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(54) **COMPOSITIONS, BIOMARKERS AND THEIR USE IN THE TREATMENT OF CANCER**

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(21) Appl. No.: **14/506,457**

(22) Filed: **Oct. 3, 2014**

Related U.S. Application Data

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Publication Classification

(51) **Int. Cl.**

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A61K 49/00	(2006.01)

(52) **U.S. Cl.**

CPC ***C12Q 1/6886*** (2013.01); ***A61K 49/0004*** (2013.01); ***A61K 31/4745*** (2013.01); *C12Q 2600/158* (2013.01); *C12Q 2600/106* (2013.01)

(57) **ABSTRACT**

The present invention relates to compositions, biomarkers, and their use in treatment of cancer. In some embodiments, the invention relates to the use of several biomarkers in methods for determining the responsiveness of a human subject to a specific compound, methods for monitoring the efficacy of the compound, or methods for treating a human subject.

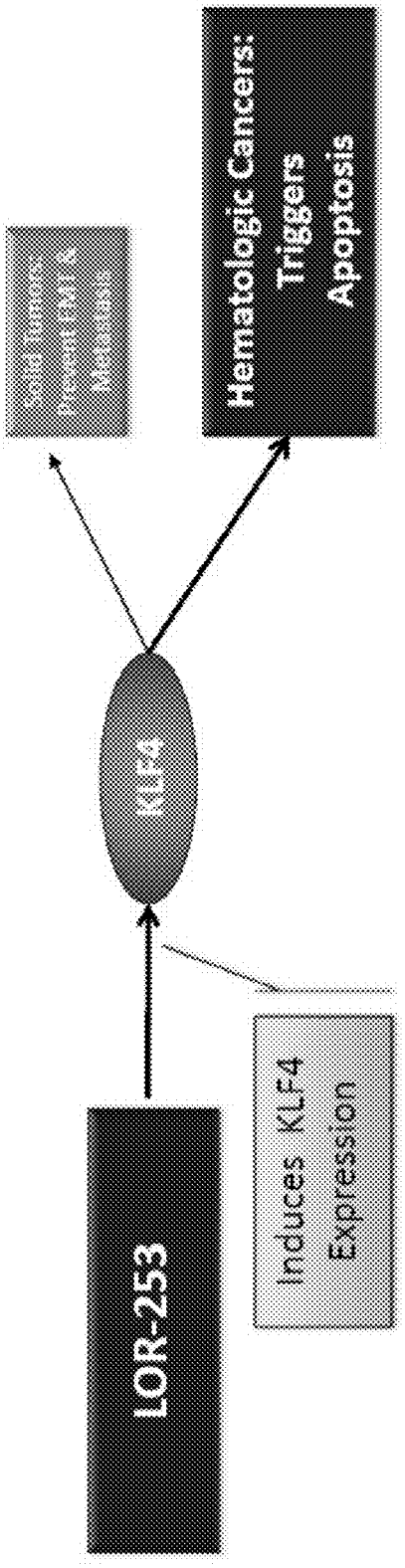

LOR-253
Induces KLF4 Expression
KLF4
Hematologic Cancers: Triggers Apoptosis

FIG. 1

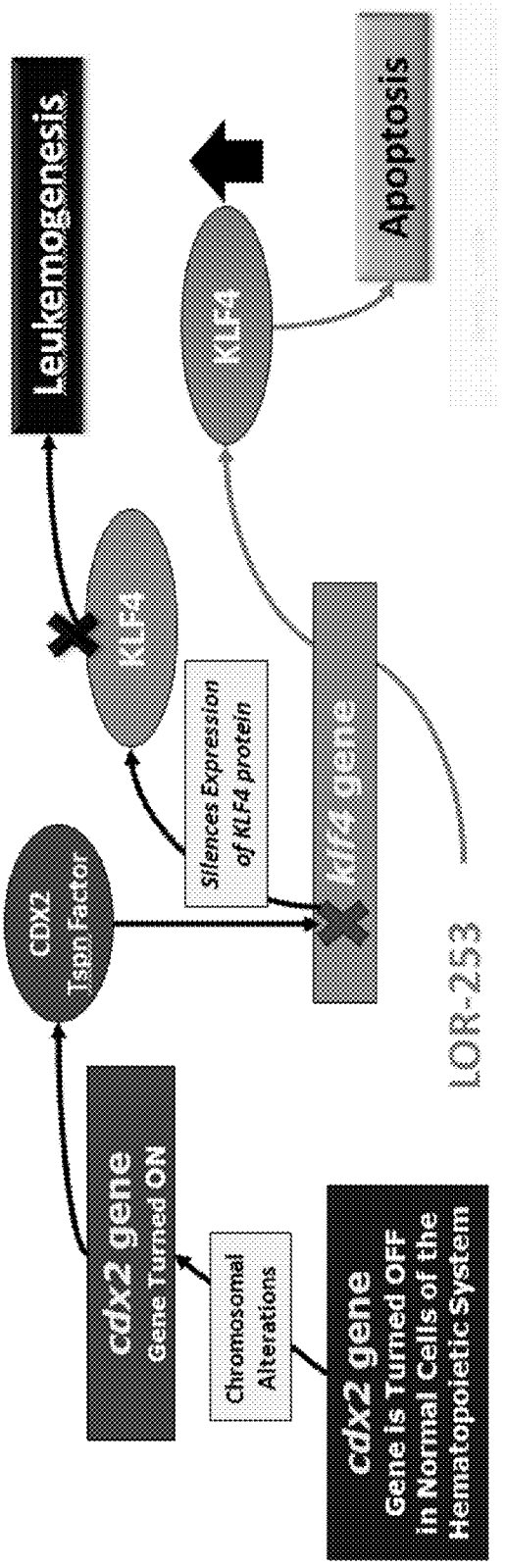

cdx2 gene
Gene Turned OFF
in Normal Cells of the
Hematopoietic System
Chromosomal
Alterations
cdx2 gene
Gene Turned ON
cdx2
Tran Factor
KLF4
Silences Expression
of KLF4 protein
Leukemogenesis
KLF4
Apoptosis
LOR-253

FIG. 2

FIG. 3

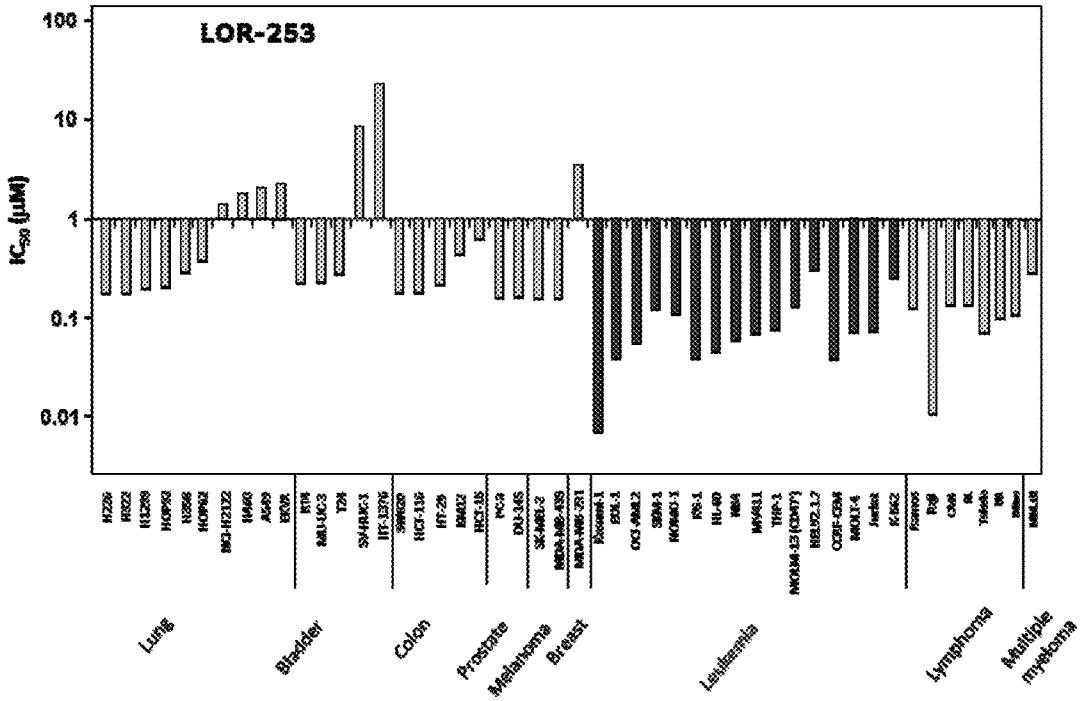
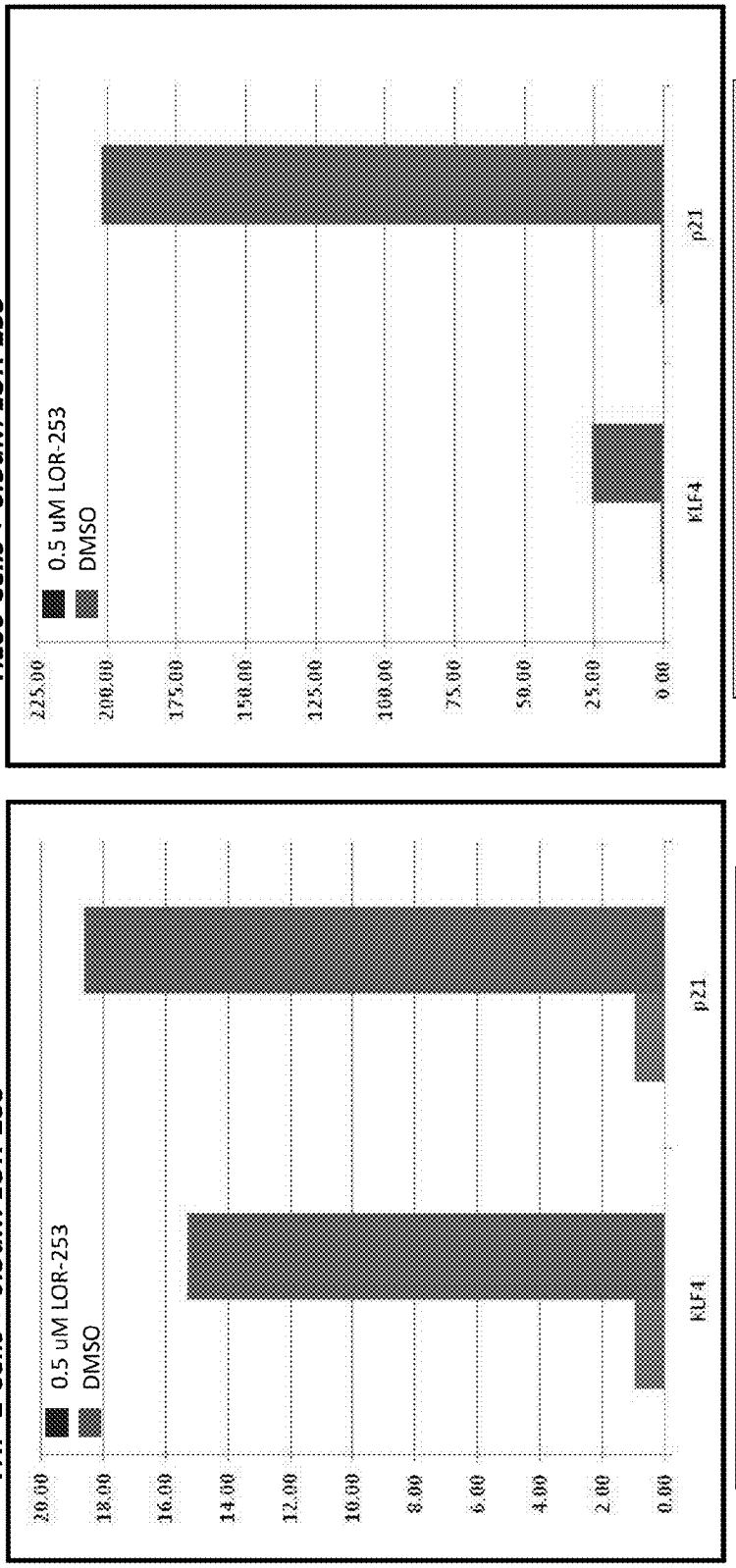

LOR-253
100
10
1
0.1
0.01
IC50 (uM)
Lung
Bladder
Colon
Prostate
Melanoma
Breast
Leukemia
Lymphoma
Multiple myeloma

FIG. 4



Fold increase (x)	KLF4	p21
DMSO	1.00	1.00
0.5 uM LOR-253	15.32	18.64

Fold increase (x)	KLF4	p21
DMSO	1.00	1.00
0.5 uM LOR-253	25.71	202.07

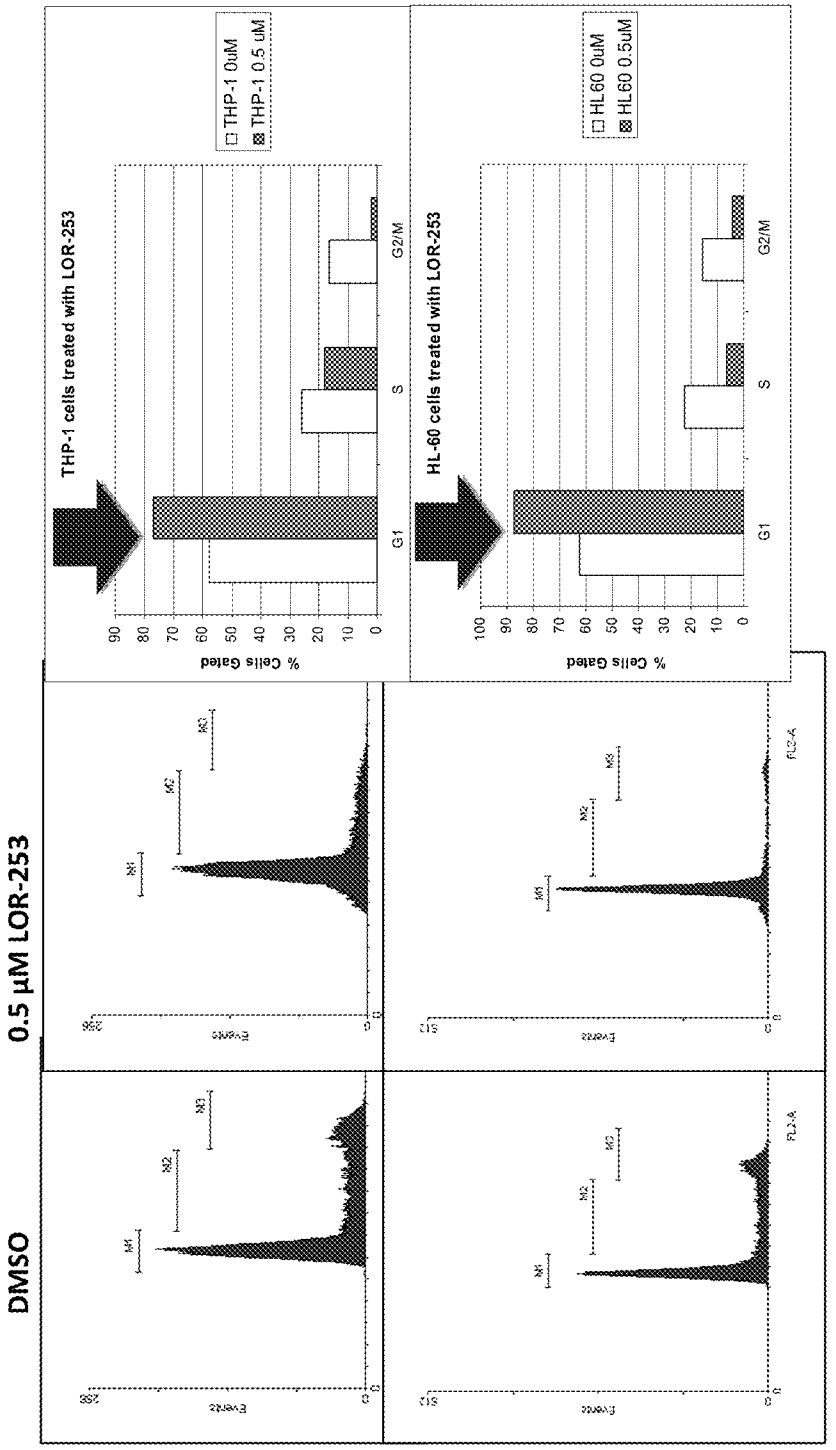

THP-1 cells treated with LOR-253
THP-1 0uM
THP-1 0.5 uM
% Cells Gated
G1
S
G2/M
HL-60 cells treated with LOR-253
HL60 0uM
HL60 0.5uM
% Cells Gated
G1
S
G2/M
DMSO
0.5 µM LOR-253
THP1
HL-60

FIG. 5

FIG. 6

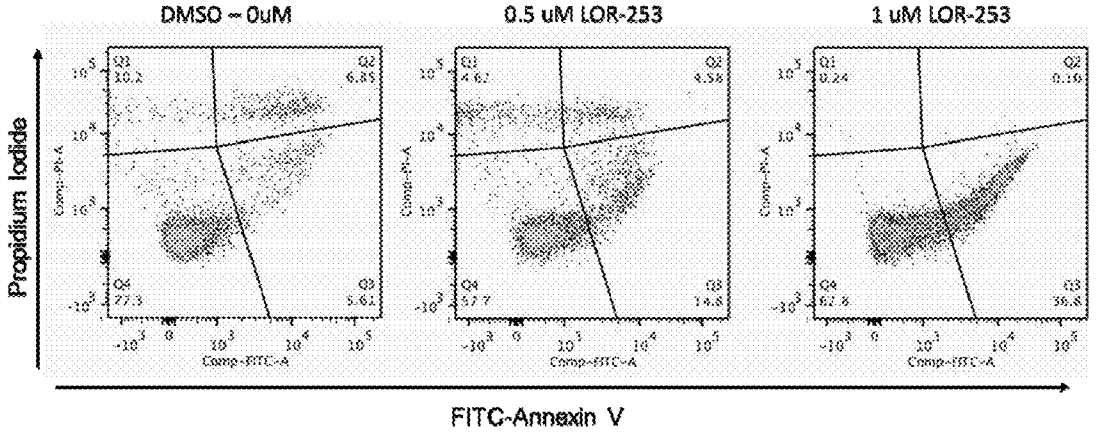

DMSO ~ 0uM
0.5 uM LOR-253
1 uM LOR-253
Propidium Iodide
FITC-Annexin V

FIG. 7

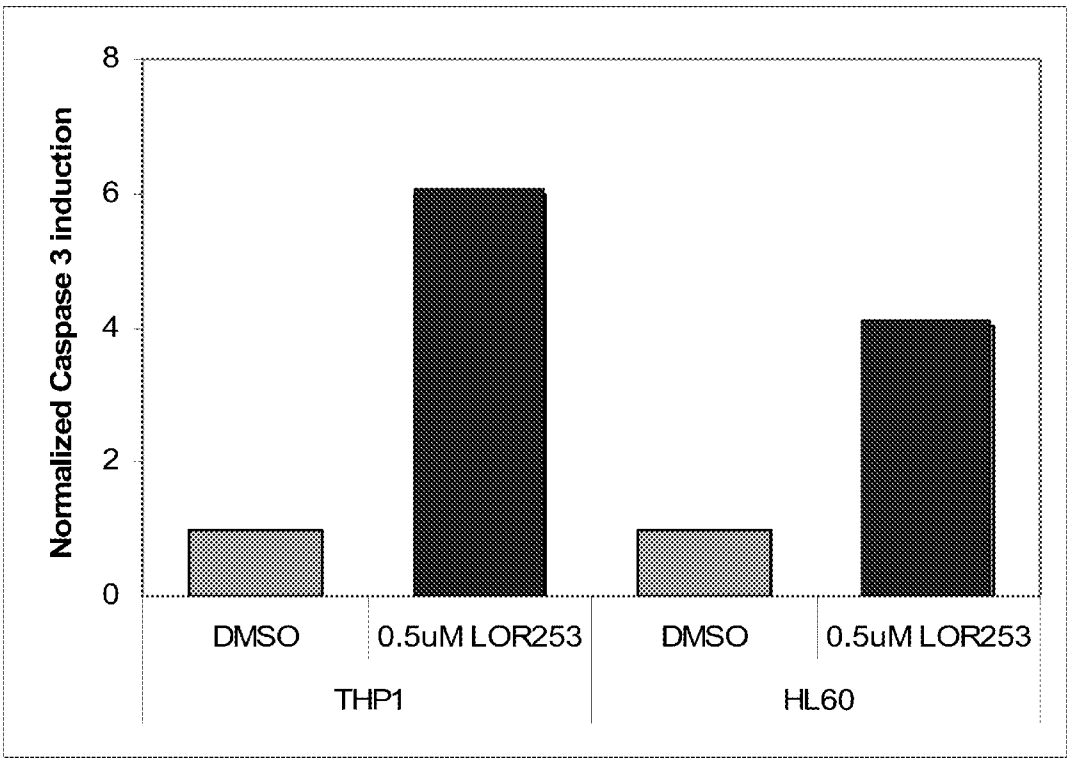

Normalized Caspase 3 induction
8
6
4
2
0
DMSO
0.5uM LOR253
DMSO
0.5uM LOR253
THP1
HL60

FIG. 8

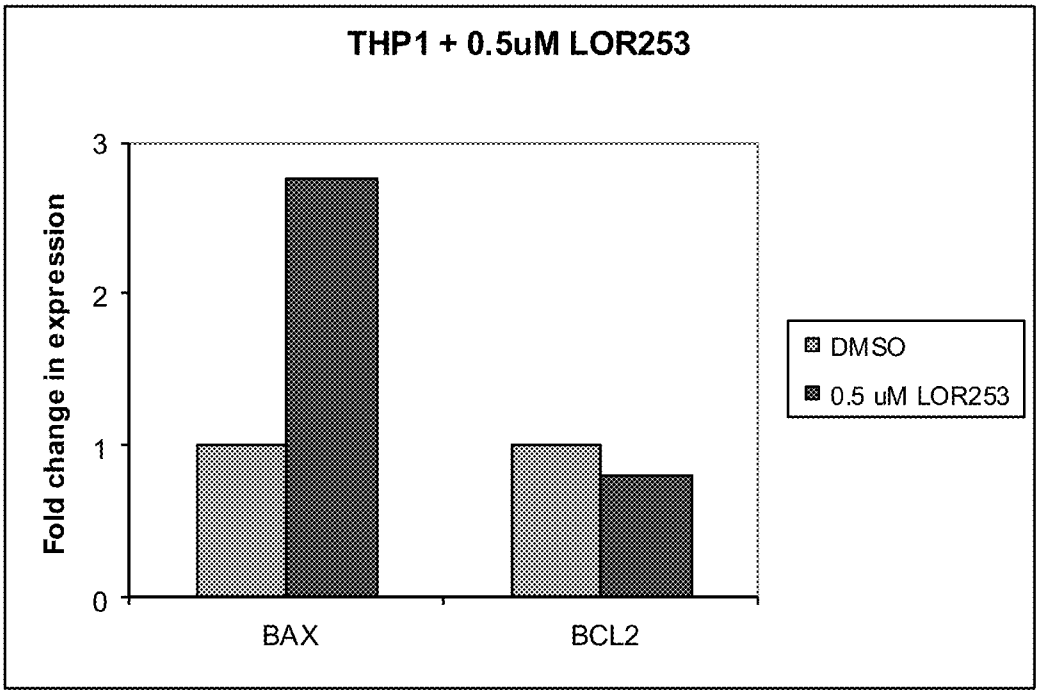

THP1 + 0.5uM LOR253
Fold change in expression
3
2
1
0
BAX
BCL2
DMSO
0.5 uM LOR253

