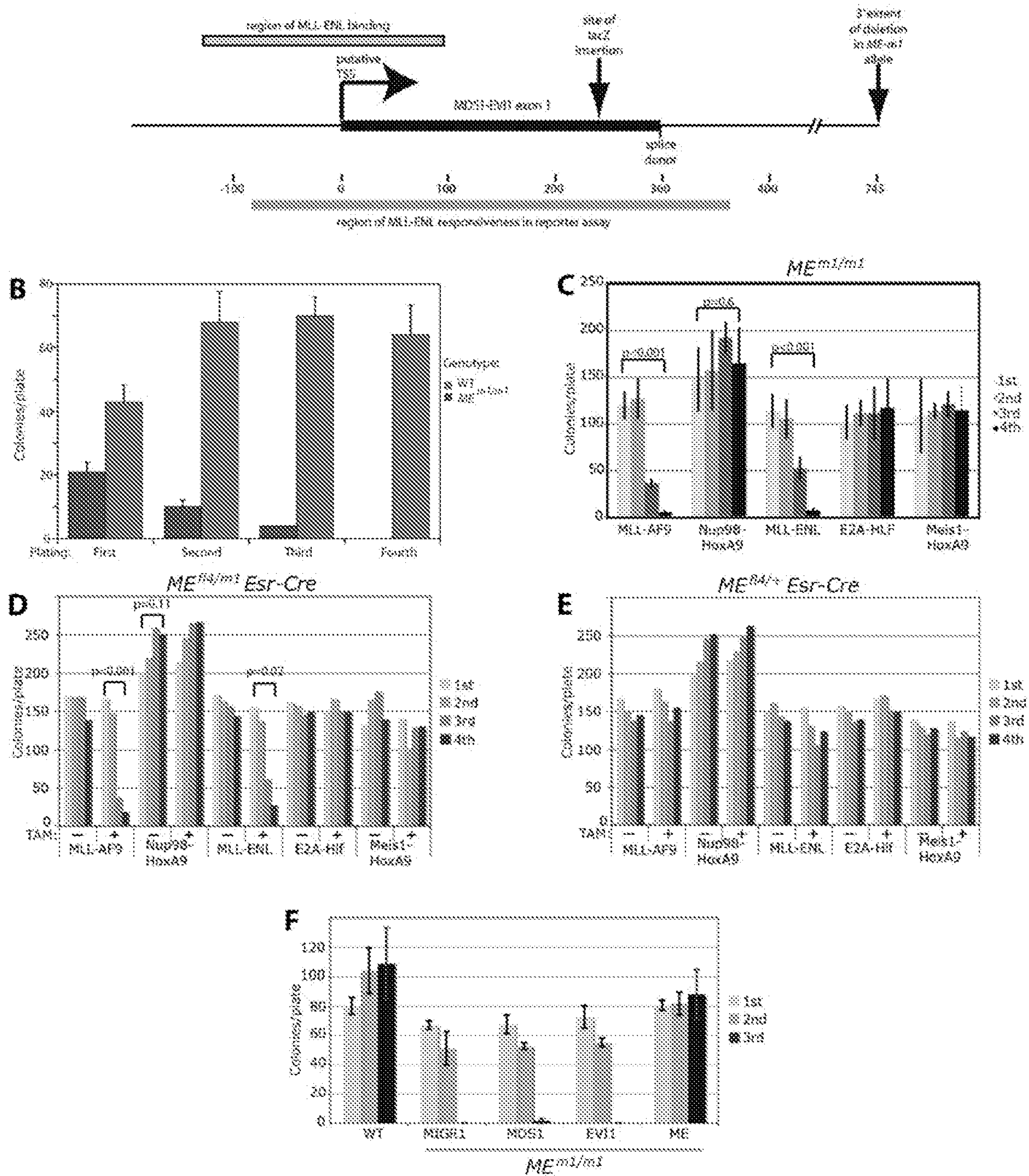
26. An inhibitor of ME protein activity selected from the group consisting of an antibody or antibody fragment that binds specifically to a PR domain of the ME protein, an anti-ME nucleic acid aptamer, a dominant negative ME fragment, or an inhibitory nucleic acid molecule that interferes with ME expression to cause a reduction in ME activity.

27. The inhibitor of ME protein activity according to claim 26, wherein the inhibitor of ME activity is an inhibitory nucleic acid molecule that interferes with ME expression to cause a reduction in ME activity.