FIG. 9

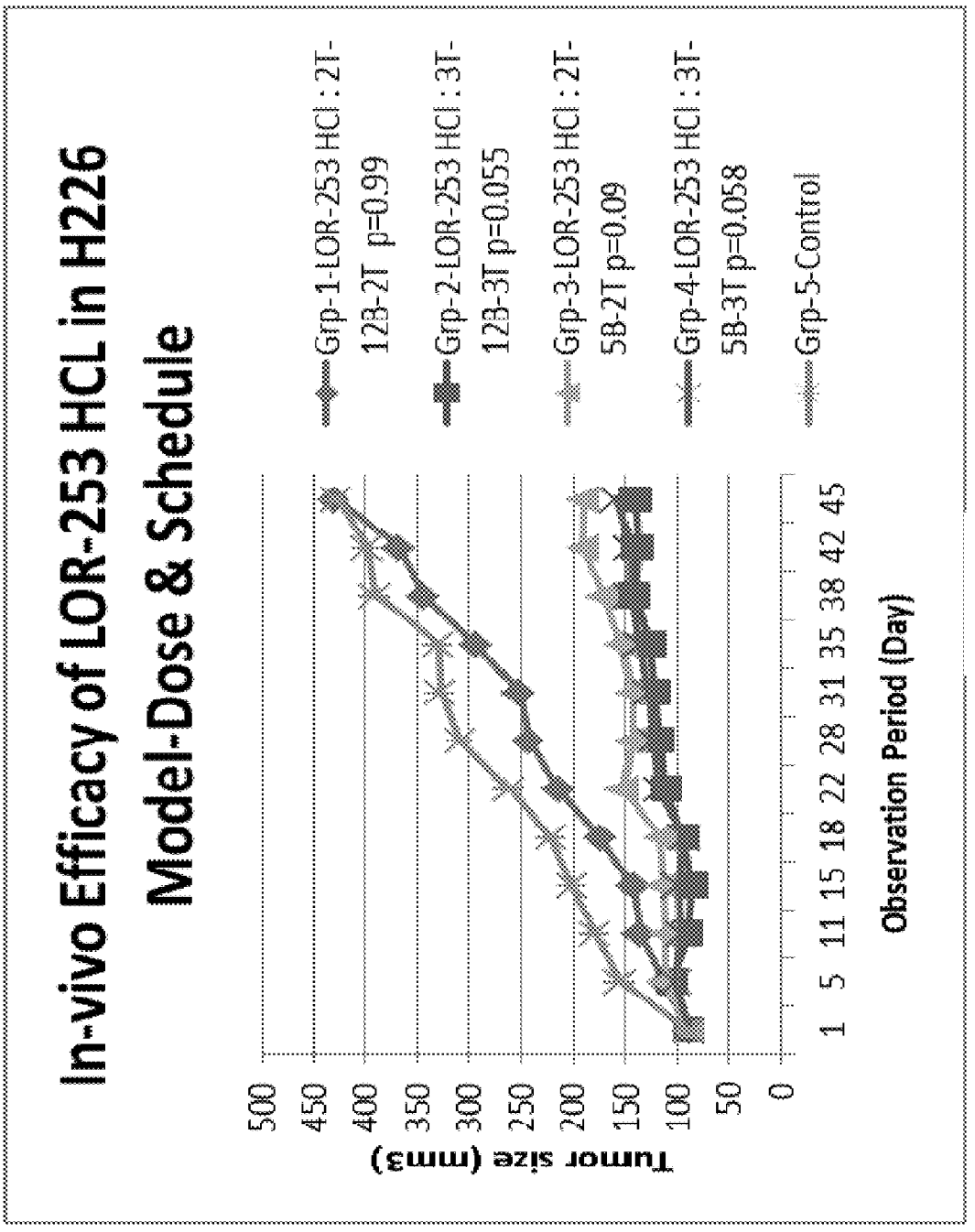

In-vivo Efficacy of LOR-253 HCL in H226
Model-Dose & Schedule
Tumor size (mm3)
500
450
400
350
300
250
200
150
100
50
0
1 5 11 15 18 22 28 31 35 38 42 45
Observation Period (Day)
Grp-1-LOR-253 HCl : 2T-12B-2T p=0.99
Grp-2-LOR-253 HCl : 3T-12B-3T p=0.055
Grp-3-LOR-253 HCl : 2T-5B-2T p=0.09
Grp-4-LOR-253 HCl : 3T-5B-3T p=0.058
Grp-5-Control

FIG. 10

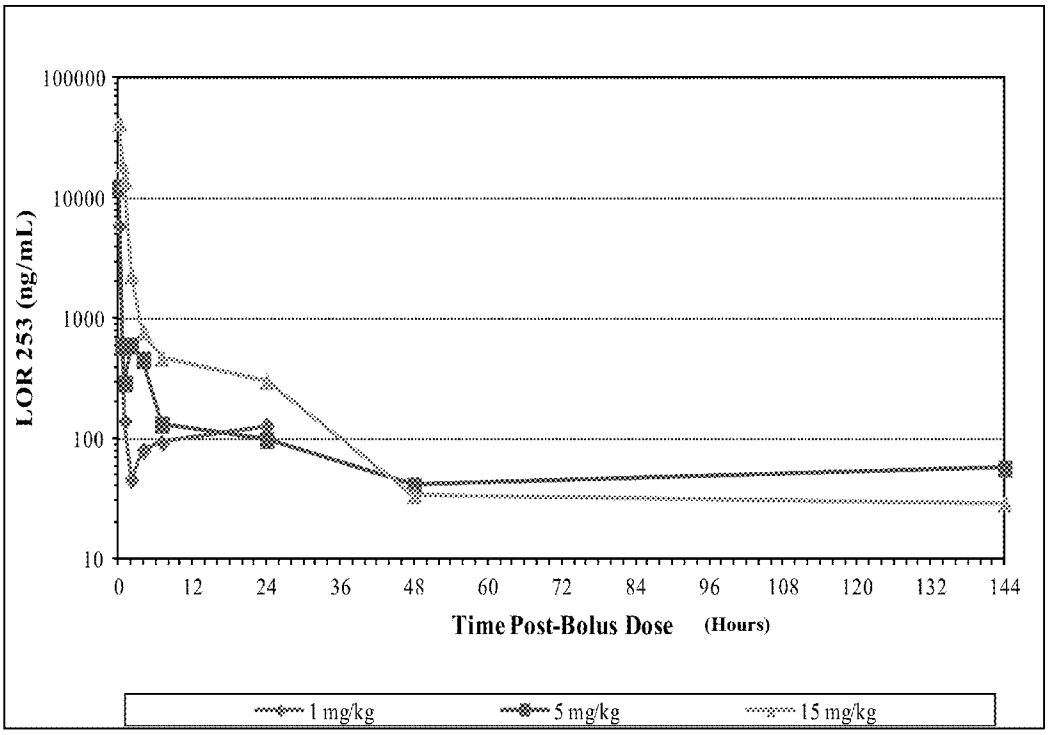
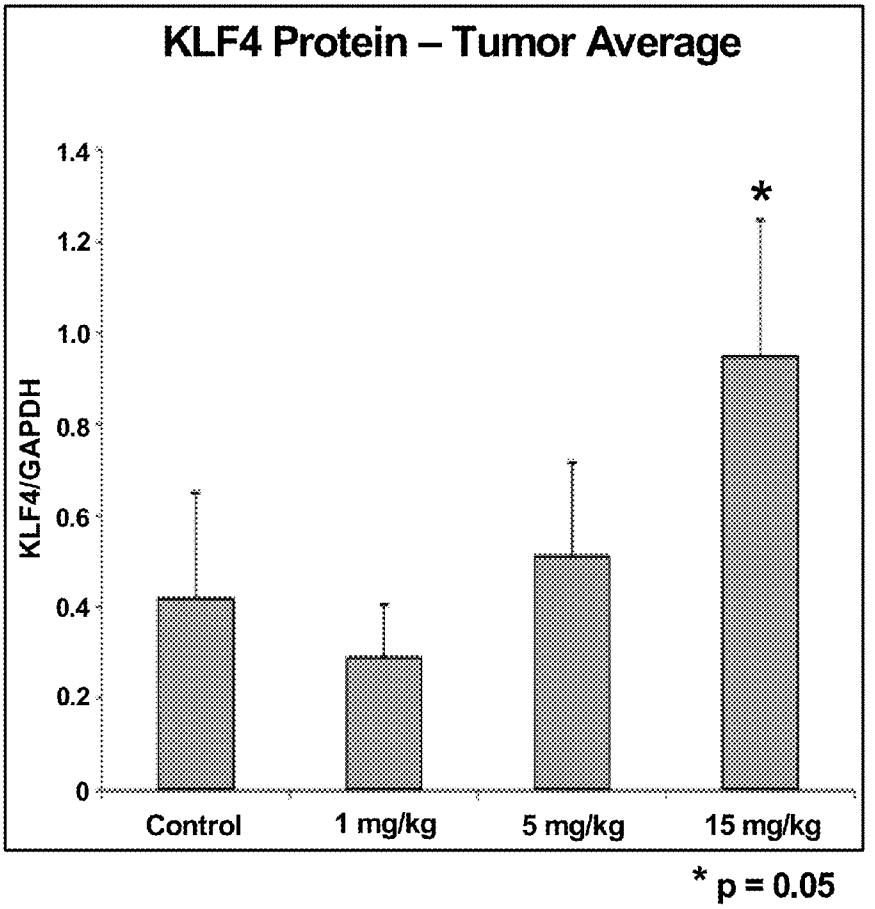

100000
10000
1000
100
10
LOR 253 (ng/mL)
0
12
24
36
48
60
72
84
96
108
120
132
144
Time Post-Bolus Dose (Hours)
1 mg/kg
5 mg/kg
15 mg/kg

FIG. 11


KLF4 Protein – Tumor Average
KLF4/GAPDH
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
*
Control
1 mg/kg
5 mg/kg
15 mg/kg
* p = 0.05

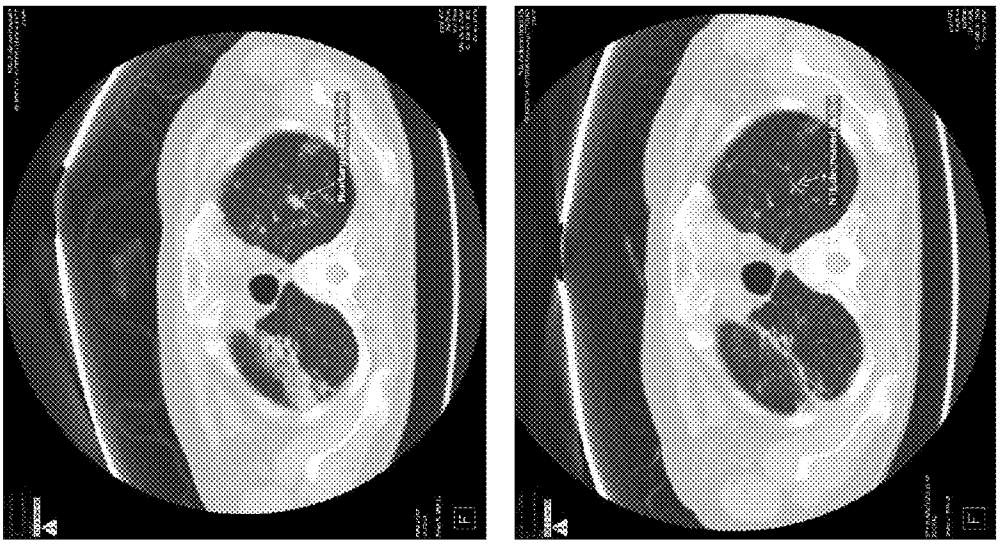
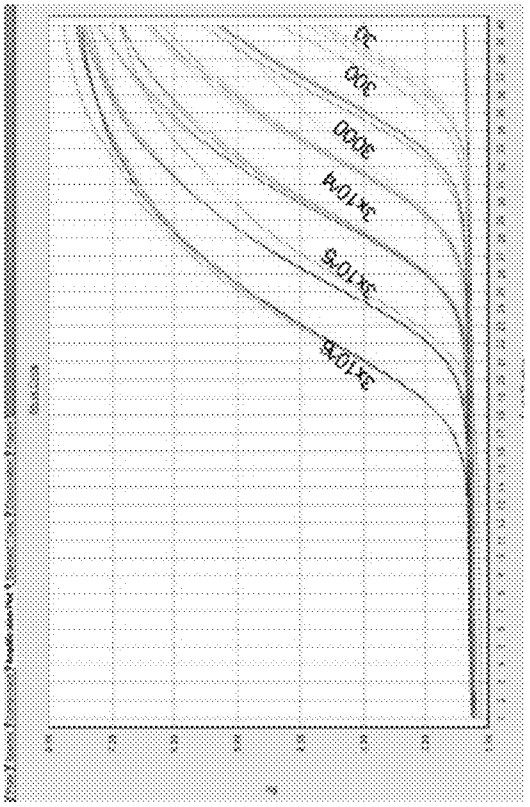


FIG. 12



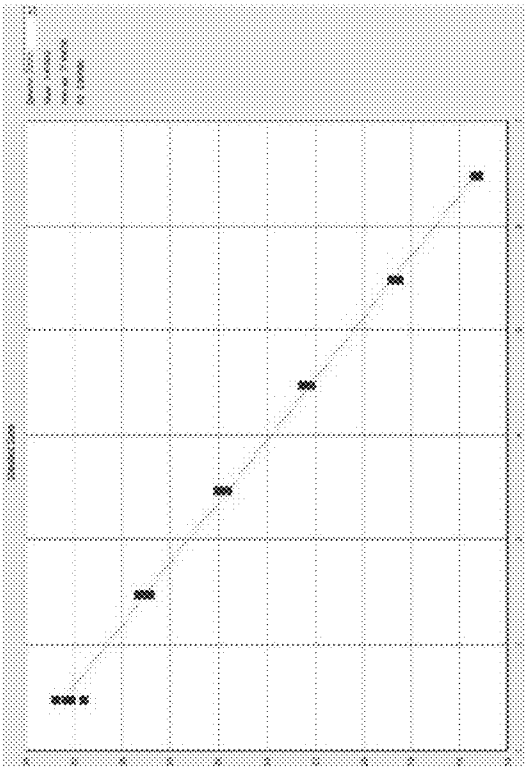


FIG. 13

FIG. 14

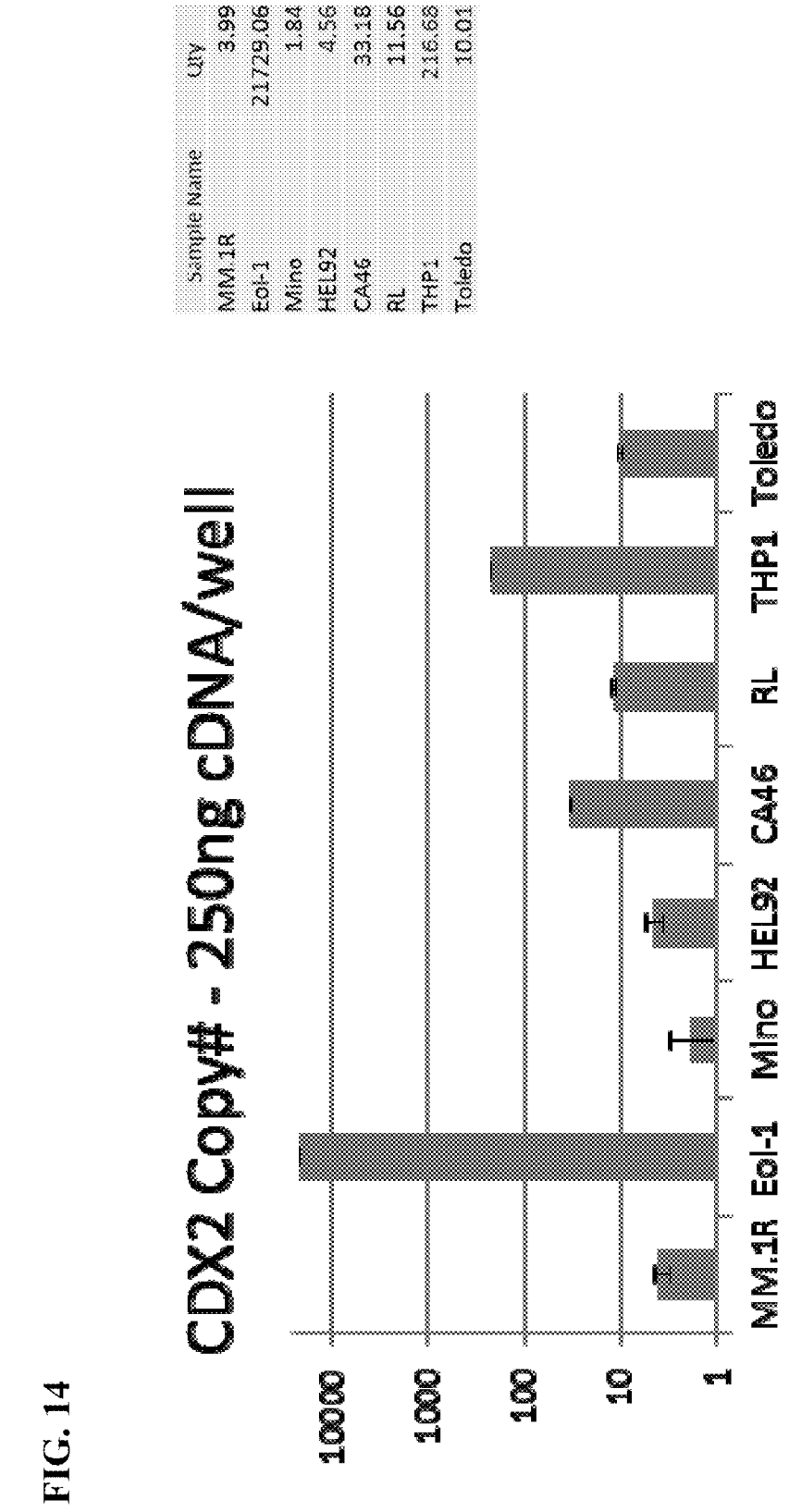

CDX2 Copy# - 250ng cDNA/well
Sample Name
Qty
MM.1R 3.99
Eol-1 21729.06
Mino 1.84
HEL92 4.56
CA46 33.18
RL 11.56
THP1 216.68
Toledo 10.01
10000
1000
100
10
1
MM.1R
Eol-1
Mino
HEL92
CA46
RL
THP1
Toledo

FIG. 15

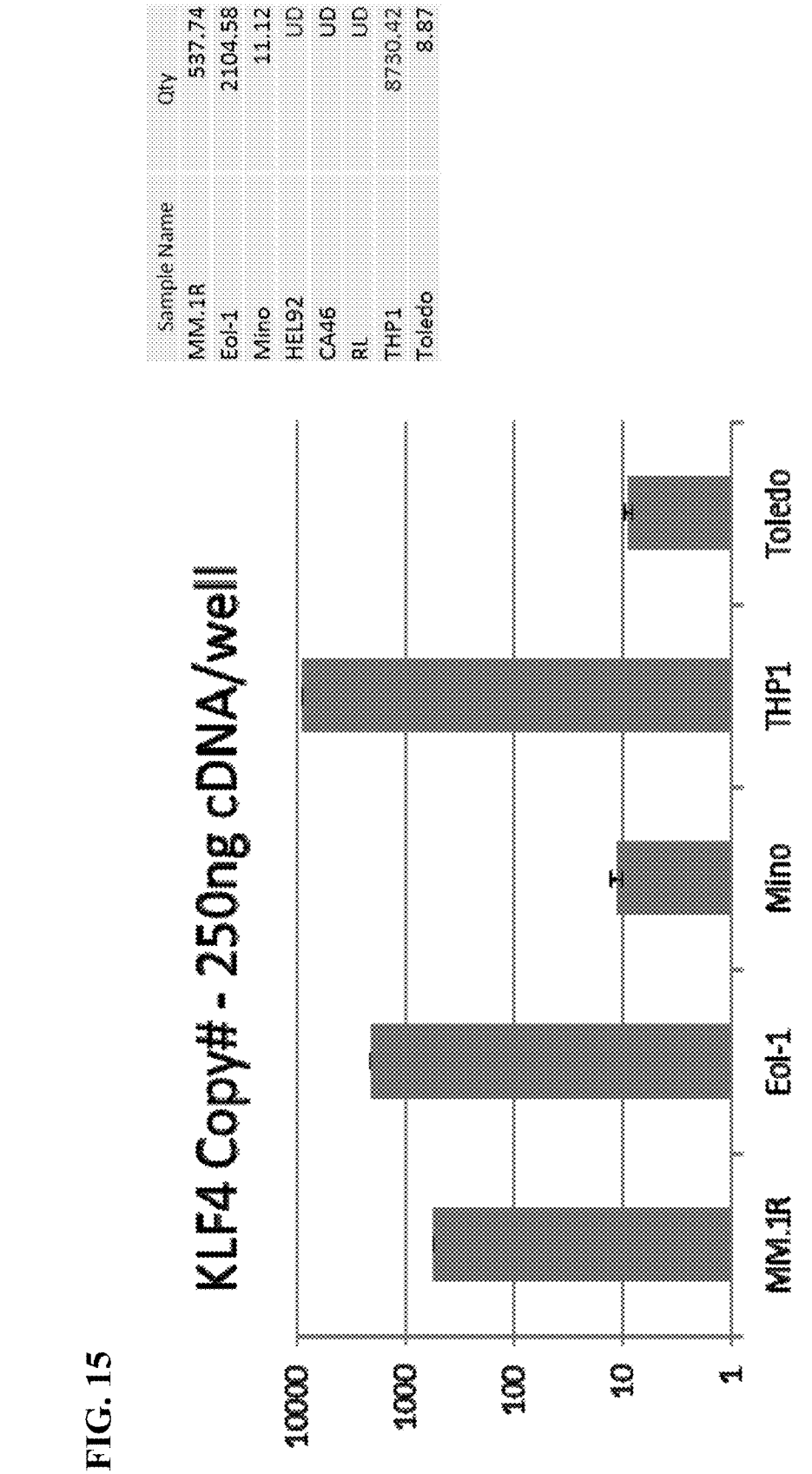
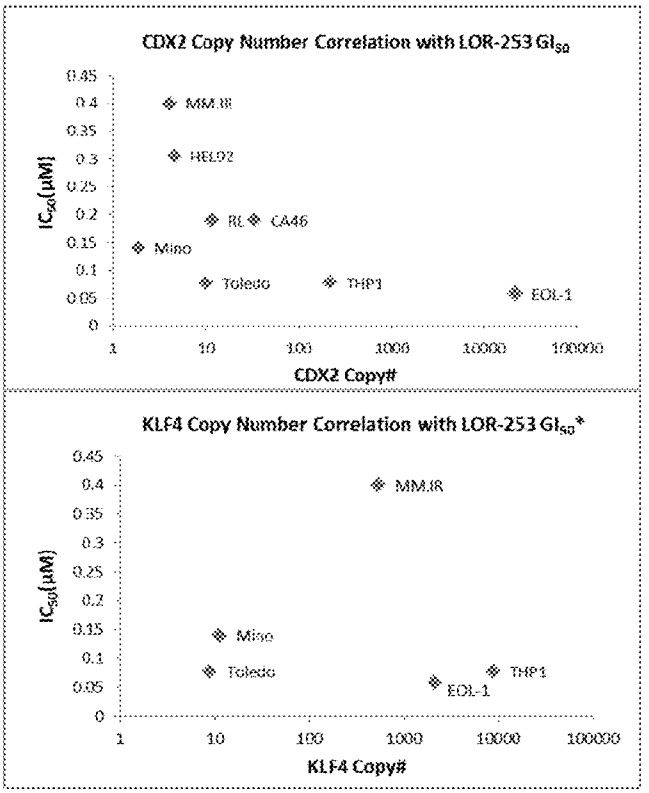
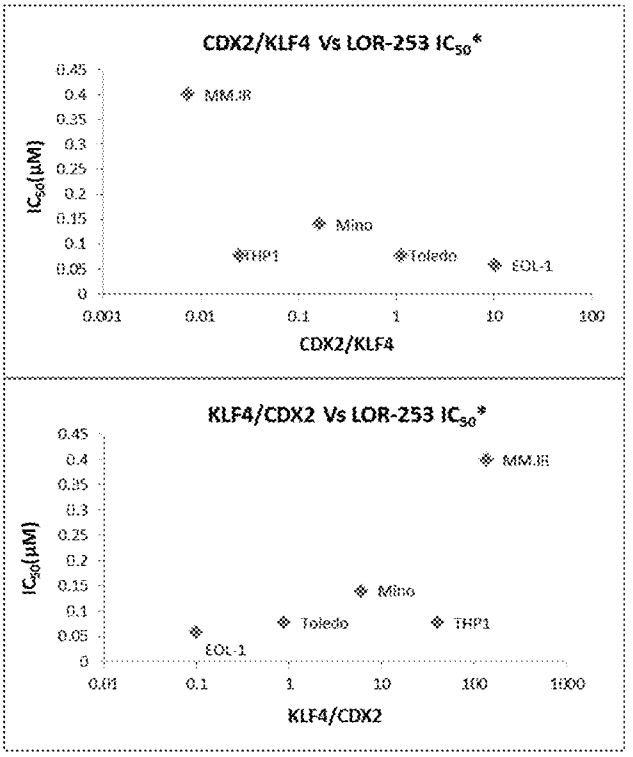

KLF4 Copy# - 250ng cDNA/well
Sample Name
Qty
MM.1R 537.74
Eol-1 2104.58
Mino 11.12
HEL92 UD
CA46 UD
RL UD
THP1 8730.42
Toledo 8.87
10000
1000
100
10
1
MM.1R
Eol-1
Mino
THP1
Toledo

FIG. 16



*KLF4 level was below the limit of detection in HEL92.1.7, CA46 and RL cell lines

FIG. 17



*KLF4 level was below the limit of detection in HEL92.1.7, CA46 and RL cell lines

FIG. 18

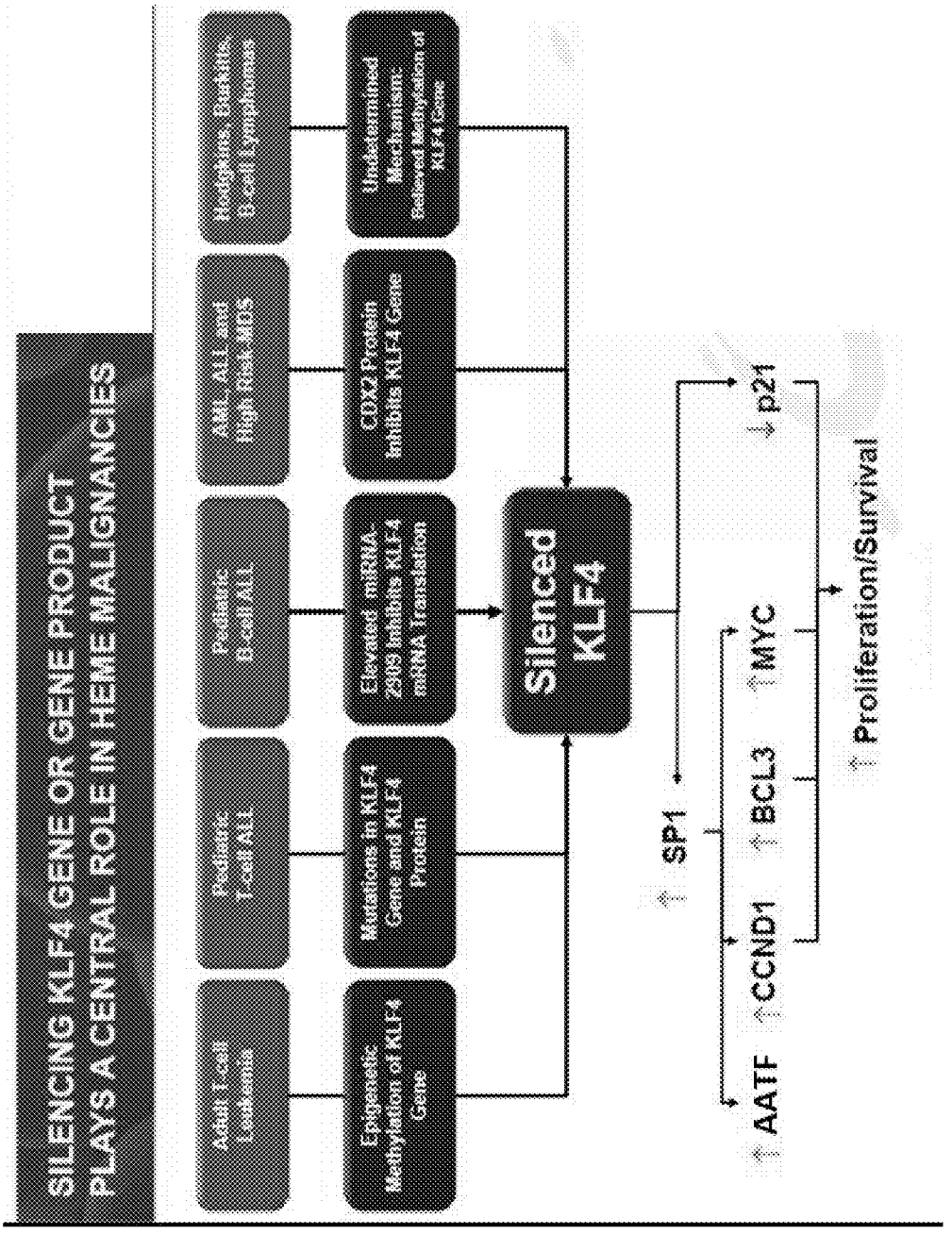

SILENCING KLF4 GENE OR GENE PRODUCT PLAYS A CENTRAL ROLE IN HEME MALIGNANCIES
Adult T-cell Leukemia
Pediatric T-cell ALL
Pediatric B-cell ALL
AML, ALL and High Risk MDS
Hodgkin's, Burkitt's, B-cell Lymphomas
Epigenetic Methylation of KLF4 Gene
Mutations in KLF4 Gene and KLF4 Protein
Elevated miRNA-2909 Inhibits KLF4 mRNA Translation
COX2 Protein Inhibits KLF4 Gene
Undetermined Mechanisms: Believed Methylation of KLF4 Gene
Silenced KLF4
↑ SP1
↑ AATF
↑ CCND1
↑ BCL3
↑ MYC
↓ p21
↑ Proliferation/Survival

FIG. 19

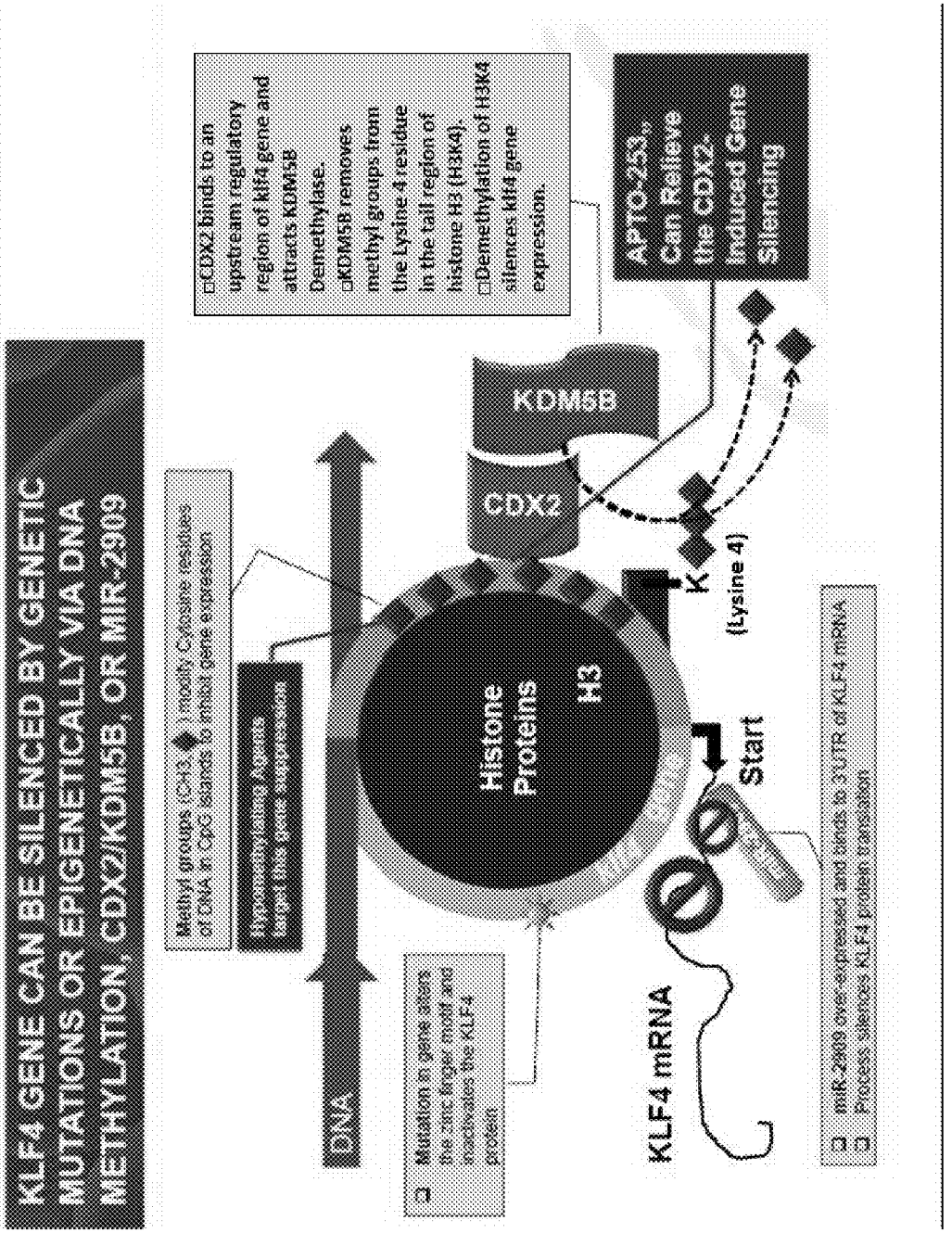

KLF4 GENE CAN BE SILENCED BY GENETIC MUTATIONS OR EPIGENETICALLY VIA DNA METHYLATION, CDX2/KDM5B, OR MIR-2909
Methyl groups (CH3, ◆) mostly Cytosine residues of DNA in CpG islands to silence gene expression
Hypermethylation Activates Target DNA gene expression
DNA
Mutation in gene alters the zinc finger motif and inactivates the KLF4 protein
Histone Proteins
H3
KDM5B
CDX2
CDX2 binds to an upstream regulatory region of klf4 gene and attracts KDM5B Demethylase.
KDM5B removes methyl groups from the Lysine 4 residue in the tail region of histone H3 (H3K4).
Demethylation of H3K4 silences klf4 gene expression.
APTO-253, Can Relieve the CDX2-Induced Gene Silencing
K
(Lysine 4)
Start
KLF4 mRNA
miR-2909 over-expressed and binds to 3'UTR of KLF4 mRNA
Process silences KLF4 protein translation

FIG. 20

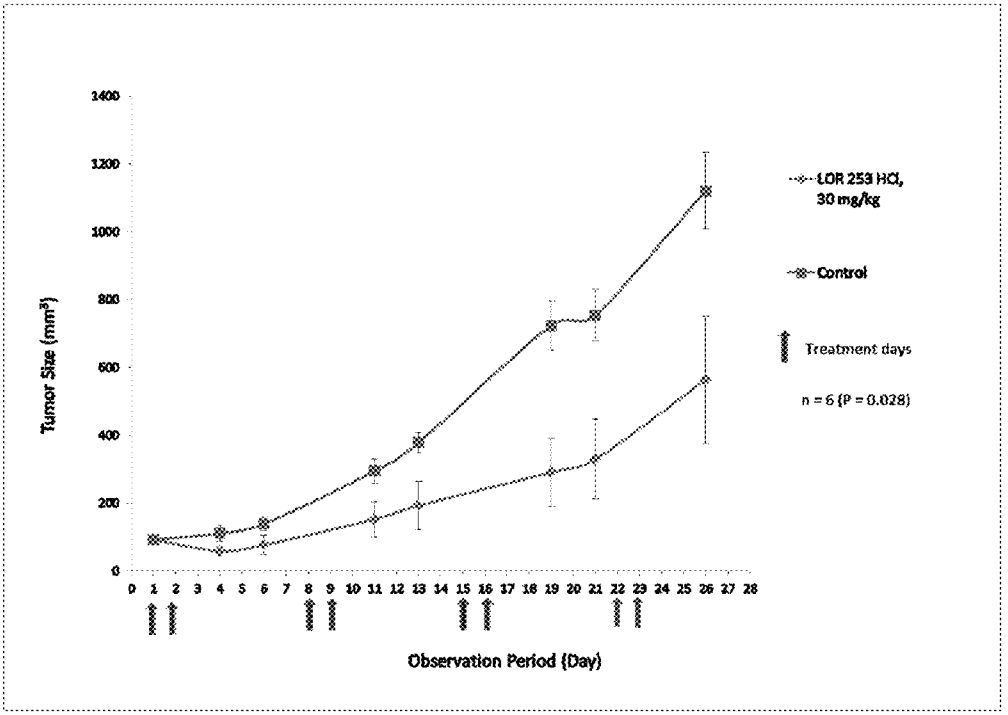

Tumor Size (mm³)
1400
1200
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Observation Period (Day)
LOR 253 HCl, 30 mg/kg
Control
Treatment days
n = 6 (P = 0.028)

FIG. 21

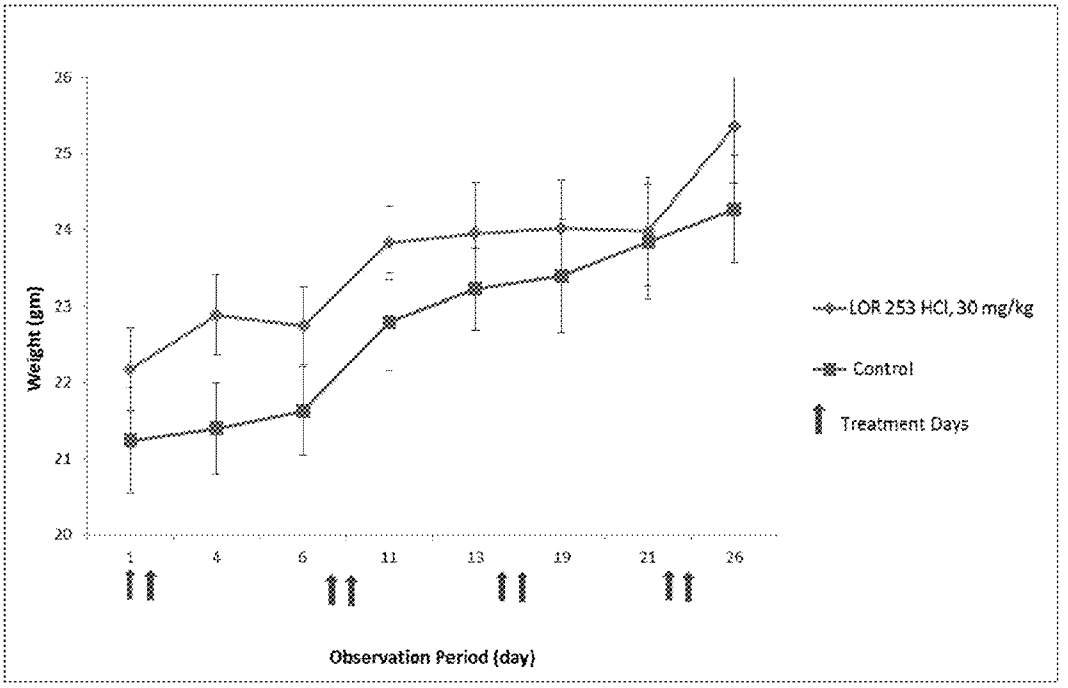

26
25
24
23
22
21
20
Weight (gm)
1
4
6
11
13
19
21
26
Observation Period (day)
LOR 253 HCl, 30 mg/kg
Control
Treatment Days

FIG. 22

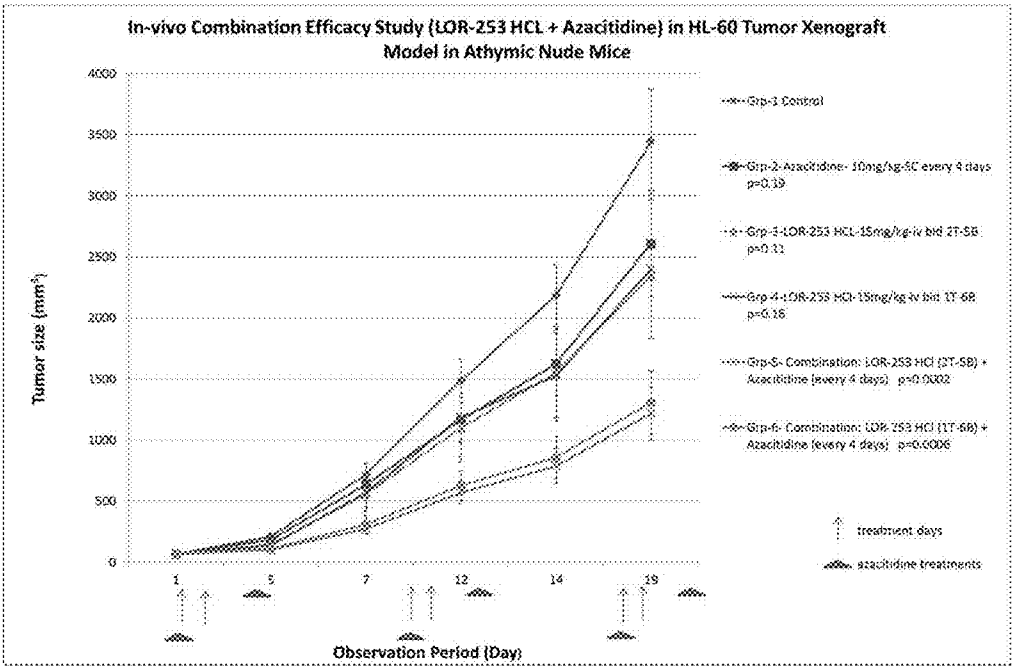

In-vivo Combination Efficacy Study (LOR-253 HCL + Azacitidine) in HL-60 Tumor Xenograft Model in Athymic Nude Mice
Tumor size (mm³)
4000
3500
3000
2500
2000
1500
1000
500
0
1
5
7
12
14
19
Observation Period (Day)
Grp-1 Control
Grp-2- Azacitidine- 10mg/kg-SC every 4 days p=0.19
Grp-3-LOR-253 HCL-15mg/kg-iv bid 2T-5B p=0.11
Grp-4-LOR-253 HCl-15mg/kg-iv bid 1T-6B p=0.16
Grp-5- Combination: LOR-253 HCl (2T-5B) + Azacitidine (every 4 days) p=0.0002
Grp-6- Combination: LOR-253 HCl (1T-6B) + Azacitidine (every 4 day(s) p=0.0006
treatment days
azacitidine treatments

FIG. 23

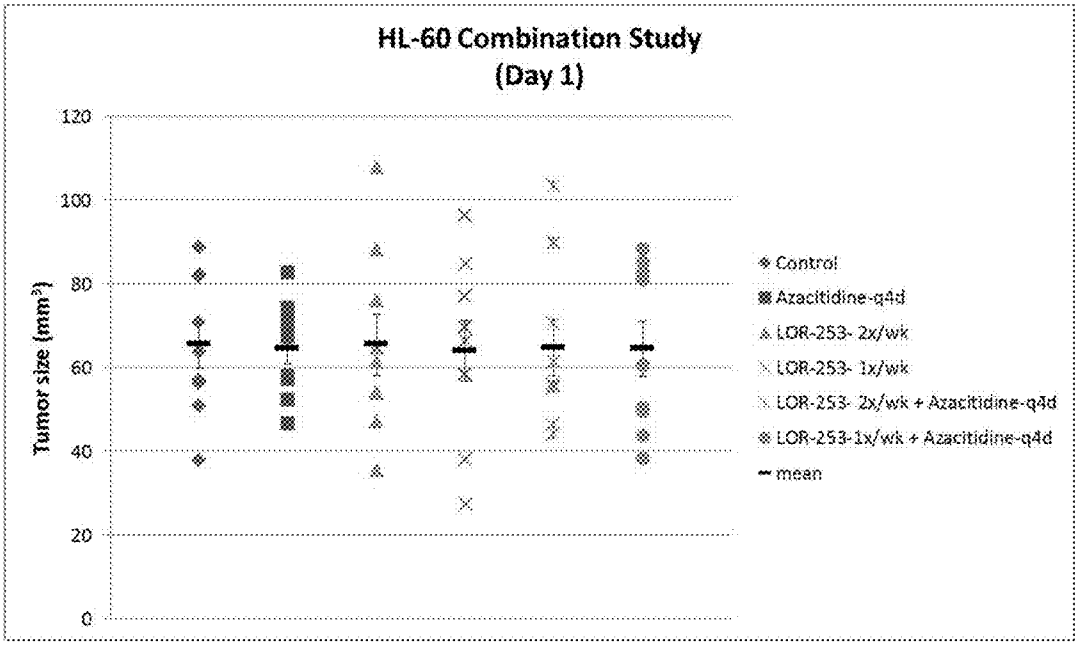

HL-60 Combination Study
(Day 1)
Tumor size (mm³)
120
100
80
60
40
20
0
Control
Azacitidine-q4d
LOR-253- 2x/wk
LOR-253- 1x/wk
LOR-253- 2x/wk + Azacitidine-q4d
LOR-253-1x/wk + Azacitidine-q4d
mean