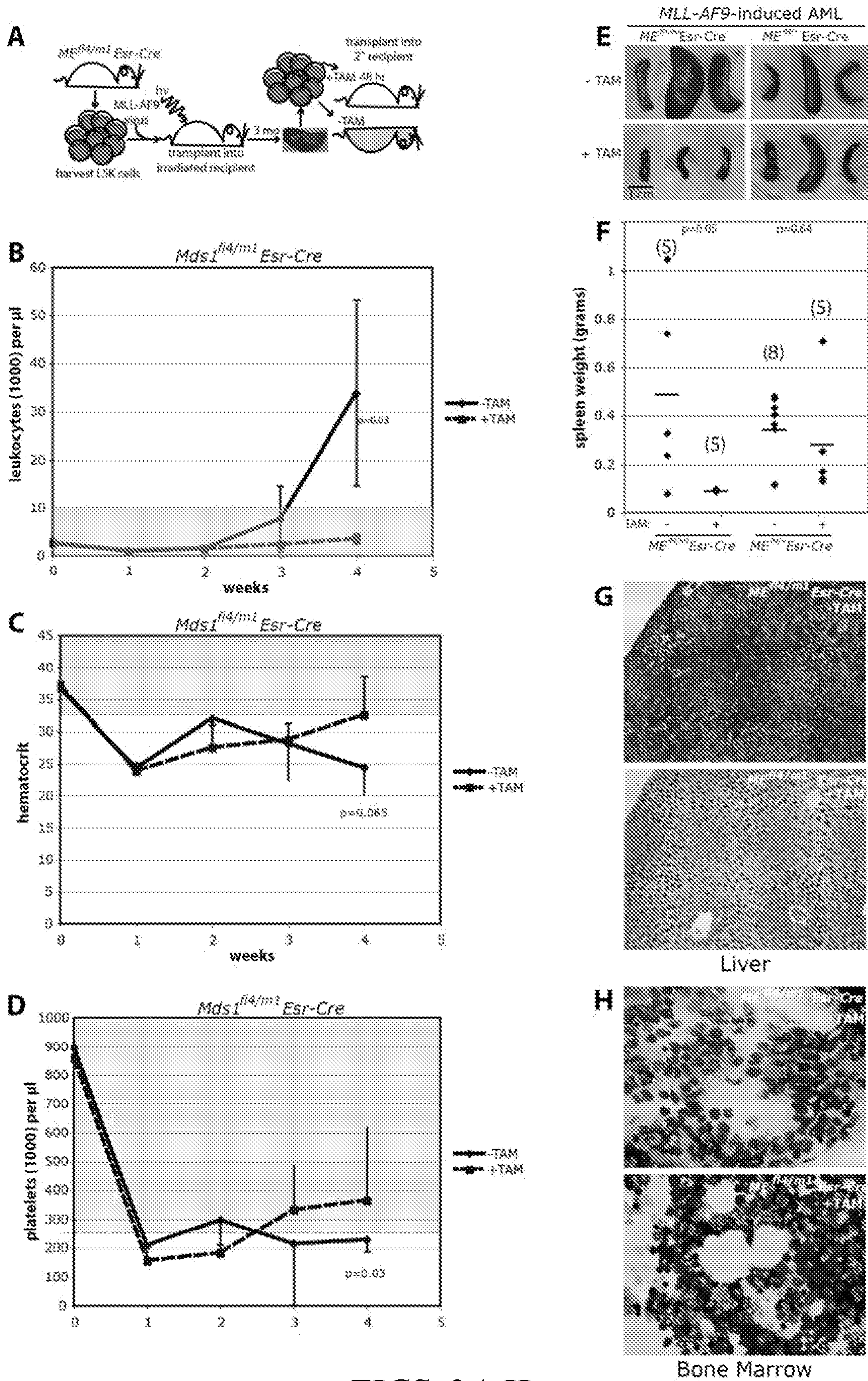
28. The inhibitor of ME protein activity according to claim 27, wherein the inhibitory nucleic acid molecule comprises an inhibitory RNA (“RNAi”) molecule.

29. The inhibitor of ME protein activity according to claim 30, wherein the RNAi molecule targets nt 511-532 of GenBank sequence M21829, nt 3139-3167 of GenBank sequence M21829, nt 494-514 of the human ME-encoding sequence, nt 403-421 of the human ME-encoding sequence (uuCUGCAUAGAUGCCAGUCAAcc, SEQ ID NO: 10), or nt 266-284 of the human ME-encoding sequence (ggGCAGGACUAGGAAUAUGGAcc, SEQ ID NO: 114).