FIG. 24

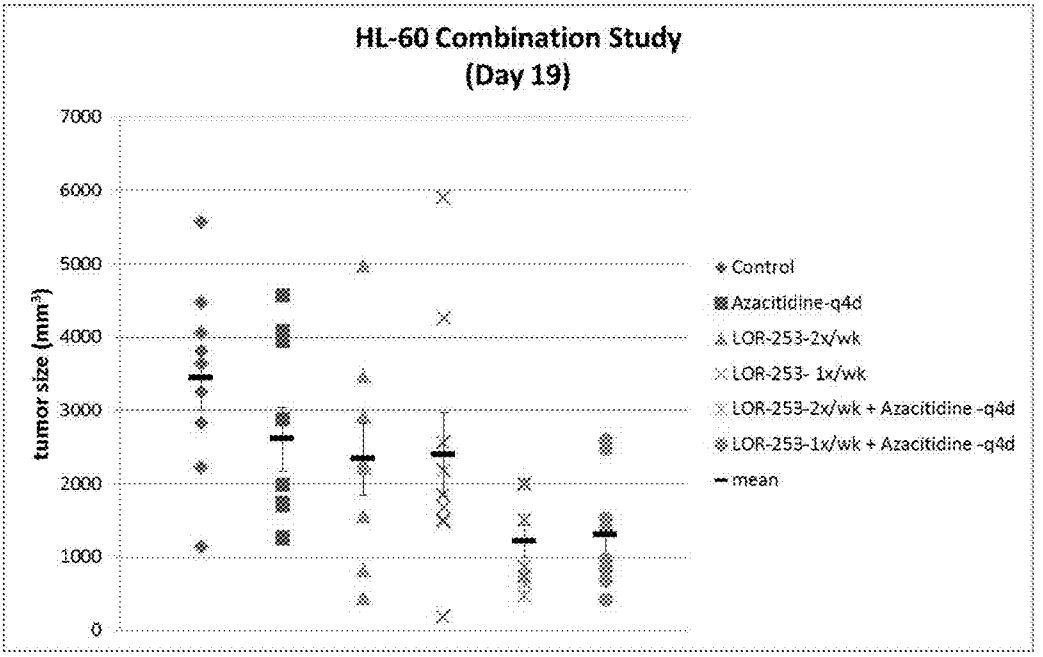

HL-60 Combination Study
(Day 19)
tumor size (mm³)
7000
6000
5000
4000
3000
2000
1000
0
Control
Azacitidine-q4d
LOR-253-2x/wk
LOR-253- 1x/wk
LOR-253-2x/wk + Azacitidine -q4d
LOR-253-1x/wk + Azacitidine -q4d
mean

FIG. 25

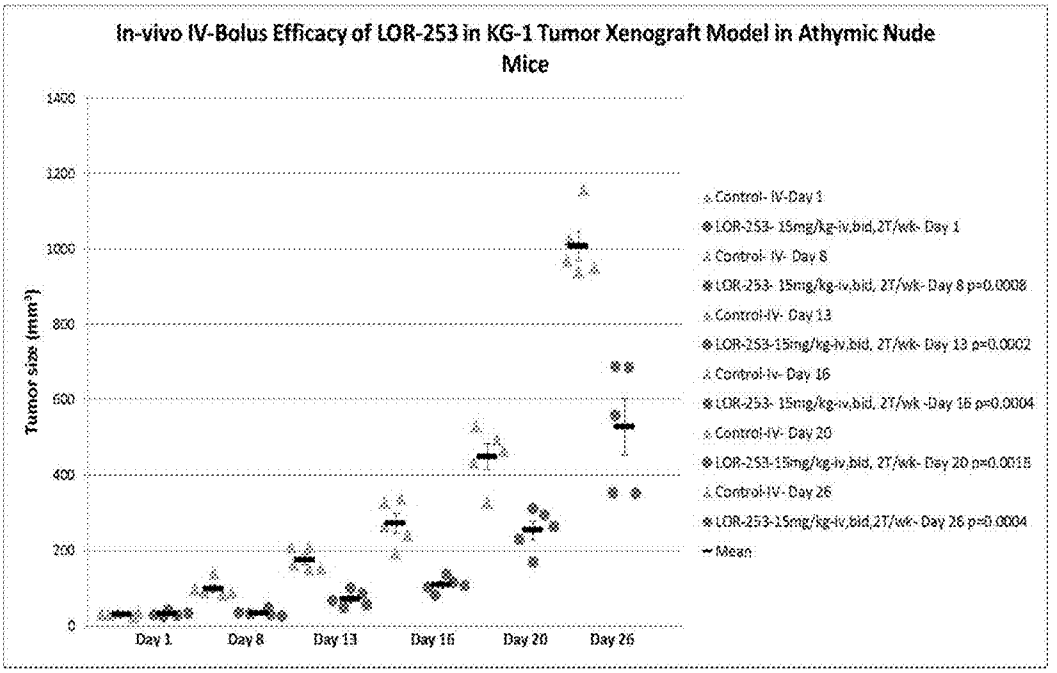

In-vivo IV-Bolus Efficacy of LOR-253 in KG-1 Tumor Xenograft Model in Athymic Nude Mice
Tumor size (mm³)
1400
1200
1000
800
600
400
200
0
Day 1
Day 8
Day 13
Day 16
Day 20
Day 26
Control- IV-Day 1
LOR-253- 15mg/kg-iv,bid,2T/wk- Day 1
Control- IV- Day 8
LOR-253- 15mg/kg-iv,bid, 2T/wk- Day 8 p=0.0008
Control-IV- Day 13
LOR-253-15mg/kg-iv,bid, 2T/wk- Day 13 p=0.0002
Control-iv- Day 16
LOR-253- 15mg/kg-iv,bid, 2T/wk -Day 16 p=0.0004
Control-IV- Day 20
LOR-253-15mg/kg-iv,bid, 2T/wk- Day 20 p=0.0018
Control-IV- Day 26
LOR-253-15mg/kg-iv,bid,2T/wk- Day 26 p=0.0004
Mean

FIG. 26

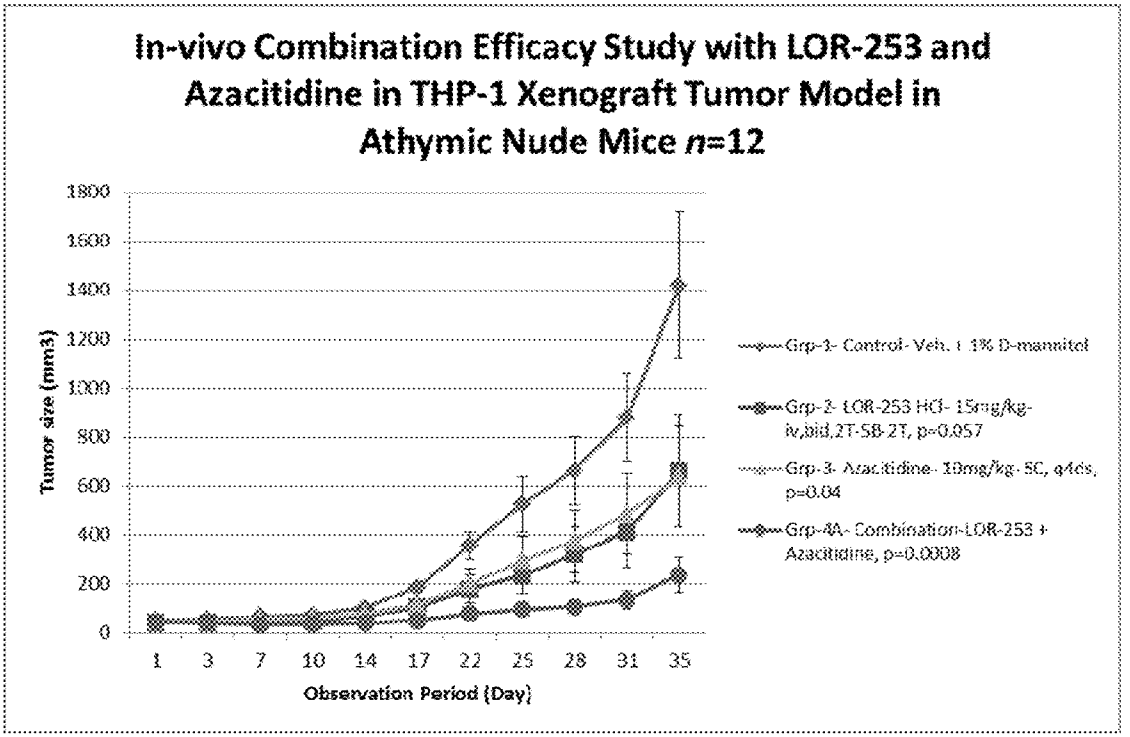

In-vivo Combination Efficacy Study with LOR-253 and Azacitidine in THP-1 Xenograft Tumor Model in Athymic Nude Mice n=12
Tumor size (mm3)
1800
1600
1400
1200
1000
800
600
400
200
0
1
3
7
10
14
17
22
25
28
31
35
Observation Period (Day)
Grp-1- Control- Veh. + 1% D-mannitol
Grp-2- LOR-253 HCl- 15mg/kg- iv,bid,2T-5B-2T, p=0.057
Grp-3- Azacitidine- 10mg/kg- SC, q4ds, p=0.04
Grp-4A- Combination-LOR-253 + Azacitidine, p=0.0008

FIG. 27

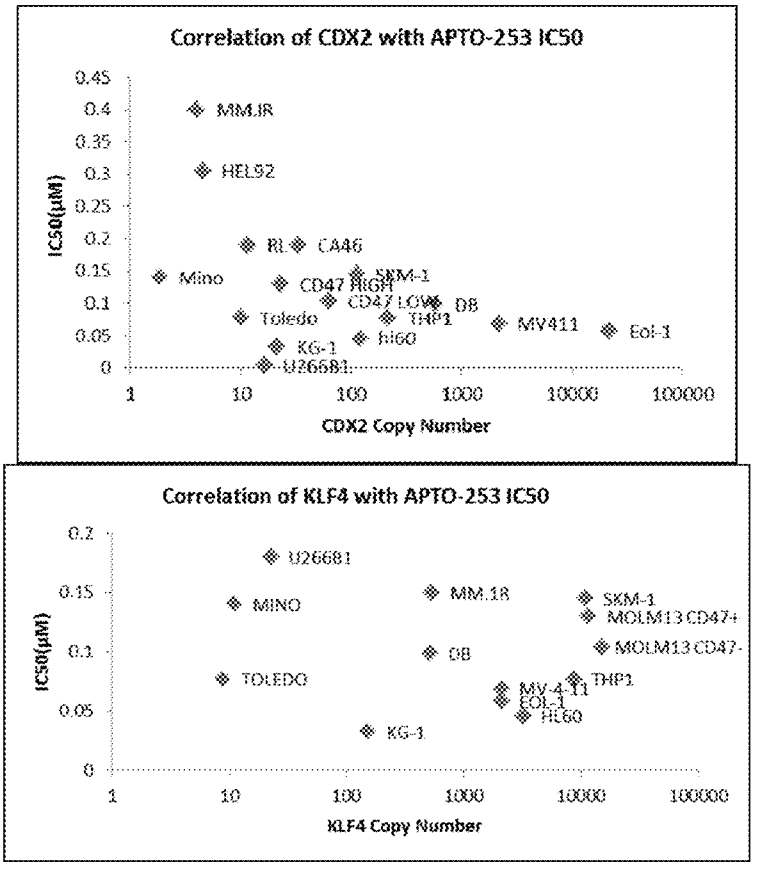
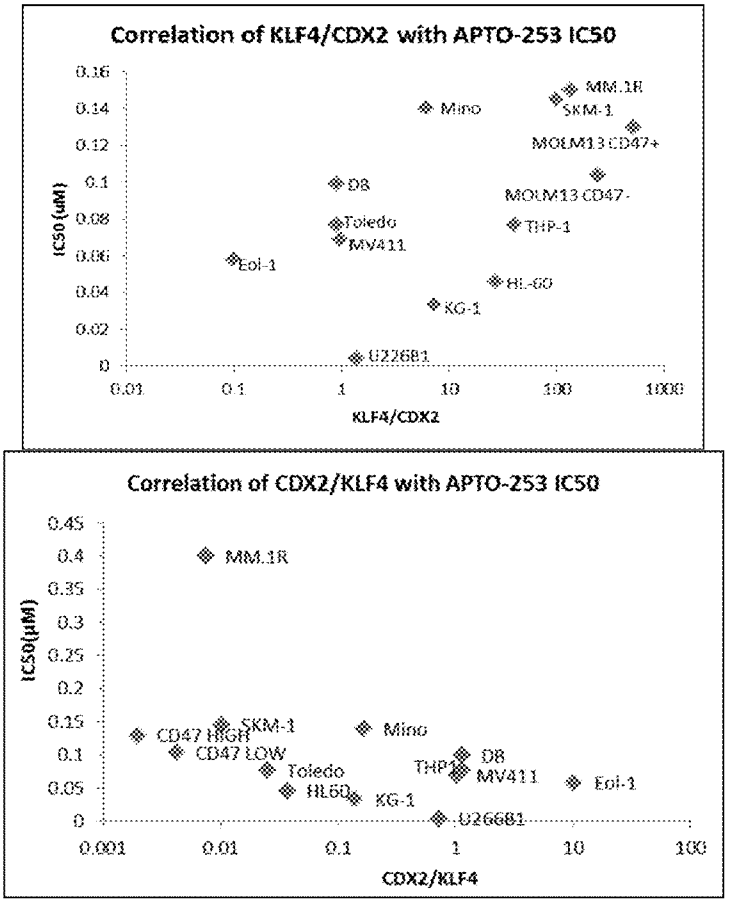

Correlation of CDX2 with APTO-253 IC50
IC50(μM)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
MM.1R
HEL92
RL
CA46
SKM-1
Mino
CD47 HIGH
CD47 LOW
OB
Toledo
THP1
MV411
Eol-1
KG-1
HI60
U26681
1
10
100
1000
10000
100000
CDX2 Copy Number
Correlation of KLF4 with APTO-253 IC50
IC50(μM)
0.2
0.15
0.1
0.05
0
U26681
MINO
MM.1R
SKM-1
MOLM13 CD47+
MOLM13 CD47-
OB
TOLEDO
THP1
MV-4-11
EOL-1
HL60
KG-1
1
10
100
1000
10000
100000
KLF4 Copy Number

FIG. 28


Correlation of KLF4/CDX2 with APTO-253 IC50
IC50 (uM)
KLF4/CDX2
MM.1R
SKM-1
Mino
MOLM13 CD47+
MOLM13 CD47-
D8
Toledo
MV411
THP-1
Eol-1
HL-60
KG-1
U22681
Correlation of CDX2/KLF4 with APTO-253 IC50
IC50(μM)
CDX2/KLF4
MM.1R
SKM-1
Mino
CD47 HIGH
CD47 LOW
Toledo
HL60
KG-1
THP1
D8
MV411
Eol-1
U26681

COMPOSITIONS, BIOMARKERS AND THEIR USE IN THE TREATMENT OF CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/887,285, filed Oct. 4, 2013, U.S. Provisional Application No. 61/919,023, filed Dec. 20, 2013 U.S. Provisional Application No. 62/017,505 filed on Jun. 26, 2014, and U.S. Provisional Application No. 62/037,868, filed Aug. 15, 2014, all of which are hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0002] The present invention relates generally to compositions, biomarkers, and methods of using them. In particular, the invention relates to the use of several biomarkers in methods for determining the responsiveness of a human subject to a compound.

BACKGROUND OF THE INVENTION

[0003] Many drugs or drug candidates have been developed for the treatment of various cancers, including some small molecule compounds. However, not all patients are responsive to such drugs or drug candidates. Certain biomarkers have been found correlative to the responsiveness of some cancer patients to certain drugs. However, there remain a large unmet medical need for determination of the responsiveness of cancer patients to many cancer drugs or drug candidates and more targeted treatment of specific cancers or specific cancer patient populations.

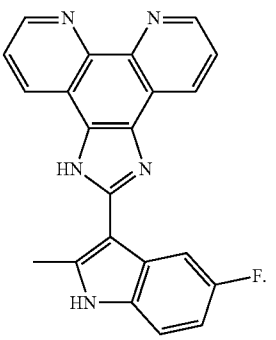
[0004] In addition, patients with specific subsets of various cancers may have specific genetic and/or phenotypic profiles, and may respond differently from patients with other subsets of the same types of cancers. Current treatments for many cancers are not very effective in patients with specific subsets of cancers, or are too toxic in such patients or in general. For example, acute myelogenous leukemia (AML) is a subset of leukemia. It is the most common form of adult leukemia (blood cancer) with a <20% survival rate after 5 years and less than 5% if patients are age >65. Current treatments for AML are blunt and harsh chemotherapies (i.e., cytarabine, anthracycliine, etc.) that are not targeted and have limiting off-target toxicities. Thus, there is a severe unmet medical need for distinguishing patients who are potentially sensitive to a certain treatment, and patients who are not. The present invention meets this need and provides compositions and methods for the effective treatment of cancer.

SUMMARY OF THE INVENTION

[0005] The present invention provides compositions and methods for determining responsiveness of a human subject to treatment. In some embodiments, biomarkers are provided. The present invention is based, in part, on the discovery that a set of biomarkers in the Caudal-related Homeobox protein CDX2—Krüppel-like factor 4 (KLF4) signaling pathway are indicative of the responsiveness of human subjects to a particular compound.

[0006] In one aspect, the invention provides methods for determining the responsiveness of a human subject to a pharmaceutical compound. In some embodiments, the methods comprise determining the level of activity of one or more biomarkers in a biological sample from the human subject. In some embodiments, at least one biomarker is KLF4, CDX2, p21, p53, SP1, MYC, BCL3, CCND1, and AATF, Caspase 3, or Annexin V.

[0007] In some embodiments, the pharmaceutical compound is a 2,4,5-trisubstituted imidazole compound described in US 2007/0123553A1, or 2-indolyl imidazo[4,5-d]phenanthroline compounds described in U.S. Pat. No. 8,148,392, or functional derivatives thereof. In some embodiments, the pharmaceutical compound is a 2-indolyl imidazo [4,5-d]phenanthroline derivative having the structure of formula I:



(I)

(a.k.a. LOR-253, or APTO-253)

[0008] In some embodiments, a lower than a normal level of KLF4 activity is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, an increased level of CDX2 activity than a normal level is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, a lower than a normal level of p21 activity is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, for the types of cancer in which KLF4 negatively regulates p53, a higher than a normal level of p53 activity is indicative that the human subject is responsive to the compound of the present invention. In some other embodiments, for the types of cancer in which KLF4 positively regulates p53, a lower than a normal level of p53 activity is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, a lower than a normal level of Caspase 3 activity is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, a higher than a normal level of SP1 activity is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, a higher than a normal level of MYC activity is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, a higher than a normal level of BCL3 activity is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, a higher than a normal level of CCND1 activity is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, a higher than a normal level of AATF activity is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, the activity of at least two biomarkers of the present invention is used to determine the responsiveness. In some embodiments, the activity is gene

copy number. In some embodiments, the activity of KLF4 and CDX2 are used to determine the responsiveness. In some embodiments, the ratio of CDX2/KLF4 and/or KLF4/CDK2 gene copy numbers is used to determine the responsiveness. In some embodiments, a lower KLF4/CDX2 ratio, or a higher CDX2/KLF4 ratio than normal level is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, a CDX2/KLF4 or KLF4/CDX2 ratio similar to that of human subjects responsive to the treatment is indicative that the human subject is responsive to the compound of the present invention.

[0009] In some embodiments, the cancer is associated with abnormal activity of the CDX2-KLF4 signaling pathway in the human subject. In some embodiments, one or more components in the CDX2-KLF4 signaling pathway have abnormal activity when compared to the activity of a control group. In some embodiments, the cancer is leukemia/lymphoma. In some embodiments, the cancer is acute myelogenous leukemia (AML). In some embodiments, the AML is elderly AML. As used herein, elderly AML patients are those patients who have, or may have AML, with an age above 60. In some embodiments, elderly AML patients are in their first relapse. In some embodiments, the AML is not elderly AML. As used herein, non-elderly AML patients are those who have, or may have AML, with an age equal to or below 60. In some embodiments, the non-elderly AML patients are in their second relapse. In some embodiments, the cancer is lymphoma, gastric cancer, multiple myeloma, myelodysplastic syndromes, or combinations thereof.

[0010] In some embodiments, the method further comprises assaying for a genetic alteration of CDX2 or determining the level of activity of CDX2 in the biological sample from the human subject, wherein the absence/presence of a genetic alteration of CDX2 or a normal/abnormal level of CDX2 activity in the subject is used alone or combined with the activity information of one or more biomarkers described herein to determine if the human subject is responsive to the compound of the present invention, such as formula I.

[0011] In another aspect, the invention provides methods for monitoring the efficacy of an anti-cancer agent. In some embodiments, the anti-cancer agent is the compound of formula I as shown above. In some embodiments, the methods comprise determining the level of activity of KLF4, CDX2, p21, p53, SP1, MYC, BCL3, CCND1, AATF, Caspase 3, and/or Annexin V in a human subject under treatment with the anti-cancer agent. In some embodiments, the anti-cancer agent comprises the compound having formula I.

[0012] In some embodiments, an increased activity level of KLF4 in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while a decrease or unchanged activity level of KLF4 during the treatment when compared to that in the same subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, a lower activity level of KLF4 in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of KLF4 toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0013] In some embodiments, a deceased activity level of CDX2 in a human subject when compared to that of CDX2 in the subject before the treatment indicates the efficacy of the anti-cancer agent, while an increase or unchanged activity level of CDX2 during the treatment when compared to that of CDX2 activity in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, a higher activity level of CDX2 in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of CDX2 activity toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0014] In some embodiments, an increased activity level of p21 in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while a decrease or unchanged activity level of p21 during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, a lower activity level of p21 in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of p21 activity toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0015] In some embodiments, for the types of cancer in which KLF4 negatively regulates p53, a decreased activity level of p53 in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while an increased or unchanged activity level of p53 during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, for the types of cancer in which KLF4 negatively regulates p53, a higher activity level of p53 in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of p53 toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0016] In some embodiments, for the types of cancer in which KLF4 positively regulates p53, an increased or unchanged activity level of p53 in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while a decreased activity level of p53 during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, for the types of cancer in which KLF4 positively regulates p53, a lower activity level of p53 in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of p53 toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0017] In some embodiments, an increased activity level of Caspase 3 in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while a decreased or unchanged activity level of Caspase 3 during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, a lower activity level of Caspase 3 in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normaliza-

tion or stabilization in the activity level of Caspase 3 toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0018] In some embodiments, an increased activity level of Annexin V in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while a decreased or unchanged activity level of Annexin V during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, a lower activity level of Annexin V in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of Annexin V toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0019] In some embodiments, a decreased activity level of SP1 in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while an increased or unchanged activity level of SP1 during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, a higher activity level of SP1 in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of SP1 toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0020] In some embodiments, a decreased activity level of MYC in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while an increased or unchanged activity level of MYC during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, a higher activity level of MYC in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of MYC toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0021] In some embodiments, a decreased activity level of BCL3 in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while an increased or unchanged activity level of BCL3 during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, a higher activity level of BCL3 in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of BCL3 toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0022] In some embodiments, a decreased activity level of CCND1 in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while an increased or unchanged activity level of CCND1 during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, a higher activity level of CCND1 in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of CCND1 toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0023] In some embodiments, a decreased activity level of AATF in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while an increased or unchanged activity level of AATF during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. In some embodiments, a higher activity level of AATF in a human subject before treatment when compared to a predetermined standard level indicates the efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the activity level of AATF toward a predetermined standard level indicates efficacy of the anti-cancer agent.

[0024] In some embodiments, activities of at least two biomarkers described herein are used to determine the responsiveness of a human subject. In some embodiments, the activity is gene copy number. In some embodiments, ratio of the activity of two biomarkers (A/B, B/A, A/C, or C/A) is used to determine the responsiveness. In some embodiments, at least one biomarker (i.e., A) is CDX2. In some embodiments, the other biomarker B is KLF4, p21, Caspase 3, or Annexin V, while the other biomarker C is p53, SP1, MYC, BCL3, CCND1, or AATF. In some embodiments, the ratio of CDX2/B, B/CDX2, CDX2/C, or C/CDX2 copy numbers is used to determine the responsiveness. In some embodiments, a lower B/CDX2 ratio, a higher CDX2/B, a higher C/CDX2, or a lower CDX2/C ratio than a predetermined standard is indicative that the human subject is responsive to the compound of the present invention. In some embodiments, an increased B/CDX2 ratio (or a decreased CDX2/B ratio) or a decreased C/CDX2 (or an increased CDX2/C ratio) in a human subject when compared to that in the subject before the treatment indicates the efficacy of the anti-cancer agent, while decreased or unchanged B/CDX2 ratio (or an increased or unchanged CDX2/B ratio) or increased or unchanged C/CDX2 ratio (or a decreased or unchanged CDX2/C ratio) during the treatment when compared to that in the subject before the treatment indicates the lack of efficacy of the anti-cancer agent. Alternatively, a normalization or stabilization in the ratio of B/CDX2, CDX2/B, C/CDX2, or CDX2/C toward a predetermined standard level indicates efficacy of the anti-cancer agent. In some embodiments, the ratio of CDX2/KLF4 and/or KLF4/CDX2 is used.

[0025] In certain embodiments of the method for monitoring the efficacy of the anti-cancer agent, the human subject is treated for AML. In certain other embodiments, the human subject is treated for lymphoma, gastric cancer, multiple myeloma, myelodysplastic syndromes, or combinations thereof.

[0026] In yet another aspect, the invention provides methods for treating a human subject. In some embodiments, the human subject is treated for AML. In certain other embodiments, the human subject is treated for lymphoma, gastric cancer, multiple myeloma, myelodysplastic syndromes, or combinations thereof. In some embodiments, the methods comprise administering to the human subject an effective amount of an anti-cancer agent or a pharmacologically acceptable salt or solvate thereof of the present invention. In some embodiments, the anti-cancer agent comprises a compound that can modulate the activity of the CDX2-KLF4

signaling pathway. In some embodiments, the anti-cancer agent comprises a compound that has at least one function selected from (1) inhibition of CDX2 activity; (2) induction of KLF4 activity; (3) induction of p21 CDK inhibitor; (4) induction of G1/S Cell cycle arrest; (5) induction of Caspase 3 enzyme; and (6) induction of Apoptosis. In some embodiments, the methods further comprise determining the level of activity of a biomarker. In some embodiments, the biomarker is KLF4, CDX2, p21, p53, SP1, MYC, BCL3, CCND1, AATF, Caspase 3, Annexin V, or any combination thereof. In some embodiments, the biomarker activity in the human subject is determined before, during, and/or after the treatment.

[0027] In certain embodiments, the methods for treating a human subject comprise assaying for a genetic alteration of CDX2 or determining the level of activity of CDX2 in the biological sample from the human subject. In some embodiments, an effective amount of the anti-cancer agent is administered to the human subject, if there is a presence of a genetic alteration of CDX2 or a higher than normal level of CDX2 activity. In some embodiments, the anti-cancer agent is the compound of formula I, or the pharmacologically acceptable salt or solvate thereof.

[0028] Additional aspects and embodiments of the invention will be apparent from the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIG. **1** is a diagram of non-limiting mechanism of how an active agent of the present invention (e.g., formula I, a.k.a. LOR-253 or APTO-253) treats cancer. Without wishing to be bound by any particular theory, in certain cancer types, such as solid tumors, KLF4 is down regulated, which is essential for accelerated cell proliferation, epithelial-mesenchymal transition (EMT) and metastasis; in certain hematologic cancers: KLF4 is down regulated, which is essential for leukemogenesis. LOR-253 induces KLF4 expression which in turn inhibits cancer cell proliferation, EMT, and metastasis, and/or triggers apoptosis.

[0030] FIG. **2** is a diagram that shows non-limiting mechanism of how CDX2 functions in certain cancers and how LOR-253 treats such cancers. Without wishing to be bound by any particular theory, in normal cells of the hematopoietic system, the CDX2 gene is turned off or expressed at a relatively low level. In certain cancers including AML, CDX2 is turned on or increased, leading to aberrantly expressed CDX2 transcription factor. CDX2 binds to the promoter region of the KLF4 gene and inhibits KLF4 expression, which is an essential step to promote leukemogenesis. LOR-253 induces KLF4 expression which in turn triggers apoptosis of the cancerous leukocytes.

[0031] FIG. **3** is a graphical representation of the effect of LOR-253 on proliferation of a number of cancer cell lines in vitro. Cells of various cancer cell lines were incubated with LOR-253 as described in the Examples, and the cell concentrations of various cell lines for 50% of maximal inhibition of cell proliferation (GI_{50}) by LOR-253 were determined and shown in the figure.

[0032] FIG. **4** is a graphical representation of the effect of LOR-253 on the expression levels of KLF4 in two cell lines, THP-1 and HL60.

[0033] FIG. **5** depicts BD FACSCalibur flow cytometer assay result for THP1 and HL-60 AML cell lines treated with DMSO or LOR-253 (left panel). FIG. **5** also shows that treatment of LOR-253 results in G1/S cell cycle arrest in THP1 and HL-60 cell lines (right panel).

[0034] FIG. **6** depicts a BD FACSCanto flow cytometry acquisition plots for THP-1 cells treated with DMSO, 0.5 or 1 uM of LOR-253. THP-1 cells treated with LOR-253 showed elevated Annexin V staining, indicating induction of apoptosis (Q3: Annexin V+/PI−).

[0035] FIG. **7** depicts Caspase 3 expression level in THP1 and HL-60 AML cell lines treated with DMSO or LOR-253.

[0036] FIG. **8** depicts fold change in expression of BAX and BCL2 in THP1 cells treated with DMSO or 0.5 μM LOR-253

[0037] FIG. **9** depicts in vivo efficacy of LOR-253 HCL in H226 xenograft model mice. Tumor sizes of H226_xenograft mice treated with LOR-253 HCL or negative control measured on the indicated days are shown.

[0038] FIG. **10** depicts the pharmacokinetic (PK) in CD-1 nude mice treated by LOR-253 at a dosage of 1, 5, or 15 mg/kg. The serum level of LOR-253 has a dose related increase.

[0039] FIG. **11** depicts the pharmacodynamic (PD) responses in mice treated for 5 consecutive days with 1, 5, and 15 mg/kg LOR-253. The KLF4 protein level was measured 16 hours after the last dose.

[0040] FIG. **12** depicts tumor shrinkage in patient with NSCLC (poorly differentiated adenocarcinoma) before the treatment (see the upper panel) and after the treatment (see the lower panel)

[0041] FIG. **13** depicts standard curve generated by serially diluted standards of known concentrations of CDX2 and KLF4 genes prepared at 3×10^2-3×10^6 copies.

[0042] FIG. **14** depicts CDX2 expression levels in MM.1R, Eol-1, Mino, HEL92.1.7, CA46, RL, THP1, and Toledo cell lines determined as copy numbers using the standard curves.

[0043] FIG. **15** depicts KLF4 expression levels in MM.1R, Eol-1, Mino, HEL92.1.7, CA46, RL, THP1, and Toledo cell lines determined as copy numbers using the standard curves.

[0044] FIG. **16** depicts correlation of CDX2 mRNA copy number or KLF4 mRNA copy number to IC50 of LOR-253 in MM.1R, Eol-1, Mino, HEL92.1.7, CA46, RL, THP1, and Toledo cell lines resulted from one study.

[0045] FIG. **17** depicts correlation of CDX2/KLF4 or KLF4/CDX2 mRNA ratio to IC50 of LOR-253 in MM.1R, Eol-1, Mino, HEL92.1.7, CA46, RL, THP1, and Toledo cell lines resulted from one study.

[0046] FIG. **18** is a diagram that shows non-limiting mechanism of how silencing KLF4 gene or gene product plays a central role in various heme malignancies. For example, epigenetic methylation of the KLF4 gene relates to adult T-cell lymphoma patients, mutations in KLF4 genes or proteins relate to pediatric T-cell ALL patients, elevated microRNA-2909 relates to pediatric B-cell ALL patients, aberrant expression of CDX2 relates to AML, ALL, and myelodysplastic syndrome (MDS) patients, which all lead to silencing of KLF4 activity (including but not limited to expressional and functional activities). Silencing of KLF4 has also been observed in various lymphomas. The lower part of the diagram illustrates that silenced KLF4 causes increased cancer cell proliferation through various "cell fate genes".

[0047] FIG. **19** is a diagram that shows non-limiting mechanism of how KLF4 gene activity can be silenced by genetic mutations or epigenetic events and how LOR-253/APTO-253 can induce KLF4 expression. Such epigenetic events include, but are not limited to, DNA hypomethylation

or demethylation, aberrant/elevated expression of CDX2 which can lead to increased presence of a demethylase KDM5B in an upstream regulatory region of the klf4 gene, and elevated amount of miR-2909. LOR-253/APTO-253 can induce KLF4 expression by relieving gene silencing caused at least by CDX2/KDM5B and/or other mechanisms

[0048] FIG. **20** depicts in vivo efficacy of LOR-253 HCL in Kasumi-1 xenograft model mice. Tumor sizes of Kasumi-1 xenograft mice treated with LOR-253 HCL or negative control measured on the indicated days are shown.

[0049] FIG. **21** depicts body weight measurements on the indicated days of Kasumi-1 tumor-bearing Mice treated with LOR-253 HCL or negative control.

[0050] FIG. **22** depicts in vivo efficacy of LOR-253 HCL as a single agent or in combination with azacitidine in HL-60 xenograft model mice. Tumor sizes of HL-60 xenograft mice treated with the indicated conditions measured on the indicated days are shown.

[0051] FIG. **23** and FIG. **24** depict the tumor sizes of individual animals at the beginning (Day 1) and end (Day 19) of the study of FIG. **17**, respectively.

[0052] FIG. **25** depicts in vivo efficacy of LOR-253 HCL in KG-1 xenograft model mice. Tumor sizes of KG-1 xenograft mice treated with LOR-253 HCL or negative control measured on the indicated days are shown.

[0053] FIG. **26** depicts in vivo efficacy of LOR-253 HCL as a single agent or in combination with azacitidine in THP-1 xenograft model mice. Tumor sizes of THP-1 xenograft mice treated with the indicated conditions measured on the indicated days are shown.

[0054] FIG. **27** depicts correlation of CDX2 mRNA copy number or KLF4 mRNA copy number to IC50 of LOR-253/APTO-253 in MM.1R, Eol-1, Mino, HEL92.1.7, CA46, RL, THP1, Toledo, U266B1, MV411, DB, HL60, CD47 LOW (or MOLM13 CD47−), CD47 HIGH (or MOLM13 CD47+), KG-1, and SKM-1 cell lines resulted from another study.

[0055] FIG. **28** depicts correlation of CDX2/KLF4 or KLF4/CDX2 mRNA ratio to IC50 of LOR-253/APTO-253 in MM.1R, Eol-1, Mino, THP1, Toledo, U266B1, MV411, DB, HL60, CD47 LOW (or MOLM13 CD47−), CD47 HIGH (or MOLM13 CD47+), KG-1, and SKM-1 cell lines resulted from another study.

DETAILED DESCRIPTION

Definitions

[0056] The verb "comprise" as is used in this description and in the claims and its conjugations are used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded.

[0057] The term "a" or "an" refers to one or more of that entity; for example, "a gene" refers to one or more genes or at least one gene. As such, the terms "a" (or "an"), "one or more" and "at least one" are used interchangeably herein. In addition, reference to "an element" by the indefinite article "a" or "an" does not exclude the possibility that more than one of the elements are present, unless the context clearly requires that there is one and only one of the elements.

[0058] The invention provides polynucleotides that can be used for biomarker assays. In some embodiments the polynucleotides are isolated, purified, chimeric, recombinant or synthetic. As used herein, the terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", "nucleic acid fragment", and "isolated nucleic acid fragment" are used interchangeably herein and encompass DNA, RNA, cDNA, whether single stranded or double stranded, as well as chemical modifications thereof. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. Nucleotides (usually found in their 5'-monophosphate form) are referred to by a single letter designation as follows: "A" for adenylate or deoxyadenylate (for RNA or DNA, respectively), "C" for cytidylate or deoxycytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deoxythymidylate, "R" for purines (A or G), "Y" for pyrimidines (C or T), "K" for G or T, "H" for A or C or T, "I" for inosine, and "N" for any nucleotide. In some embodiments, the sequences of the polynucleotides are derived from gene markers of the present invention.

[0059] The invention also provides proteins or polypeptides that can be used for biomarker assays. In some embodiments the proteins or polypeptides are isolated, purified, chimeric, recombinant or synthetic. As used herein, the term "polypeptide" or "protein" refers to amino acid polymers of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labelling component. Also included are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, eic), as well as other modifications known in the art. Polypeptides can occur as single chains or associated chains. Polypeptides of the invention can take various forms (e.g. native, fusions, glycosylated, non-glycosylated, lipidated, non-lipidated, phosphorylated, non-phosphorylated, myristoylated, non-myristoylated, monomeric, multimeric, particulate, denatured, etc). In some embodiments, the sequences of the proteins or polypeptides are derived from gene markers of the present invention.

[0060] Single letter amino acid abbreviations used herein have their standard meaning in the art, and all peptide sequences described herein are written according to convention, with the N-terminal end to the left and the C-terminal end to the right.

[0061] The invention provides probes and primers that are derived from the nucleic acid sequences of the biomarker genes. The term "probe" as used herein refers to an oligonucleotide which is capable of specific annealing to the amplification target. The term "primer" as used herein refers to an oligonucleotide which is capable of annealing to the amplification target allowing a DNA polymerase to attach, thereby serving as a point of initiation of DNA synthesis when placed under conditions in which synthesis of primer extension product is induced, i.e., in the presence of nucleotides and an agent for polymerization such as DNA polymerase and at a suitable temperature and pH. The (amplification) primer is preferably single stranded for maximum efficiency in amplification. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of

the agent for polymerization. The exact lengths of the primers will depend on many factors, including temperature and composition (A/T vs. G/C content) of primer. A pair of bi-directional primers consists of one forward and one reverse primer as commonly used in the art of DNA amplification such as in PCR amplification.

[0062] The terms "array" or "matrix" refer to an arrangement of addressable locations or "addresses" on a device. The locations can be arranged in two-dimensional arrays, three-dimensional arrays, or other matrix formats. The number of locations may range from several to at least hundreds of thousands. Most importantly, each location represents a totally independent reaction site. A "nucleic acid array" refers to an array containing nucleic acid probes, such as oligonucleotides or larger portions of genes. The nucleic acid on the array is preferably single-stranded. Arrays wherein the probes are oligonucleotides are referred to as "oligonucleotide arrays" or "oligonucleotide chips." A "microarray," also referred to herein as a "biochip" or "biological chip," is an array of regions having a density of discrete regions of at least about 100/cm^2, and preferably at least about 1000/cm^2. The regions in a microarray have typical dimensions, for example, diameters, in the range of between about 10-250 μm, and are separated from other regions in the array by about the same distance. None limiting examples of compositions and methods for making and using arrays are described in U.S. Pat. Nos. 5,202,231, 5,695,940, 5,525,464, 5,445,934, 5,744,305, 5,677,195, 5,800,992, 5,871,928, 5,795,716, 5,700,637, 6,054,270, 5,807,522, and 6,110,426, each of which is incorporated by reference herein in its entirety for all purposes. As used herein, when the level of a biomarker goes toward the level of a predetermined standard level, it is called normalization

[0063] As used herein, the term "marker" or "biomarker" encompasses a broad range of intra- and extra-cellular events as well as whole-organism physiological changes. A marker may represent essentially any aspect of cell function, for example, but not limited to, levels or rate of production of signaling molecules, transcription factors, metabolites, gene transcripts as well as post-translational modifications of proteins. Marker may include partial and/or whole genome analysis of transcript levels, rates, and/or stability, and partial and/or whole proteome analysis of protein levels, activity and/or modifications. A signature may refer to a gene or gene product which is up- or down-regulated in a subject to be treated compared to clinically normal subjects. A signature may also refer to a gene or gene product which is up- or down-regulated in a treated subject having the disease compared to an untreated subject. That is, the gene or gene product is sufficiently specific to the treated cell that it may be used, optionally with other genes or gene products, to identify, predict, or detect efficacy of a small molecule. Thus, in some embodiments, a signature is a gene or gene product that is characteristic of efficacy of a compound in a diseased cell or the response of that diseased cell to treatment by the compound.

[0064] As used herein, the term "sample" or "biological sample" refers to a sample obtained from an organism or from components (e.g., cells) of an organism. The sample may be of any biological tissue or fluid. The sample may be a sample which is derived from a patient. Such samples include, but are not limited to, sputum, blood, blood cells (e.g., white blood cells), tissue or biopsy samples (e.g., tumor biopsy), urine, peritoneal fluid, and pleural fluid, patient derived xenografts (PDXs), or cells therefrom. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes. Methods for obtaining biological samples are well known in the art and any standard methods for obtaining biological samples can be employed. Biological samples that find use with the methods of the present invention include but are not limited to blood (including but not limited to serum, blood, plasma, whole blood and derivatives thereof), skin, hair, hair follicles, saliva, oral mucous, vaginal mucous, sweat, tears, epithelial tissues, urine, semen, seminal fluid, seminal plasma, prostatic fluid, pre-ejaculatory fluid (Cowper's fluid), excreta, biopsy, ascites, cerebrospinal fluid, lymph, and tissue extract sample or biopsy samples. (See, e.g., *Clinical Proteomics: Methods and Protocols*, Vol. 428 in Methods in Molecular Biology, Ed. Antonia Vlahou (2008).) In some embodiments, the biological sample is selected from blood (including but not limited to serum, blood, plasma, whole blood and derivatives thereof), skin, hair, hair follicles, saliva, oral mucous, vaginal mucous, sweat, tears, epithelial tissues, urine, semen, seminal fluid, seminal plasma, prostatic fluid, pre-ejaculatory fluid (Cowper's fluid), excreta, biopsy, ascites, cerebrospinal fluid, lymph, and tissue extract sample or biopsy sample. In some embodiments, the biological sample is a blood sample.

[0065] In some embodiments, a collection of activity profiles of a panel of biomarkers is provided. As used herein, the term "activity profile" refers to a set of data representing distinctive features or characteristics of one or more biomarkers. Such features or characteristics include, but are not limited to, transcript abundance, transcript stability, transcription rate, translation rate, post-translation modification, protein abundance, protein stability, and/or protein enzymatic activity, etc. In some embodiments, the activity profile comprises data related to gene expression level of each biomarker. In some embodiments, the collection comprising activity profiles is obtained from a specific population of subjects. In some embodiments, the specific population of subjects consists of clinically normal subjects. In some embodiments, the population consists of patents responsive to one or more anti-cancer agents of the present invention. In some embodiments, the population consists of patients not responsive to one or more anti-cancer agents of the present invention.

[0066] In some embodiments, the collection comprises activity profiles that are statistically homogeneous in one or more aspects, e.g., statistically homogeneous in one or more quantitative or semi-quantitative parameters describing the features and characteristics of the activity profiles. In some embodiments, the quantitative parameters include, but are not limited to, transcript abundance, transcript stability, transcription rate, translation rate, post-translation modification, protein abundance, protein stability, and/or protein enzymatic activity, etc. Whether a group of activity profiles are statistically homogeneous or not in one or more aspects can be determined by any suitable statistic test and/or algorithm known to one skilled in the art.

[0067] In some embodiments, one or more of the biomarkers increase its activity in response to the treatment. In some embodiments, one or more of the biomarkers decrease its activity in response to the treatment. In some embodiments, one or more of the biomarkers remains its activity in response to the treatment. As used herein, the activity of a biomarker can be a parameter at genomic DNA level, transcriptional level, post-transcriptional level, translational level, post-translational level, including, but not limited to gene activity,

RNA activity, and protein activity. The gene activity can be gene copy number, gene amplification number, or promoter activity, etc. RNA activity can be mRNA abundance, synthesis rate, and/or stability, etc. Protein activity can be protein abundance, synthesis rate, stability, enzymatic activity, phosphorylation rate, modifications, binding activity, etc.

[0068] As used herein, when the level of a biomarker goes toward the level of a predetermined standard level, it is called normalization.

[0069] As used herein, when the level of a biomarker reduces its speed of going away from the level of a predetermined standard level, it is called stabilization.

[0070] The term "CDX2-KLF4 signaling pathway" as used herein refers to a group of biological molecules that work together to control one or more cellular functions through CDX2 and/or KLF4, or by affecting the expression or activity of CDX2 or KLF4 as described herein, directly or indirectly. Sometimes the expression level and/or activity of CDX2 and/or KLF4 is also referred to as "CDX2-KLF4 axis".