30. A pharmaceutical composition comprising an inhibitor of ME protein activity according to one of claims 26 to 29.

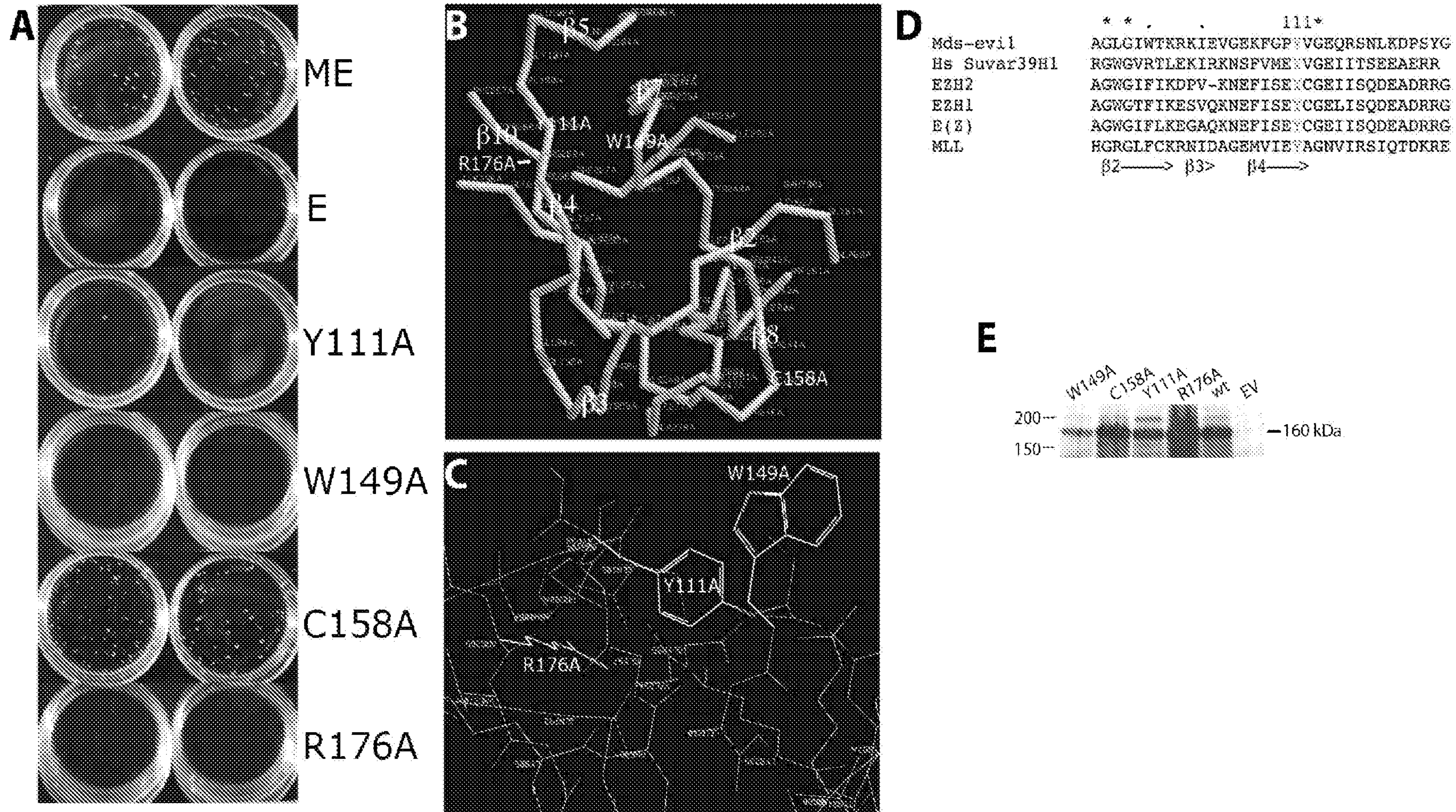


FIGS. 1A-F



FIGS. 2A-H

FIGS. 3A-C



FIGS. 4A-E

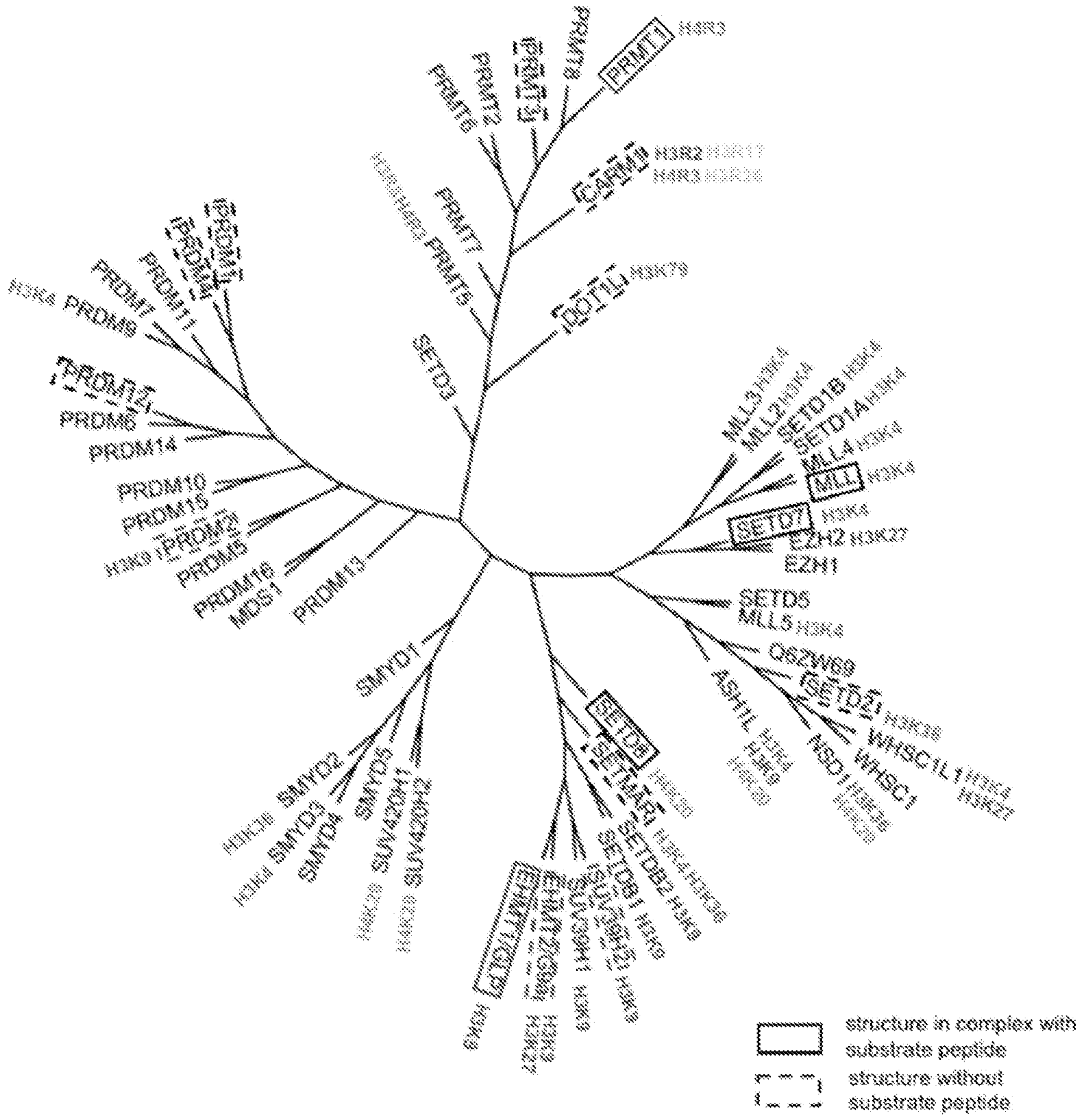
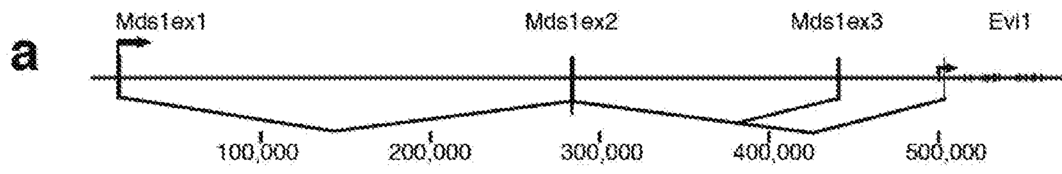
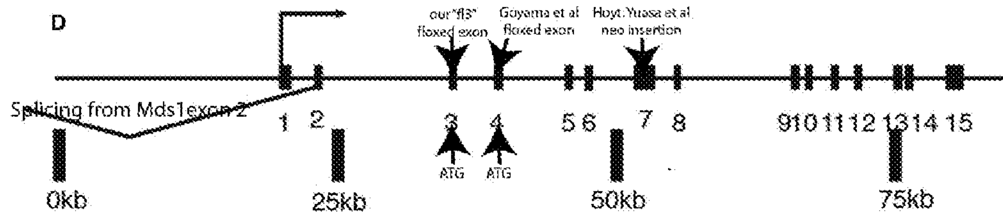
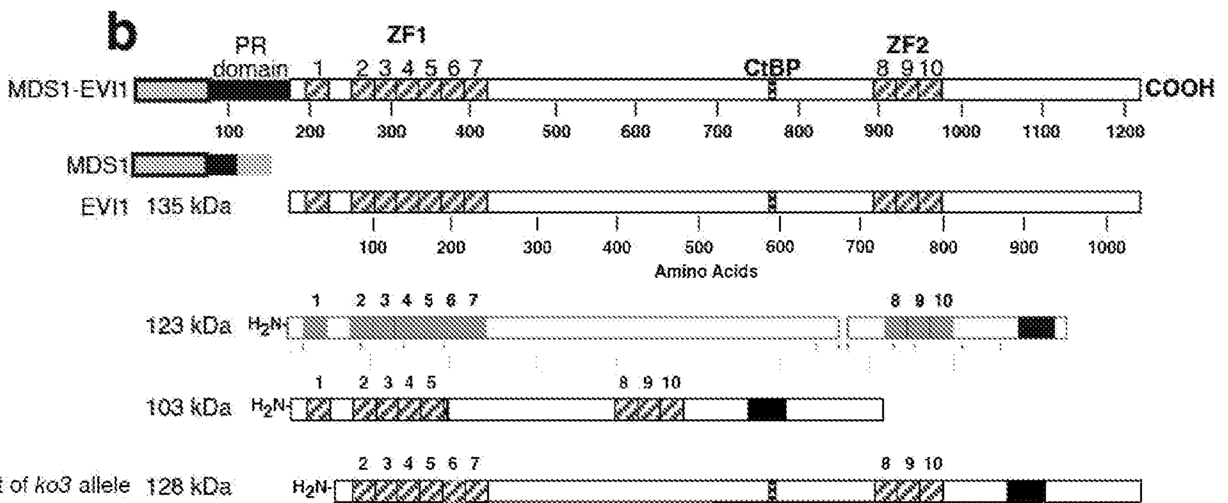