[0071] As used herein, the term "component in the CDX2-KLF4 signaling pathway", referrers to CDX2, KLF4, other genes, gene products (including but not limited to RNA and protein), or other biological molecules that can modulate the activity of CDX2 and/or KLF4, directly or indirectly, or genes, gene products, or other biological molecules that can be modulated by CDX2 and/or KLF4, directly or indirectly. The modulation can either increase or decrease the activity level of a given gene. Such components include, but are not limited to those described in Lengerke et al. ("Caudal genes in blood development and leukemia", Ann NY Acad. Sci. 2012 August; 1266:47-54.), Scholl et al. ("The homeobox gene CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis", *J. Clin. Invest.* 117: 1037-1048 (2007).), Yoon et al. ("Krüppel-like Factor 4 Mediates p53-dependent G1/S Cell Cycle Arrest in Response to DNA Damage", J Biol Chem Vol. 278, No. 4, January 24, pp. 2101-2105, 2003), Faber et al. ("CDX2-driven leukemogenesis involves KLF4 repression and deregulated PPARγ signaling", J Clin Invest. 2013 Jan. 2; 123(1):299-314.), Rouhi et al. ("Deregulation of the CDX2-KLF4 axis in acute myeloid leukemia and colon cancer", Oncotarget. 2013 February; 4(2):174-175.), Lengerke et al. ("BMP and Wnt specify hematopoietic fate by activation of the Cdx-Hox pathway", Cell Stem Cell 2008 Jan. 10; 2(1):72-82.), Saandi et al. ("Regulation of the tumor suppressor homeogene Cdx2 by HNF4α in intestinal cancer", Oncogene. 2013 Aug. 8; 32(32):3782-8.), Malik et al., (miR-2909-mediated regulation of KLF4: a novel molecular mechanism for differentiating between B-cell and T-cell pediatric acute lymphoblastic leukemias. Mol Cancer. 13:175, 2014), and Rowland et al. ("KLF4, p21 and context-dependent opposing forces in cancer", Nat Rev Cancer. 2006 January; 6(1):11-23), each of which is incorporated herein by reference in its entirety for all purposes. In some embodiments, such components include, but are not limited to CDX2, KLF4, KDM5B, miR-2909, p53, p21, Caspase-3, Annexin V, BAX, BCL2, BCL3, BMP, Wnt, HNF4α, Fgf, SP1, MYC, CCND1, AATF, and Hox genes. KLF4 negatively regulates (or suppresses) the activity of SP1, MYC, BCL3, CCND1, and AATF, directly or indirectly, while positively regulates the activity of p21. In addition, KLF4 negatively regulates the activity of p53 in some cancer types (e.g. breast cancer, as described by Rowland et al., The KLF4 tumour suppressor is a transcriptional repressor of p53 that acts as a context-dependent oncogene. Nat Cell Biol. 2005. 7:1074-82), but positively regulates the activity of p53 in some other cancer types (e.g., colon cancer and multiple myeloma, as described by Ghaleb et al. (Krüppel-like factor 4 exhibits antiapoptotic activity following gamma-radiation-induced DNA damage. Oncogene. 2007. 26:2365-73), and Schoenhals et al. (Krüppel-like factor 4 blocks tumor cell proliferation and promotes drug resistance in multiple myeloma. Haematologica. 2013. 98:1442-9)). Many of the genes regulated by KLF4 are referred to as "cell fate genes". An illustration of some possible effects of silenced KLF4 gene expression or activity on some of the genes modulated by KLF4 can be found in FIG. **13**. Some other components in the CDX2-KLF4 signaling pathway can modulate the expression of KLF4. Examples of such components include, but are not limited to, CDX2, KDM5B (a demethylase), and an miRNA "miR-2909". An illustration of some possible mechanisms of how these KLF4 modulators can affect KLF4 gene expression or activity in various cancer types can be found in FIG. **13** and FIG. **14**. A component in the CDX2-KLF4 signaling pathway can be used as a biomarker according to the methods described herein for treating cancers, especially for treating cancers by an anti-cancer agent of the present invention, such as LOR-253/APTO-253.

[0072] As used herein, the term "modulate the CDX2-KLF4 signaling pathway" refers to the process in which one or more component in the CDX2-KLF4 signaling pathway is modulated by an agent or an event (including a mutation). In some embodiments, such modulation leads to increased, decreased, normalized, and/or stabilized activity of one or more components in the CDX2-KLF4 signaling pathway.

[0073] As used herein, the term "baseline level" of the activity of a biomarker refers to the activity level of a given biomarker in a human subject before any treatment.

[0074] The term lower alkyl refers to $(C_1\text{-}C_6)$alkyl. A lower alkyl includes methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, pentyl, 3-pentyl, hexyl, $(C_3\text{-}C_6)$cycloalkyl (e.g., cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl), $(C_3\text{-}C_6)$cycloalkyl$(C_1\text{-}C_6)$alkyl (e.g., cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl, 2-cyclopropylethyl, 2-cyclobutylethyl, 2-cyclopentylethyl, or 2-cyclohexylethyl), $(C_1\text{-}C_6)$alkoxy (e.g., methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, pentoxy, 3-pentoxy, or hexyloxy) $(C_2\text{-}C_6)$alkenyl (e.g., vinyl, allyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1,-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, or 5-hexenyl), $(C_2\text{-}C_6)$ alkynyl (e.g., ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 3-butynyl, 1-pentynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl), $(C_1\text{-}C_6)$alkanoyl (e.g., acetyl, propanoyl or butanoyl), halo$(C_1\text{-}C_6)$alkyl (e.g., iodomethyl, bromomethyl, chloromethyl, fluoromethyl, trifluoromethyl, 2-chloroethyl, 2-fluoroethyl, 2,2,2-trifluoroethyl, or pentafluoroethyl), hydroxy$(C_1\text{-}C_6)$alkyl (e.g., hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropyl, 2-hydroxypropyl, 3-hydroxypropyl, 1-hydroxy butyl, 4-hydroxybutyl, 1-hydroxypentyl, 5-hydroxypentyl, 1-hydroxyhexyl, or 6-hydroxyhexyl), $(C_1\text{-}C_6)$alkoxycarbonyl (e.g., methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, or hexyloxycarbonyl), $(C_1\text{-}C_6)$alkylthio (e.g., methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, pentylthio, or hexylthio),

and/or (C_2-C_6)alkanoyloxy (e.g., acetoxy, propanoyloxy, butanoyloxy, isobutanoyloxy, pentanoyloxy, or hexanoyloxy).

[0075] A compound described herein or its function derivative can be used according to the present invention. The term "derivative" as used herein includes derivatives, analogs, prodrugs, and unnatural precursors of a given compound.

[0076] The biomarkers of the present invention can be used to determine treatment efficacy of the anti-cancer agents of the present invention for a human subject. As used herein, the term "treatment efficacy" and variants thereof are generally indicated by alleviation of one or more signs or symptoms associated with the disease and can be readily determined by one skilled in the art. "Treatment efficacy" may also refer to the prevention or amelioration of signs and symptoms of toxicities typically associated with standard or non-standard treatments of a disease. Determination of treatment efficacy is usually indication and disease specific and can include any methods known or available in the art for determining that a treatment is providing a beneficial effect to a subject. For example, evidence of treatment efficacy can include but is not limited to general improvements in the overall health of the subject, such as but not limited to enhancement of patient life quality, increase in predicted subject survival rate, decrease in depression, decreasing the severity and/or frequency one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (e.g., preventing or delaying the worsening of the disease), delay or slowing the progression of the disease, ameliorating the disease state, etc. In some embodiments of the invention, the treatment efficacy is clinical efficacy or statistically significant.

[0077] The terms "treating" and "treatment" as used herein refer to an approach for obtaining beneficial or desired results including clinical results, and may include even minimal changes or improvements in one or more measurable markers of the disease or condition being treated. A treatment is usually effective to reduce at least one symptom of a condition, disease, disorder, injury or damage. Exemplary markers of clinical improvement will be apparent to persons skilled in the art. Examples include, but are not limited to, one or more of the following: decreasing the severity and/or frequency one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (e.g., preventing or delaying the worsening of the disease), delay or slowing the progression of the disease, ameliorating the disease state, decreasing the dose of one or more other medications required to treat the disease, and/or increasing the quality of life, etc.

[0078] "Prophylaxis," "prophylactic treatment," or "preventive treatment" refers to preventing or reducing the occurrence or severity of one or more symptoms and/or their underlying cause, for example, prevention of a disease or condition in a subject susceptible to developing a disease or condition (e.g., at a higher risk, as a result of genetic predisposition, environmental factors, predisposing diseases or disorders, or the like).

[0079] The term "disorder" or "disease" used interchangeably herein, refers to any alteration in the state of the body or one of its organs and/or tissues, interrupting or disturbing the performance of organ function and/or tissue function (e.g., causes organ dysfunction) and/or causing a symptom such as discomfort, dysfunction, distress, or even death to a subject afflicted with the disease.

[0080] By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be incorporated into a pharmaceutical composition administered to a patient without causing any significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. When the term "pharmaceutically acceptable" is used to refer to a pharmaceutical carrier or excipient, it is implied that the carrier or excipient has met the required standards of toxicological and manufacturing testing or that it is included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug administration.

[0081] The term "effective amount" refers to the amount of one or more compounds that renders a desired treatment outcome. An effective amount may be comprised within one or more doses, i.e., a single dose or multiple doses may be required to achieve the desired treatment endpoint.

[0082] The term "therapeutically effective amount" as used herein, refers to the level or amount of one or more agents needed to treat a condition, or reduce or prevent injury or damage, optionally without causing significant negative or adverse side effects.

[0083] A "prophylactically effective amount" refers to an amount of an agent sufficient to prevent or reduce severity of a future disease or condition when administered to a subject who is susceptible and/or who may develop a disease or condition.

[0084] According to the methods of the present invention, the term "subject," and variants thereof as used herein, includes any subject that has, is suspected of having, or is at risk for having a disease or condition. Suitable subjects (or patients) include mammals, such as laboratory animals (e.g., mouse, rat, rabbit, guinea pig), farm animals, and domestic animals or pets (e.g., cat, dog). Non-human primates and, preferably, human patients, are included. A subject "at risk" may or may not have detectable disease, and may or may not have displayed detectable disease prior to the diagnostic or treatment methods described herein. "At risk" denotes that a subject has one or more so-called risk factors, which are measurable parameters that correlate with development of a condition described herein, which are described herein. A subject having one or more of these risk factors has a higher probability of developing a condition described herein than a subject without these risk factor(s). One example of such a risk factor is an increase or decrease in a biomarker of the present invention as compared to a clinically normal sample.

[0085] As used herein, the term "predetermined standard level" or "predetermined activity profiles" refers to standardized data or data set representing the average, representative features or characteristics of one or more biomarkers in a specific population. Such features or characteristics include, but are not limited to, gene copy number, gene amplification, transcript abundance, transcript stability, transcription rate, translation rate, post-translation modification, protein abundance, protein stability, and/or protein enzymatic activity, etc. In some embodiments, the specific population of subjects are consisting of about 5, about 10, about 20, about 50, about 100, about 200, about 300, about 400, about 500, about 1000, about 5000, about 10K, or more individual subjects. The predetermined activity profile can be a standardized data or data set collected before, during, or after the specific population of subjects has been all exposed to a drug. In some embodiments, the specific population is consisting of subjects responsive to a given drug.

[0086] As used herein, a subject is "responsive" to a drug for treating when the level of one or more of the biomarkers of the present invention increases or decreases toward a predetermined standard level when the subject is exposed to a the drug, or when the drug modifies the speed of level changes of one or more biomarkers of the present invention compared to a placebo. For methods related to detection, quantitation and comparison of biomarker levels, see, e.g., *Current Protocols in Molecular Biology*, Ed. Ausubel, Frederick M. (2010); *Current Protocols in Protein Science Last*, Ed. Coligan, John E., et al. (2010); *Current Protocols in Nucleic Acid Chemistry*, Ed. Egli, Martin (2010); *Current Protocols in Bioinformatics*, Ed. Baxevanis, Andreas D. (2010); and *Molecular Cloning: A Laboratory Manual*, Third Edition, Sambrook, Joseph (2001), all of which are incorporated herein by reference in their entirety.

[0087] In certain embodiments, when measuring biomarkers or other indicators of treatment, an "increased" or "decreased" amount or level may include a "statistically significant" amount. In some embodiments, the administration of an anti-cancer agent such as LOR-253 provides "statistically significant" therapeutic effect or clinical efficacy for treating the cancer. In some embodiments, such statistically significant therapeutic effect or clinical efficacy includes slower cancer cell proliferation or tumor growth caused by the anti-cancer agent as compared to a control vehicle. A result is typically referred to as statistically significant if it is unlikely to have occurred by chance. The significance level of a test or result relates traditionally to the amount of evidence required to accept that an event is unlikely to have arisen by chance. In certain cases, statistical significance may be defined as the probability of making a decision to reject the null hypothesis when the null hypothesis is actually true (a decision known as a Type I error, or "false positive determination"). This decision is often made using the p-value: if the p-value is less than the significance level, then the null hypothesis is rejected. The smaller the p-value, the more significant the result. Bayes factors may also be utilized to determine statistical significance (see, e.g., Goodman S., *Ann Intern Med.* 130:1005-13, 1999). In some embodiments, an "increased" or "decreased" amount or level is about 1.1×, 1.2×, 1.3×, 1.4×, 1.5×, 2×, 2.5×, 3×, 3.5×, 4×, 4.5×, 5×, 6×, 7×, 8×, 9×, 10×, 15×, 20×, 25×, 30×, 40×, or 50× more or less the amount of a predetermined standard, or the amount of a determined time point relative to a previous or earlier timepoint.

[0088] The phrase "pharmaceutically acceptable salt(s)", as used herein, unless otherwise indicated, includes salts of acidic or basic groups which may be present in a compound. Compounds that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of such basic compounds are those that form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions, such as the acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bistosylate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edislyate, estolate, esylate, ethylsuccinate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, phospate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodode, and valerate salts.

Cancers

[0089] The cancers which can be treated in accordance with one embodiment of the present invention thus include, but are not limited to, leukaemias; adenocarcinomas and carcinomas, including squamous cell carcinomas. Carcinomas are also frequently referred to as "solid tumours," as described above, and examples of commonly occurring solid tumours that can be treated in accordance with the present invention include, but are not limited to, anal cancer, bladder cancer, colon cancer, colorectal cancer, duodenal cancer, gastric (stomach) cancer, lung (non-small cell) cancer, oesophageal cancer, prostate cancer, rectal cancer and small intestine cancer. Accordingly, one embodiment of the present invention provides for the use of a compound of Formula I in the treatment of a cancer selected from the group of leukemia, bladder cancer, lung (non-small cell) cancer, prostate cancer and a cancer of the GI tract, wherein cancers of the GI tract include, but are not limited to, anal cancer, colon cancer, colorectal cancer, duodenal cancer, gastric (stomach) cancer, oesophageal cancer, rectal cancer and small intestine cancer.

[0090] The term "leukaemia" or "leukemia" refers broadly to progressive, malignant diseases of the blood-forming organs. Leukaemia is typically characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow but can also refer to malignant diseases of other blood cells such as erythroleukaemia, which affects immature red blood cells. Leukaemia is generally clinically classified on the basis of (1) the duration and character of the disease—acute or chronic; (2) the type of cell involved—myeloid (myelogenous), lymphoid (lymphogenous) or monocytic, and (3) the increase or non-increase in the number of abnormal cells in the blood—leukaemic or aleukaemic (subleukaemic). Leukaemia includes, for example, acute leukaemia, chronic leukaemia, adult leukaemia, pediatric/child leukaemia, lymphocytic leukemia, myeloid leukemia, acute lymphocytic leukemia (ALL), acute nonlymphocytic leukaemia, chronic lymphocytic leukaemia, acute granulocytic leukaemia, chronic granulocytic leukaemia, acute promyelocytic leukaemia, chronic myelogenous leukemia (CML), T-cell leukaemia, B-cell leukaemia, adult T-cell leukaemia, pediatric T-cell ALL, pediatric B-cell ALL, aleukaemic leukaemia, aleukocythemic leukaemia, basophylic leukaemia, blast cell leukaemia, bovine leukaemia, chronic myelocytic leukaemia, leukaemia cutis, embryonal leukaemia, eosinophilic leukaemia, Gross' leukaemia, hairy-cell leukaemia, hemoblastic leukaemia, hemocytoblastic leukaemia, histiocytic leukaemia, stem cell leukaemia, acute monocytic leukaemia, leukopenic leukaemia, lymphatic leukaemia, lymphoblastic leukaemia, lymphocytic leukaemia, lymphogenous leukaemia, lymphoid leukaemia, lymphosarcoma cell leukaemia, mast cell leukaemia, megakaryocytic leukaemia, micromyeloblastic leukaemia, monocytic leukaemia, myeloblastic leukaemia, myelocytic leukaemia, myeloid granulocytic leukaemia, myelomonocytic leukaemia, Naegeli leukaemia, plasma cell leukaemia, plasmacytic leukaemia, promyelocytic leukaemia, Rieder cell leukaemia, Schilling's leukaemia, stem cell leukaemia, subleukaemic leukaemia, and undifferentiated cell leukaemia.

[0091] The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. The term "carcinoma" also encompasses adenocarcinomas. Adenocarcinomas are carcinomas that originate in cells that make organs which have glandular (secretory) properties or that originate in cells that line hollow viscera, such as the gastrointestinal tract or bronchial epithelia, and include adenocarcinomas of the lung and prostate.

[0092] Methods of the present invention can be applied in the treatment of early stage cancers including early neoplasias that may be small, slow growing, localized and/or non-aggressive, for example, with the intent of curing the disease or causing regression of the cancer, as well as in the treatment of intermediate stage and in the treatment of late stage cancers including advanced and/or metastatic and/or aggressive neoplasias, for example, to slow the progression of the disease, to reduce metastasis or to increase the survival of the patient. Similarly, the combinations may be used in the treatment of low grade cancers, intermediate grade cancers and or high grade cancers.

[0093] Methods of the present invention can also be used in the treatment of indolent cancers, recurrent cancers including locally recurrent, distantly recurrent and/or refractory cancers (i.e. cancers that have not responded to treatment), metastatic cancers, locally advanced cancers and aggressive cancers. Thus, an "advanced" cancer includes locally advanced cancer and metastatic cancer and refers to overt disease in a patient, wherein such overt disease is not amenable to cure by local modalities of treatment, such as surgery or radiotherapy. The term "metastatic cancer" refers to cancer that has spread from one part of the body to another. Advanced cancers may also be unresectable, that is, they have spread to surrounding tissue and cannot be surgically removed.

[0094] Methods of the present invention can also be used in the treatment of drug resistant cancers, including multidrug resistant tumours. As is known in the art, the resistance of cancer cells to chemotherapy is one of the central problems in the management of cancer.

[0095] One skilled in the art will appreciate that many of these categories may overlap, for example, aggressive cancers are typically also metastatic. "Aggressive cancer," as used herein, refers to a rapidly growing cancer. One skilled in the art will appreciate that for some cancers, such as breast cancer or prostate cancer the term "aggressive cancer" will refer to an advanced cancer that has relapsed within approximately the earlier two-thirds of the spectrum of relapse times for a given cancer, whereas for other types of cancer, nearly all cases present rapidly growing cancers which are considered to be aggressive. The term can thus cover a subsection of a certain cancer type or it may encompass all of other cancer types.

[0096] In some embodiments, the cancer is leukemia/lymphoma. In some embodiments, the cancer is acute myelogenous leukemia (AML). In some embodiments, the cancer is lymphoma, gastric cancer, multiple myeloma, myelodysplastic syndromes, or combinations thereof. In some other embodiments, the cancer is T-cell leukemia, e.g., adult T-cell leukemia associated with epigenetic methylation of KLF4 gene. In some other embodiments, the cancer is ALL, e.g., pediatric ALL. In some other embodiments, the cancer is pediatric T-cell ALL associated with one or more mutations in KLF4 gene or protein. In some other embodiments, the cancer is pediatric B-cell ALL associated with elevated miRNA-2902. In some other embodiments, the cancer is AML, ALL or MDS, e.g., high risk MDS, all of which associated with higher than normal CDX2 activity. In some other embodiments, the cancer is Hodgkins, Burkitts, or B-cell lymphomas, all of which associated with methylation of KLF4 gene.

[0097] Acute myeloid leukemia (AML): also known as acute myelogenous leukemia or acute nonlymphocytic leukemia (ANLL), is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. The symptoms of AML are caused by replacement of normal bone marrow with leukemic cells, which causes a drop in red blood cells, platelets, and normal white blood cells. These symptoms include fatigue, shortness of breath, easy bruising and bleeding, and increased risk of infection. Several risk factors and chromosomal abnormalities have been identified, but the specific cause is not clear. As an acute leukemia, AML progresses rapidly and is typically fatal within weeks or months if left untreated. Several risk factors for developing AML include, but are not limited to, preleukemic blood disorders, such as myelodysplastic syndrome or myeloproliferative disease; exposure to anticancer chemotherapy; radiation, such as high amounts of ionizing radiation exposure; and genetic reasons, such as those described in Taylor et al. ("The hereditary basis of human leukemia". In Henderson E S, Lister T A, Greaves M F. Leukemia (6th ed.). Philadelphia: W B Saunders. p. 210. ISBN 0-7216-5381-2), Horwitz et al. ("Anticipation in familial leukemia". Am. J. Hum. Genet. 59 (5): 990-8. PMC 1914843. PMID 8900225), Crittenden ("An interpretation of familial aggregation based on multiple genetic and environmental factors". Ann. N.Y. Acad. Sci. 91 (3): 769-80), and Horwitz ("The genetics of familial leukemia". Leukemia 11 (8): 1347-59). The World Health Organization (WHO) classification of acute myeloid leukemia attempts to be more clinically useful and to produce more meaningful prognostic information than the FAB criteria. Each of the WHO categories contains numerous descriptive subcategories of interest to the hematopathologist and oncologist; however, most of the clinically significant information in the WHO schema is communicated via categorization into one of the subtypes listed below:

Name	Description	ICD-O (International Classification of Diseases for Oncology)
Acute myeloid leukemia with recurrent genetic abnormalities	Includes: AML with translocations between chromosome 8 and 21 [t(8; 21)] (ICD-O 9896/3); RUNX1/RUNX1T1 AML with inversions in chromosome 16 [inv(16)] (ICD-O 9871/3); CBFB/MYH11	Multiple

-continued

Name	Description	ICD-O (International Classification of Diseases for Oncology)
	APL with translocations between chromosome 15 and 17 [t(15; 17)] (ICD-O 9866/3); RARA; PML AML with translocations in chromosomes 9 and 11 [t(9; 11)]; MLLT3-MLL Patients with AML in this category generally have a high rate of remission and a better prognosis compared to other types of AML.	
AML with multilineage dysplasia	This category includes patients who have had a prior myelodysplastic syndrome (MDS) or myeloproliferative disease (MPD) that transforms into AML. This category of AML occurs most often in elderly patients and often has a worse prognosis.	M9895/3
AML and MDS, therapy-related	This category includes patients who have had prior chemotherapy and/or radiation and subsequently develop AML or MDS. These leukemias may be characterized by specific chromosomal abnormalities, and often carry a worse prognosis.	M9920/3
AML not otherwise categorized	Includes subtypes of AML that do not fall into the above categories.	M9861/3

[0098] The French-American-British (FAB) classification system divides AML into eight subtypes, M0 through to M7, based on the type of cell from which the leukemia developed and its degree of maturity. Although the WHO classification (see above) may be more useful, the FAB system is still widely used.

Type	Name	Cytogenetics	Percentage of adult AML patients
M0	acute myeloblastic leukemia, minimally differentiated		5%
M1	acute myeloblastic leukemia, without maturation		15%
M2	acute myeloblastic leukemia, with granulocytic maturation	t(8; 21)(q22; q22), t(6; 9)	25%
M3	promyelocytic, or acute promyelocytic leukemia (APL)	t(15; 17)	10%
M4	acute myelomonocytic leukemia	inv(16)(p13q22), del(16q)	20%
M4eo	myelomonocytic together with bone marrow eosinophilia	inv(16), t(16; 16)	5%
M5	acute monoblastic leukemia (M5a) or acute monocytic leukemia (M5b)	del (11q), t(9; 11), t(11; 19)	10%
M6	acute erythroid leukemias, including erythroleukemia (M6a) and very rare pure erythroid leukemia (M6b)		5%
M7	acute megakaryoblastic leukemia	t(1; 22)	5%

[0099] Previous methods for treating AML are described in Bishop J ("The treatment of adult acute myeloid leukemia". Semin Oncol 24 (1): 57-69. 1997), Weick et al. ("A randomized investigation of high-dose versus standard-dose cytosine arabinoside with daunorubicin in patients with previously untreated acute myeloid leukemia: a Southwest Oncology Group study" (PDF). Blood 88 (8): 2841-51, 1996), Bishop et al. ("A randomized study of high-dose cytarabine in induction in acute myeloid leukemia" Blood 87 (5): 1710-7, 1996), Huang et al. ("Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia". Blood 72 (2): 567-72, 1988), Tallman et al. ("All-trans-retinoic acid in acute promyelocytic leukemia". N. Engl. J. Med. 337 (15): 1021-8, 1997), Fenaux et al. ("A randomized comparison of all transretinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. The European APL Group". Blood 94 (4): 1192-200, 1999), Estey E ("Treatment of acute myelogenous leukemia". Oncology (Williston Park) 16 (3): 343-52, 355-6; discussion 357, 362, 365-6, 2002), and Cassileth et al. ("Maintenance chemotherapy prolongs remission duration in adult acute nonlymphocytic leukemia". J Clin Oncol 6 (4): 583-7, 1988).

[0100] Acute lymphocytic leukemia (ALL): also known as acute lymphoblastic leukemia, is an acute leukemia that is characterized by dysregulated proliferation and accumulation of leukemic lymphocytes/lymphoblasts (immature white blood cells such as early B- and T-lymphocyte progenitors) in the bone marrow and various extramedullary sites. ALL is the most common type of cancer in children, and it is a relatively uncommon cancer in adults. Risk factors for developing ALL include, but are not limited to, genetic disorders/mutations and various epigenetic modifications such as those described by Iacobucci et al. ("Cytogenetic and molecular predictors of outcome in acute lymphocytic leukemia: recent developments", Curr Hematol Malig Rep. 2012 June; 7(2):133-43.) and Florean et al. ("Epigenomics of leukemia: from mechanisms to therapeutic applications". Epigenomics. 2011 October; 3(5):581-609).

[0101] Chronic myelogenous leukemia (CML): also known as chronic myeloid leukemia, is a chronic leukemia

that is characterized by dysregulated/increased proliferation of various blood cells, predominantly myeloid cells in peripheral blood, and their precursors in the bone marrow, resulting in their accumulation in the blood. It is less frequent than chronic lymphocytic leukemia (CLL) in adults in the Western world, and the median age of CML onset is 50-60 years. Risk factors for developing CML also include, but are not limited to, genetic disorders/mutations and various epigenetic modifications such as those described by Florean et al. The disease course is triphasic, starting with an early phase, also known as chronic phase (CP) disease. Then, leukemia stem cells can acquire additional genetic defects.

[0102] Adult T-cell leukaemia (ATLL): also known as adult T-cell lymphoma, is an uncommon lymphoproliferative disorder of mature CD4+ T cells that is caused by the retrovirus human T-lymphotrophic virus type 1 (HTLV-1) as reviewed by Qayyum et al. ("Adult T-cell leukemia/lymphoma". Arch Pathol Lab Med. 2014 February; 138(2):282-6). Presently, about 20 million people worldwide are HTLV-1 carriers, with most infected individuals residing in endemic areas such as southern Japan, Africa, the Caribbean basin, and Latin America. The lifelong viral carrier state and long latency (20-40 years) are common after HTLV-1 infection, therefore this type of leukemia/lymphoma is almost exclusively found in adults and is extremely rare in children. The lifetime risk of progression to ATLL in an HTLV-1-positive patient is 2.1% for women and 6.6% for men. The mean age of onset is 60 years old (range, 20-80 years old). The overwhelming majority of ATLL cases occur in patients infected during the early years of life, presumably because of a less efficient immune response in this age group. In addition, the prolonged infection may increase chances of accruing subsequent mutations, and ultimately malignant transformation. Major paths of viral transmission are breast feeding, blood exposure, and unprotected sex. The World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues in 2008 subclassified ATLL into 4 distinct variants according to the Shimoyama classification: acute (60%), lymphomatous (20%), chronic (15%), and smoldering (5%). There are no absolutely necessary features for each variant, and overlap is seen. The acute variant manifests as marked leukocytosis with atypical lymphocytes and eosinophilia. Symptoms include hypercalcemia with or without osteolytic lesions, renal dysfunction and neuropsychiatric disturbances, elevated lactate dehydrogenase level, central nervous ring-enhancing lesions, and secondary respiratory complications. The lymphomatous variant is an aggressive advanced disease resembling acute-onset subtype, and marked lymphadenopathy without leukemia is a prominent feature of this variant. The chronic variant typically presents with skin rash, leukocytosis with absolute lymphocytosis, mild lymphadenopathy, and hypercalcemia. The smoldering variant is asymptomatic and is characterized by normal white blood cell count with less than 5% circulating atypical lymphoid cells and without associated hypercalcemia or organomegaly, although skin and pulmonary involvement often occur. Progression from the smoldering variant to the acute variant can occur.

[0103] Lymphoma: Lymphoma is a type of blood cancer that occurs when B or T lymphocytes, the white blood cells that form a part of the immune system and help protect the body from infection and disease, divide faster than normal cells or live longer than they are supposed to. Typically, lymphoma presents as a solid tumor of lymphoid cells. The current WHO classification, published in 2001 and updated in 2008, is the latest classification of lymphoma and is based upon the foundations laid within the "Revised European-American Lymphoma classification" (REAL):

[0104] A. Mature B-cell neoplasms:

- **[0105]** Chronic lymphocytic leukemia/Small lymphocytic lymphoma
- **[0106]** B-cell prolymphocytic leukemia
- **[0107]** Lymphoplasmacytic lymphoma (such as Waldenström macroglobulinemia)
- **[0108]** Splenic marginal zone lymphoma
- **[0109]** Plasma cell neoplasms:
  - **[0110]** Plasma cell myeloma
  - **[0111]** Plasmacytoma
  - **[0112]** Monoclonal immunoglobulin deposition diseases
  - **[0113]** Heavy chain diseases
- **[0114]** Extranodal marginal zone B cell lymphoma, also called MALT lymphoma
- **[0115]** Nodal marginal zone B cell lymphoma (NMZL)
- **[0116]** Follicular lymphoma
- **[0117]** Mantle cell lymphoma
- **[0118]** Diffuse large B cell lymphoma
- **[0119]** Mediastinal (thymic) large B cell lymphoma
- **[0120]** Intravascular large B cell lymphoma
- **[0121]** Primary effusion lymphoma
- **[0122]** Burkitt lymphoma/leukemia

[0123] B. Mature T cell and natural killer (NK) cell neoplasms

- **[0124]** T cell prolymphocytic leukemia
- **[0125]** T cell large granular lymphocytic leukemia
- **[0126]** Aggressive NK cell leukemia
- **[0127]** Adult T cell leukemia/lymphoma
- **[0128]** Extranodal NK/T cell lymphoma, nasal type
- **[0129]** Enteropathy-type T cell lymphoma
- **[0130]** Hepatosplenic T cell lymphoma
- **[0131]** Blastic NK cell lymphoma
- **[0132]** Mycosis fungoides/Sezary syndrome
- **[0133]** Primary cutaneous CD30-positive T cell lymphoproliferative disorders
  - **[0134]** Primary cutaneous anaplastic large cell lymphoma
  - **[0135]** Lymphomatoid papulosis
- **[0136]** Angioimmunoblastic T cell lymphoma
- **[0137]** Peripheral T cell lymphoma, unspecified
- **[0138]** Anaplastic large cell lymphoma

[0139] C. Hodgkin Lymphoma

- **[0140]** Classical Hodgkin lymphomas:
  - **[0141]** Nodular sclerosis
  - **[0142]** Mixed cellularity
  - **[0143]** Lymphocyte-rich
  - **[0144]** Lymphocyte depleted or not depleted
- **[0145]** Nodular lymphocyte-predominant Hodgkin lymphoma

[0146] D. Immunodeficiency-associated lymphoproliferative disorders

- **[0147]** Associated with a primary immune disorder
- **[0148]** Associated with the Human Immunodeficiency Virus (HIV)
- **[0149]** Post-transplant
- **[0150]** Associated with methotrexate therapy
- **[0151]** Primary central nervous system lymphoma occurs most often in immuno-compromised patients, in particular those with AIDS, but it can occur in the immunocompetent as well. It has a poor prognosis, particularly in those with AIDS. Treatment can consist of corticosteroids, radiotherapy, and chemotherapy, often with methotrexate.

Subtypes of lymphoma with relative incidence, histopathology, immunophenotype, overall t-year survival are show below (*Robbins basic pathology* (8th ed.). Philadelphia: Saunders/Elsevier. 2007. pp. Table 12-8.):

Lymphoma type	Relative incidence[13]	Histopathology[13]	Immunophenotype	Overall 5-year survival	Other comments
Precursor T-cell leukemia/lymphoma	40% of lymphomas in childhood.	Lymphoblasts with irregular nuclear contours, condensed chromatin, small nucleoli and scant cytoplasm without granules.	TdT, CD2, CD7		It often presents as a mediastinal mass because of involvement of the thymus. It is highly associated with NOTCH1 mutations. Most common in adolescent males.
Follicular lymphoma	40% of lymphomas in adults	Small "cleaved" cells (centrocytes) mixed with large activated cells (centroblasts). Usually nodular ("follicular") growth pattern	CD10, surface Ig	72-77%	Occurs in older adults. Usually involves lymph nodes, bone marrow and spleen. Associated with t(14; 18) translocation overexpressing Bcl-2. Indolent
Diffuse large B cell lymphoma	40 to 50% of lymphomas in adults	Variable. Most resemble B cells of large germinal centers. Diffuse growth pattern.	Variable expression of CD10 and surface Ig	60%	Occurs in all ages, but most commonly in older adults. Often occurs outside lymph nodes. Aggressive.
Mantle cell lymphoma	3 to 4% of lymphomas in adults	Lymphocytes of small to intermediate size growing in diffuse pattern	CD5	50% to 70%	Occurs mainly in adult males. Usually involves lymph nodes, bone marrow, spleen and GI tract. Associated with t(11; 14) translocation overexpressing cyclin D1. Moderately aggressive.
B-cell chronic lymphocytic leukemia/lymphoma	3 to 4% of lymphomas in adults	Small resting lymphocytes mixed with variable number of large activated cells. Lymph nodes are diffusely effaced	CD5, surface immunoglobulin	50%	Occurs in older adults. Usually involves lymph nodes, bone marrow and spleen. Most patients have peripheral blood involvement. Indolent.
MALT lymphoma	~5% of lymphomas in adults	Variable cell size and differentiation. 40% show plasma cell differentiation. Homing of B cells to epithelium creates lymphoepithelial lesions.	CD5, CD10, surface Ig		Frequently occurs outside lymph nodes. Very indolent. May be cured by local excision.
Burkitt's lymphoma	<1% of lymphomas in the United States	Round lymphoid cells of intermediate size with several nucleoli. Starry-sky appearance by diffuse spread with interspersed apoptosis.	CD10, surface Ig	50%	Endemic in Africa, sporadic elsewhere. More common in immunocompromised and in children. Often visceral involvement. Highly aggressive.
Mycosis fungoides	Most common cutaneous lymphoid malignancy	Usually small lymphoid cells with convoluted nuclei that often infiltrate the epidermis, creating Pautrier microabscesseses.	CD4	75%	Localized or more generalized skin symptoms. Generally indolent. In a more aggressive variant, Sezary's disease, there is skin erythema and peripheral blood involvement.

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Lymphoma type	Relative incidence[13]	Histopathology[13]	Immunophenotype	Overall 5-year survival	Other comments
Peripheral T-cell lymphoma-Not-Otherwise-Specified	Most common T cell lymphoma	Variable. Usually a mix small to large lymphoid cells with irregular nuclear contours.	CD3		Probably consists of several rare tumor types. It is often disseminated and generally aggressive.
Nodular sclerosis form of Hodgkin lymphoma	Most common type of Hodgkin's lymphoma	Reed-Sternberg cell variants and inflammation. usually broad sclerotic bands that consists of collagen.	CD15, CD30		Most common in young adults. It often arises in the mediastinum or cervical lymph nodes.
Mixed-cellularity subtype of Hodgkin lymphoma	Second most common form of Hodgkin's lymphoma	Many classic Reed-Sternberg cells and inflammation	CD15, CD30		Most common in men. More likely to be diagnosed at advanced stages than the nodular sclerosis form. Epstein-Barr virus involved in 70% of cases.

[0152] Gastric cancer: a.k.a. stomach cancer, which refers to cancer arising from any part of the stomach. Stomach cancer is often either asymptomatic (producing no noticeable symptoms) or it may cause only nonspecific symptoms (symptoms which are not specific to just stomach cancer, but also to other related or unrelated disorders) in its early stages. It can be diagnosed by gastroscopic exam, upper GI series, or computed tomography or CT scanning. It is previously treated by surgery, chemotherapy, and radiation.

[0153] Colorectal cancer: a.k.a. colon cancer, rectal cancer, bowel cancer or colorectal adenocarcinoma, is a cancer from uncontrolled cell growth in the colon or rectum (parts of the large intestine), or in the appendix. Greater than 75-95% of colon cancer occurs in people with little or no genetic risk. Other risk factors include older age, male gender, high intake of fat, alcohol or red meat, obesity, smoking and a lack of physical exercise. Approximately 10% of cases are linked to insufficient activity. The risk for alcohol appears to increase at greater than one drink per day. Colorectal cancer is a disease originating from the epithelial cells lining the colon or rectum of the gastrointestinal tract, most frequently as a result of mutations in the Wnt signaling pathway that artificially increase signaling activity. The mutations can be inherited or are acquired, and most probably occur in the intestinal crypt stem cell. Genes in the Wnt signaling pathway that are related to colorectal cancer include, but are not limited to, APC, β-catenin, AXIN1, AXIN2, TCF7L2, or NKD1. Beyond the defects in the Wnt-APC-beta-catenin signaling pathway, other mutations must occur for the cell to become cancerous. The p53 protein, produced by the TP53 gene, normally monitors cell division and kills cells if they have Wnt pathway defects. Eventually, a cell line acquires a mutation in the TP53 gene and transforms the tissue from an adenoma into an invasive carcinoma. Other apoptotic proteins commonly deactivated in colorectal cancers are TGF-β and DCC. Other oncogenes overexpressed in colorectal cancer include, genes encoding the proteins KRAS, RAF, and PI3K, which normally stimulate the cell to divide in response to growth factors, can acquire mutations that result in over-activation of cell proliferation. In addition to the oncogenic and inactivating imutations described for the genes above, non-hypermutated samples also contain mutated CTNNB1, FAM123B, SOX9, ATM, and ARID1A. Progressing through a distinct set of genetic events, hypermutated tumors display mutated forms of ACVR2A, TGFBR2, MSH3, MSH6, SLC9A9, TCF7L2, and BRAF. The common theme among these genes, across both tumor types, is their involvement in WNT and TGF-β signaling pathways, which in turn results in increased activity of MYC, a central player in colorectal cancer.

[0154] Multiple myeloma: a.k.a. plasma cell myeloma or Kahler's disease is a cancer of plasma cells, a type of white blood cell normally responsible for producing antibodies. It can be symptomatic myeloma, asymptomatic myeloma and MGUS (monoclonal gammopathy of undetermined significance). Myeloma is diagnosed with blood tests (serum protein electrophoresis, serum free kappa/lambda light chain assay), bone marrow examination, urine protein electrophoresis, and X-rays of commonly involved bones. It is previously treated by steroids, chemotherapy, proteasome inhibitors, immunomodulatory drugs (IMiDs) such as thalidomide or lenalidomide, and stem cell transplants.

[0155] Myelodysplastic syndromes (MDS): Myelodysplastic syndrome ("MDS") refers to a diverse group of hematopoietic stem cell disorders, which are hematological (blood-related) medical conditions with ineffective production (or dysplasia) of the myeloid class of blood cells. MDS is characterized by a cellular marrow with impaired morphology and maturation (dysmyelopoiesis), peripheral blood cytopenias, and a variable risk of progression to acute leukemia, resulting from ineffective blood cell production. *The Merck Manual* 953 (17th ed. 1999) and List et al., 1990, *J. Clin. Oncol.* 8:1424. Some types of MDS, referred to as

"low-risk MDS", progress slowly and may cause mild to moderate anemia, or decrements to other types of cells. Some other types of MDS are called "high-risk MDS" and may cause severe problems. In patients with high-risk MDS, immature cells called blast cells make up more than five percent of the cells in the marrow and do not develop into normal red cells, white cells and platelets, often causing more severe deficiency in those cells/platelets. When MDS patients develop more than 20 percent blast cells, they are reclassified as having AML with trilineage dysplasia (AML-TLD).

[0156] The initial hematopoietic stem cell injury can be from causes such as, but not limited to, cytotoxic chemotherapy, radiation, virus, chemical exposure, and genetic predisposition. A clonal mutation predominates over bone marrow, suppressing healthy stem cells. In the early stages of MDS, the main cause of cytopenias is increased programmed cell death (apoptosis). As the disease progresses and converts into leukemia, gene mutation rarely occurs and a proliferation of leukemic cells overwhelms the healthy marrow. The disease course differs, with some cases behaving as an indolent disease and others behaving aggressively with a very short clinical course that converts into an acute form of leukemia. Patients with MDS can develop severe anemia and require blood transfusions. In some cases, the disease worsens and the patient develops cytopenias (low blood counts) caused by progressive bone marrow failure.

[0157] According to French-American-British classification published in 1976, which was revised in 1982, cases were classified into five categories:

ICD-O	Name	Description
M9980/3	Refractory anemia (RA)	characterized by less than 5% primitive blood cells (myeloblasts) in the bone marrow and pathological abnormalities primarily seen in red cell precursors
M9982/3	Refractory anemia with ring sideroblasts (RARS)	also characterized by less than 5% myeloblasts in the bone marrow, but distinguished by the presence of 15% or greater red cell precursors in the marrow being abnormal iron-stuffed cells called "ringed sideroblasts"
M9983/3	Refractory anemia with excess blasts (RAEB)	characterized by 5-20% myeloblasts in the marrow
M9984/3	Refractory anemia with excess blasts in transformation (RAEB-T)	characterized by 21-30% myeloblasts in the marrow (>30% blasts is defined as acute myeloid leukemia)
M9945/3	Chronic myelomonocytic leukemia (CMML), not to be confused with chronic myelogenous leukemia or CML	characterized by less than 20% myeloblasts in the bone marrow and greater than 1*109/L monocytes (a type of white blood cell) circulating in the peripheral blood.

[0158] The World Health Organization (WHO) modified this classification, introducing several new disease categories and eliminating others. Most recently the WHO has evolved a new classification scheme (2008) which is based more on genetic findings:

Old system	New system
Refractory anemia (RA)	Refractory cytopenia with unilineage dysplasia (Refractory anemia, Refractory neutropenia, and Refractory thrombocytopenia)
Refractory anemia with ringed sideroblasts (RARS)	Refractory anemia with ring sideroblasts (RARS) Refractory anemia with ring sideroblasts - thrombocytosis (RARS-t) (provisional entity) which is in essence a myelodysplastic/myeloproliferative disorder and usually has a JAK2 mutation (janus kinase) - New WHO classification 2008
	Refractory cytopenia with multilineage dysplasia (RCMD) includes the subset Refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS). RCMD includes patients with pathological changes not restricted to red cells (i.e., prominent white cell precursor and platelet precursor (megakaryocyte) dysplasia.
Refractory anemia with excess blasts (RAEB)	Refractory anemia with excess blasts I and II. RAEB was divided into *RAEB-I (5-9% blasts) and RAEB-II (10-19%) blasts, which has a poorer prognosis than RAEB-I. Auer rods may be seen in RAEB-II which may be difficult to distinguish from acute myeloid leukemia.
Refractory anemia with excess blasts in transformation (RAEB-T)	The category of RAEB-T was eliminated; such patients are now considered to have acute leukemia. 5q-syndrome, typically seen in older women with normal or high platelet counts and isolated deletions of the long arm of chromosome 5 in bone marrow cells, was added to the classification.
Chronic myelomonocytic leukemia (CMML)	CMML was removed from the myelodysplastic syndromes and put in a new category of myelodysplastic-myeloproliferative overlap syndromes. 5q-syndrome Myelodysplasia unclassifiable (seen in those cases of megakaryocyte dysplasia with fibrosis and others) Refractory cytopenia of childhood (dysplasia in childhood) - New WHO classification 2008

[0159] Signs and symptoms of MDS include, but are not limited to, anemia (low red blood cell count or reduced hemoglobin), with chronic tiredness, shortness of breath, chilled sensation, sometimes chest pain; neutropenia (low neutrophil count), with increased susceptibility to infection; thrombocytopenia (low platelet count), with increased susceptibility to bleeding and ecchymosis (bruising), as well as subcutaneous hemorrhaging resulting in purpura or petechia; splenomegaly or rarely hepatomegaly; abnormal granules in cells, abnormal nuclear shape and size; and/or chromosomal abnormalities, including chromosomal translocations and abnormal chromosome number.

[0160] Previous treatment methods for MDS include, but are not limited to, bone marrow transplantation, use of hematopoietic growth factors or cytokines to stimulate blood cell development in a recipient, such as erythropoietin (EPO), granulocyte macrophage colony stimulating factor (GM-CSF), and granulocyte colony stimulating factor (G-CSF) (Metcalf, 1985, *Science* 229:16; Dexter, 1987, *J. Cell Sci.* 88:1; Golde and Gasson, 1988, *Scientific American*, July: 62; Tabbara and Robinson, 1991, *Anti-Cancer Res.* 11:81; Ogawa, 1989, *Environ. Health Presp.* 80:199; and Dexter, 1989, *Br. Med. Bull.* 45:337.). Unfortunately, bone transplantation is painful for donor and recipient, and hematopoietic growth factors have not proven effective in many clinical settings.