FIG. 5







FIGS. 6A-B

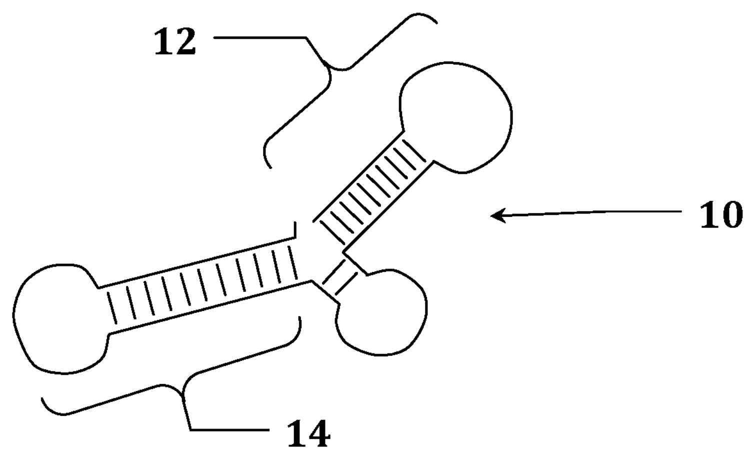


FIG. 7

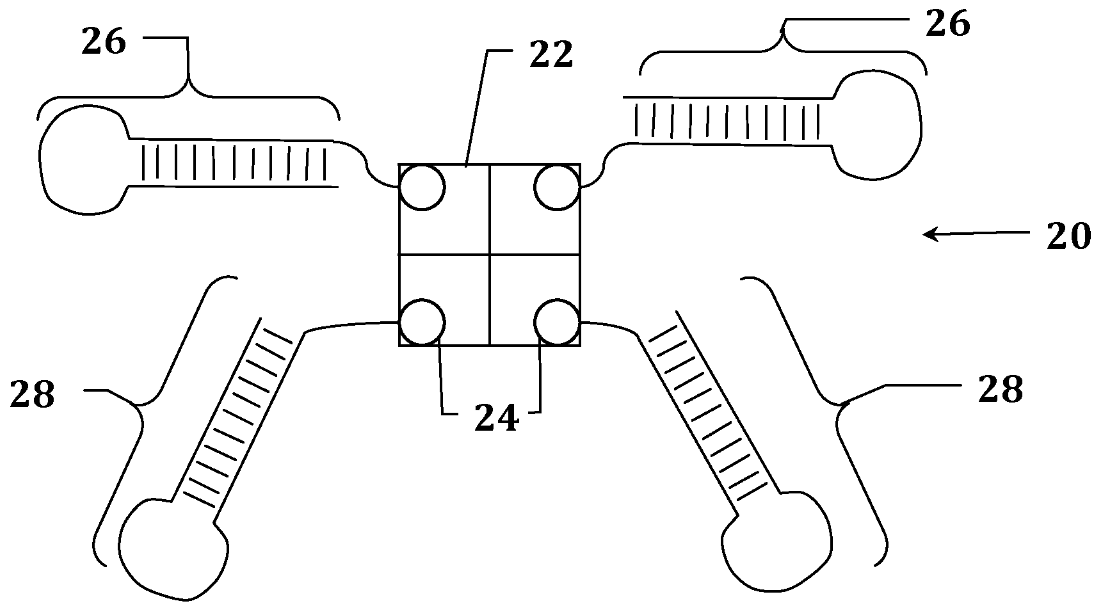


FIG. 8

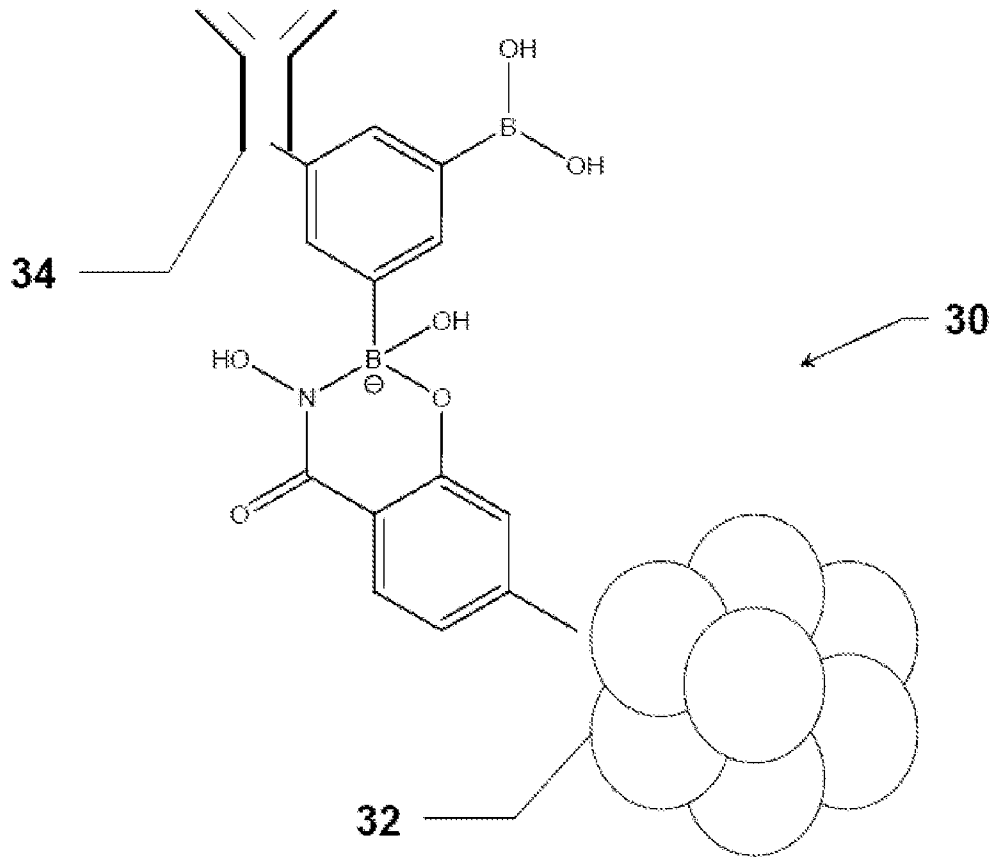


FIG. 9