[0161] In some embodiments, the histology of the cancer is determined before, during or after the treatment. Any suitable test can be used to determine the histology of the cancer. Such test and examination include, but are not limited to, common signs and symptoms of esophaeal cancer, including but not limited to, backwards movement of food through the esophagus and possibly mouth (regurgitation), chest pain not related to eating, difficulty swallowing solids or liquids, heartburn, vomiting blood, hoarseness, chronic cough, hiccups, pneumonia, bone pain, bleeding into the esophagus, and weight loss, medical history and physical exam, imaging tests, chest X-ray, computed tomography (CT) scan, magnetic resonance imaging (MRI) scan, positron emission tomography (PET) scan, bone scan, sputum cytology, needle biopsy, bronchoscopy, endobronchial ultrasound, endoscopic esophageal ultrasound, mediastinoscopy and mediastinotomy, thoracentesis, thoracoscopy, immunohistochemistry, molecular tests, blood tests, barium swallow, endoscopic ultrasound, esophagogastroduodenoscopy (EGD) and biopsy, or any suitable methods derived from thereof.

Biomarkers

[0162] The present invention provides biomarkers associated with cancers, which can be used in many ways to help the cancer treatment. In some embodiments, the biomarkers are those components in the CDX2-KLF4 signaling pathway. In some embodiments, the activity level of one or more components in the CDX2-KLF4 signaling pathway is measured, and compared to a control. In some embodiments, the activity level is measured before, during, or after the treatment by an anti-cancer agent of the present invention. In some embodiments, when the activity level is measured before, during, or after the treatment, the control is the normal activity level of a biomarker in a patient or a group of patients who are responsive to an anti-cancer agent of the present invention. In some embodiments, when the activity level is measured during or after the treatment, the control is activity level of a biomarker in the same patient subjected to the treatment, before the patient receives the treatment.

[0163] CDX2, a.k.a. Caudal Type Homeobox 2, CDX3, Caudal Type HomeoBox Transcription Factor 2, Caudal-Type Homeobox Protein 2, or Homeobox Protein CDX-2, is a member of the caudal-related homeobox transcription factor gene family. The encoded protein is a major regulator of intestine-specific genes involved in cell growth and differentiation. This protein also plays a role in early embryonic development of the intestinal tract. Aberrant expression of this gene is associated with intestinal inflammation and tumorigenesis. Diseases associated with CDX2 include atrophic gastritis, and signet ring cell adenocarcinoma, and among its related super-pathways are Transcription Role of VDR in regulation of genes involved in osteoporosis and Cytoskeleton remodeling Regulation of actin cytoskeleton by Rho GTPases. GO annotations related to this gene include transcription regulatory region sequence-specific DNA binding and sequence-specific DNA binding transcription factor activity. An important paralog of this gene is CDX1. It is involved in the transcriptional regulation of multiple genes expressed in the intestinal epithelium, and important in broad range of functions from early differentiation to maintenance of the intestinal epithelial lining of both the small and large intestine. DNA and protein sequences of human CDX2 were previously reported, see GenBank Nos. NC_000013.10, NC_018924.2, NT_024524.14, NP_001256.3, ENSP00000370408, and Uniprot No. Q99626, each of which is incorporated herein by reference in its entirety for all purposes. Such sequences can be utilized to design procedures for detection and analysis of the level of CDX2 activity by ways known to one skilled in the art. CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis (Scholl et al., The Journal of Clinical Investigation, 17(4): 1037-1048), with an mRNA copy numbers varied between about 27 copies to about 89,000 copies. As used herein, the phrase "CDX2 gene is turned on" or "CDX2 activity is on" refers to that the mRNA copies of CDX2 in a human subject is at least about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 copies. Otherwise, the CDX2 gene is considered as being turned off.

[0164] Krüppel-Like Factor 4 (KLF4), a.k.a. Gut, EZF, GKLF, Epithelial Zinc Finger Protein EZF, Gut-Enriched Krueppel-Like Factor, Endothelial Krüppel-Like Zinc Finger Protein, or Krueppel-Like Factor 4, is associated with diseases including, but are not limited to, leukemia, skin squamous cell carcinoma, and familial adenomatous polyposis. An important paralog of this gene is KLF1. KLF4 can act both as activator and as repressor. It binds the 5'-CACCC-3' core sequence, such as the promoter region of its own gene. It regulates the expression of key transcription factors during embryonic development, and plays an important role in maintaining embryonic stem cells, and in preventing their differentiation. It is required for establishing the barrier function of the skin and for postnatal maturation and maintenance of the ocular surface. It is also involved in the differentiation of epithelial cells and may also function in skeletal and kidney development. It further contributes to the down-regulation of p53/TP53 transcription, and induction of p21. DNA and protein sequences of human KLF4 were previously reported, see GenBank Nos. NC_000009.11, NT_008470.19, NC_018920.2, and Uniprot No. O43474, each of which is incorporated herein by reference in its entirety for all pur-

poses. Such sequences can be utilized to design procedures for detection and analysis of the level of KLF4 activity by ways known to one skilled in the art.

[0165] p21, a.k.a. Cyclin-Dependent Kinase Inhibitor 1A, Cip1, CDJN1, CIP1, WAFT, CAP20, MDA-6, SDI1, CDK-interacting protein 1, CDK-interaction protein 1, cyclin-dependent kinase inhibitor 1, DNA synthesis inhibitor, Melanoma Differentiation Associated Protein, p21CIP, wild type P53-activated fragment, mDA6, or PCI1, encodes a potent cyclin-dependent kinase inhibitor. The encoded protein binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes, and thus functions as a regulator of cell cycle progression at G1. The expression of this gene is tightly controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli. This protein can interact with proliferating cell nuclear antigen (PCNA), a DNA polymerase accessory factor, and plays a regulatory role in S phase DNA replication and DNA damage repair. This protein was reported to be specifically cleaved by CASP3-like spases, which thus leads to a dramatic activation of CDK2, and may be instrumental in the execution of apoptosis following caspase activation. Multiple alternatively spliced variants have been found for this gene. DNA and protein sequences of human p21 were previously reported, see GenBank Nos. NC_000006.11, NT_007592.15, NC_018917.2 and Uniprot No. P38936, each of which is incorporated herein by reference in its entirety for all purposes. Such sequences can be utilized to design procedures for detection and analysis of the level of p21 activity by ways known to one skilled in the art.

[0166] Some other biomarkers provided by the present invention are H3K4 demethylase Jarid1b (KDM5B, a.k.a. Plu-1 or Rbp2-h1), microRNA miR-2909, tumor suppressor p53 (a.k.a TP53 or tumor protein (EC:2.7.1.37)), cysteine-aspartic acid protease Caspase-3, Annexin V, B-cell CLL/Lymphoma 2 (BCL2), B-cell CLL/Lymphoma 3 (BCL3), BCL2-associated X protein (BAX), bone morphogenetic proteins (BMPs), Wnt (a.k.a. murine int-1), hepatocyte nuclear factor 4a (HNF4α), fibroblast growth factors (Fgf), homeobox (Hox), transcription factor SP1, transcription factor MYC, cyclin D1 (CCND1, a.k.a. PRAD1), and apoptosis-antagonizing transcription factor (AATF).

[0167] In some embodiments, the activity level of one or more biomarkers of the present invention is determined, for example, by a method known to skill in the art, including but not limited to those described herein. Additionally, the levels of more than one biomarker can be determined in order to generate a composite of the level of more than one biomarker. In some embodiments, the methods of the present invention comprise determining a composite level of a panel of selected biomarkers. The composite can contain any of the biomarkers described by the present application.

[0168] The biomarkers can be any in vitro or in vivo information for the activity of genes described herein, including, but not limited to gene amplification, gene expression, RNA levels, protein activity, pathway activation or pathway signaling. The information can reflect increased, decreased, or unchanged level of the activity of a selected biomarker when compared to a control group. In one embodiment, biomarkers include any form of mutations at the DNA, RNA, or protein level that are associated with gene activity. In another embodiment, biomarkers include any measurement directly or indirectly associated with activity. Such information is collectively referred as biomarker profile herein.

[0169] In some embodiments the gene copy number of a given biomarker in a human subject is at least 1.5×, 2×, 3×, 4×, 5×, 6×, 7×, 8×, 9×, 10×, 100×, 1000×, 10000× or more of the normal copy number in a control group. In some embodiments, the increase is determined by comparing to one or more standard levels or by comparing to levels known in the art as standard levels.

[0170] The biomarker profile can be determined by any suitable methods known to one skilled in the art. In some embodiments, a biological sample is taken from a subject and analyzed. In some embodiments, the biological sample is then assayed for activity of one or more biomarkers, such as gene amplification number, RNA, mRNA, cDNA, cRNA, protein, etc.

[0171] In some embodiments, mRNA from a biological sample is directly used in determining the level of activity of one or more biomarkers. In some embodiments, the level is determined by hybridization. In some embodiments, the RNA is transformed into cDNA (complementary DNA) copy using methods known in the art. In some particular embodiments, the cDNA is labeled with a fluorescent label or other detectable label. The cDNA is then hybridized to a substrate containing a plurality of probes of interest. A probe of interest typically hybridizes under stringent hybridization conditions to at least one DNA sequence of a gene signature. In certain embodiments, the plurality of probes are capable of hybridizing to the sequences derived from the gene biomarkers under the hybridization conditions. In some embodiments, the conditions comprise using 6×SSC (0.9 M NaCl, 0.09 M sodium citrate, pH 7.4) at 65° C. The probes may comprise nucleic acids. The term "nucleic acid" encompasses known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, peptide-nucleic acids (PNAs). Methods for detecting can include but are not limited to RT-PCR, northern blot analyses, gene expression analyses, microarray analyses, gene expression chip analyses, hybridization techniques (including FISH), expression beadchip arrays, and chromatography as well as any other techniques known in the art. Methods for detecting DNA can include but are not limited to PCR, real-time PCR, digital PCR, hybridization (including FISH), microarray analyses, SNP detection assays, SNP genotyping assays and chromatography as well as any other techniques known in the art

[0172] In some embodiments, the method comprises detecting the protein expression level of a biomarker. The protein expression level can be determined by any suitable methods known to one skilled in the art. Any suitable methods of protein detection, quantization and comparison can be used, such as those described in Tschesche (Methods in Protein Biochemistry, ISBN Walter de Gruyter, 2011, ISBN 3110252368, 9783110252361), Goluch et al. (Chip-based detection of protein cancer markers, ProQuest, 2007, ISBN 0549463453, 9780549463450), Speicher (Proteome Analysis: Interpreting the Genome, Elsevier, 2004, ISBN 0080515304, 9780080515304), Albala et al. (Protein Arrays, Biochips and Proteomics, CRC Press, 2003, ISBN

0203911121, 9780203911129), Walker (The Protein Protocols Handbook, Springer, 2002, ISBN 0896039404, 9780896039407), Fung (Protein Arrays: Methods and Protocols, Springer, 2004, ISBN 1592597599, 9781592597598), and Bienvenut (Acceleration and Improvement of Protein Identification by Mass Spectrometry, Springer, 2005, ISBN 1402033184, 9781402033186), each of which is incorporated by reference in its entirety for all purposes. In some embodiments, the protein expression level of biomarkers are detected and measured by immunohistochemistry (IHC), western blot, protein immunestaining, protein immuneprecipitation, immuneeletrophoresis, immunoblotting, BCA assay, spectrophotometry, mass spectrometry or enzyme assay, or combinations thereof. For additional methods related to detection, quantitation and comparison of biomarker levels, see, e.g., Current Protocols in Molecular Biology, Ed. Ausubel, Frederick M. (2010); Current Protocols in Protein Science Last, Ed. Coligan, John E., et al. (2010); Current Protocols in Nucleic Acid Chemistry, Ed. Egli, Martin (2010); Current Protocols in Bioinformatics, Ed. Baxevanis, Andreas D. (2010); and Molecular Cloning: A Laboratory Manual, Third Edition, Sambrook, Joseph (2001), all of which are incorporated herein by reference in their entirety.

[0173] In some embodiments, antibody to a given biomarker is used. In some embodiments, a kit for detection is used. Such antibodies and kits are available from EMD Millipore, OriGene Custom Assay Services, R&D Systems for biochemical assays, GenScript Custom Assay Services, Enzo Life Sciences for kits & assays, Cloud-Clone Corp. ELISAs, or Cloud-Clone Corp. CLIAs. The term "antibody" as used herein is intended to include monoclonal antibodies, polyclonal antibodies, and chimeric antibodies. The antibody may be from recombinant sources and/or produced in transgenic animals. The term "antibody fragment" as used herein is intended to include Fab, Fab', F(ab')2, scFv, dsFv, ds-scFv, dimers, minibodies, diabodies, and multimers thereof and biospecific antibody fragments. Antibodies can be fragmented using conventional techniques. For example, F(ab')2 fragments can be generated by treating the antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments. Papain digestion can lead to the formation of Fab fragments. Fab, Fab' and F(ab')2, scFv, dsFv, ds-scFv, dimers, minibodies, diabodies, biospecific antibody fragments and other fragments can also be synthesized by recombinant techniques.

[0174] Immunoassays carried out in accordance with the present invention may be homogeneous assays or heterogeneous assays. In a homogeneous assay the immunological reaction usually involves the specific antibody, a labeled analyte, and the sample of interest. The signal arising from the label is modified, directly or indirectly, upon the binding of the antibody to the labeled analyte. Both the immunological reaction and detection of the extent thereof can be carried out in a homogeneous solution. Immunochemical labels which may be employed include free radicals, radioisotopes, fluorescent dyes, enzymes, bacteriophages, or coenzymes.

[0175] In a heterogeneous assay approach, the reagents are usually the sample, the antibody, and means for producing a detectable signal. Samples as described above may be used. The antibody can be immobilized on a support, such as a bead (such as protein A and protein G agarose beads), plate or slide, and contacted with the specimen suspected of containing the antigen in a liquid phase. The support is then separated from the liquid phase and either the support phase or the liquid phase is examined for a detectable signal employing means for producing such signal. The signal is related to the presence of the analyte in the sample. Means for producing a detectable signal include the use of radioactive labels, fluorescent labels, or enzyme labels. For example, if the antigen to be detected contains a second binding site, an antibody which binds to that site can be conjugated to a detectable group and added to the liquid phase reaction solution before the separation step. The presence of the detectable group on the solid support indicates the presence of the antigen in the test sample. Examples of suitable immunoassays include, but are not limited to immunoblotting, immunoprecipitation, immunofluorescence methods, chemiluminescence methods, electrochemiluminescence (ECL) or enzyme-linked immunoassays.

[0176] Those skilled in the art will be familiar with numerous specific immunoassay formats and variations thereof which may be useful for carrying out the method disclosed herein. See generally E. Maggio, Enzyme-Immunoassay, (1980) (CRC Press, Inc., Boca Raton, Fla.); see also U.S. Pat. No. 4,727,022, U.S. Pat. No. 4,659,678, U.S. Pat. No. 4,376,110, U.S. Pat. No. 4,275,149, U.S. Pat. No. 4,233,402,U.S. Pat. No. 4,230,767, each of which is herein incorporated by reference in its entirety for all purposes.

[0177] Antibodies can be conjugated to a solid support suitable for a diagnostic assay (e.g., beads such as protein A or protein G agarose, microspheres, plates, slides or wells formed from materials such as latex or polystyrene) in accordance with known techniques, such as passive binding. Antibodies as described herein may likewise be conjugated to detectable labels or groups such as radiolabels (e.g., 35S, 125I, 131I), enzyme labels (e.g., horseradish peroxidase, alkaline phosphatase), and fluorescent labels (e.g., fluorescein, Alexa, green fluorescent protein, rhodamine) in accordance with known techniques.

[0178] Antibodies can also be useful for detecting post-translational modifications of proteins, polypeptides, mutations, and polymorphisms, such as tyrosine phosphorylation, threonine phosphorylation, serine phosphorylation, glycosylation (e.g., O-GlcNAc). Such antibodies specifically detect the phosphorylated amino acids in a protein or proteins of interest, and can be used in immunoblotting, immunofluorescence, and ELISA assays described herein. These antibodies are well-known to those skilled in the art, and commercially available. Post-translational modifications can also be determined using metastable ions in reflector matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) (Wirth, U. et al. (2002) Proteomics 2(10): 1445-51).

[0179] In some embodiments, detection reagents can be immobilized on a solid matrix such as a porous strip to form at least one detection site. In some embodiments, polynucleotide or polypeptide arrays or microarrays containing a plurality of detection agents that hybridize to nucleotide or polypeptide of the biomarkers are utilized. Alternatively, the substrate array can be on, e.g., a solid substrate, e.g., a "chip" as described in U.S. Pat. No. 5,744,305. Alternatively, the substrate array can be a solution array.

[0180] Non-limiting examples for compositions and methods for detecting the components in the CDX2-KLF4 signaling pathway are described in U.S. Pat. Nos. 4,762,706, 5,081,230, 5,300,631, 5,443,956, 7,695,926, 7,785,817, 7,479,376, 7,364,868 and U.S. Patent Publication Nos. 20050196793, 20110281277, 20120251509, 20050186642, 20140011279, 20110171221, 20040235073, 20130011411, and

20130034862, each of which is incorporated herein by reference in its entirety for all purposes.

[0181] The present invention also provides kits for detecting and/or measuring activity level of one or more biomarkers of the present invention. In some embodiments, the kits are manufactured based on the detecting/measuring methods described herein or methods known to one skilled in the art. In some embodiments, the kits comprise packaging material and at least one vial comprising agent for detecting/measuring. In some embodiments, the kits comprise packaging material and at least one vial comprising agent for detecting/measuring the activity of at least one component in the CDX2-KLF4 signaling pathway. In some embodiments, the kits comprise packaging material and at least one vial comprising agent for detecting/measuring the activity of CDX2, KLF4, Caspase-3, Annexin V, p53, and/or p21. Optionally, the kits further comprise additional components selected from buffers, preservatives, aqueous diluent. In some embodiments, the kits comprise a sample that can be used as a control, such as a sample obtained from one or more human subjects who are responsive to a treatment by an anti-cancer agent of the present invention. In some embodiments, control is a sample obtained from one or more human subjects who are not responsive to a treatment by an anti-cancer agent of the present invention. Alternatively, the kits comprise an instruction, which provides standard activity level of human subjects who are responsive to a treatment by an anti-cancer agent of the present invention (e.g., a predetermined range of activity level of one or more biomarkers of the present invention in the responsive human subjects), and/or the standard activity level of human subjects who are not responsive to a treatment by an anti-cancer agent of the present invention (e.g., a predetermined range of activity level of one or more biomarkers of the present invention in the non-responsive human subjects). By using the kits of the present invention, one can make a determination regarding if the activity level of a given biomarker in the human subject being tested is statistically distinguishable from the standard activity level. Such determination can be used in combination with methods described herein.

Anti-Cancer Compositions

[0182] Anti-cancer compositions that can be utilized in the present invention comprise at least one active agent. In some embodiments, the active agent can modulate the activity of the CDX2-KLF4 signaling pathway. As used herein, the term "modulate" refers to that the compositions can increase, decrease, eliminate, enhance, delay, reduce, or block the activity of a component in the CDX2-KLF4 signaling pathway. In some embodiments, the compounds can decrease the CDX2 activity, and/or increase the KLF4 activity in a human subject. In some embodiments, the compounds can increase or decrease the activity of one or more upstream or downstream components in the signaling pathway. In some embodiments, the compounds can increase the activity of one or more downstream components that are positively regulated by KLF4 (e.g., p21), or decrease the activity of one or more downstream components in the signaling pathway that are negatively regulated by KLF4 (e.g., SP1). In some embodiments, the compounds can decrease the activity of one or more downstream components that are positively regulated by CDX2, or increase the activity of one or more downstream components in the signaling pathway that are negatively regulated by CDX2.

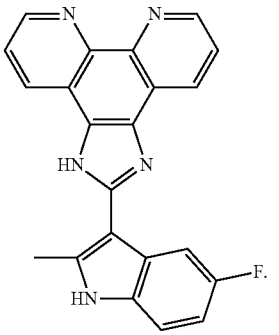
[0183] The active agents can be chemical compounds or compositions, biological molecules, or combinations thereof. In some embodiments, the active agents are small molecules. As used herein, the term "small molecule" refers to a molecule having a molecular weight of less than 500 MW, wherein the drug is a non-peptidyl or peptide agent. In some embodiments, the active agents are antibodies. In some embodiments, the active agents are antibodies. In some embodiments, the active agents are polynucleotides, such as siRNA.

[0184] In some embodiments, the active agents contain one or more entities that can inhibit or decrease the activity of CDX2, e.g., at DNA, RNA, protein level, or combinations thereof. In one embodiment, the anti-CDX2 agent is an anti-CDX2 antibody. According to the present invention, an anti-CDX2 antibody at least comprises one or more anti-CDX2 CDRs. In some embodiments, the anti-CDX2 agents are small interference RNA molecules, such as those disclosed by Wang et al. ("siRNA targeting of Cdx2 inhibits growth of human gastric cancer MGC-803 cells", *World J Gastroenterol*. Apr. 28, 2012; 18(16): 1903-1914.)

[0185] In some embodiments, the active agents contain one or more entities that can increase the activity of KLF4, or increase the activity of an entity in the CDX2-KLF4 signaling pathway that is positively regulated by KLF4, e.g., at DNA, RNA, protein level, or combinations thereof. In some embodiments, the active agent of the present invention is a 2-indolyl imidazo[4,5-d]phenanthroline derivative, such as those described in U.S. Pat. No. 8,148,392 or U.S. Patent Publication No. 2007/0123553A1, each of which is herein incorporated by reference in its entirety for all purposes.

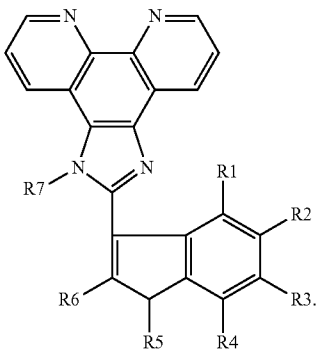
[0186] In some embodiments, the compound has the structure of formula I:

(I)



[0187] In some embodiments, the compound has the structural formula (II), or a salt thereof:

(II)

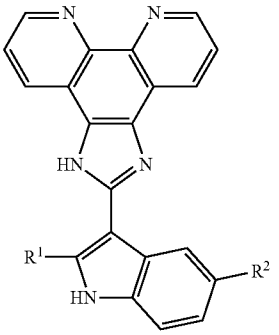


wherein: R1, R2, R3, R4, R6 and R7 are independently selected from hydrogen, halogen, hydroxyl, thiol, lower alkyl, substituted lower alkyl, lower alkenyl, substituted lower alkenyl, lower alkynyl, substituted lower alkynyl, alkoxy, alkylthio, acyl, aryloxy, amino, amido, carboxyl, aryl, substituted aryl, heterocycle, substituted heterocycle, heteroalkyl, substituted heteroalkyl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, nitro, or cyano or —S(O)1-2R wherein R is alkyl, substituted alkyl, aryl, substituted aryl, heterocycle, heteroaryl, substituted heterocycle, or substituted heteroaryl; and wherein R5 is H, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, acyl, —CH2-aryl, —CH2— heteroaryl.

[0188] In some embodiments, R1, R2, R3, R4 are independently hydrogen; halogen; C1-C4 alkyl; C1-C4 alkoxy; or C6-C14 aryl; R5 is hydrogen; C1-C4 alkyl; C1-C4 alkyl substituted with C6-C14 aryl; or C4-C6 cycloalkyl; R6 is hydrogen; halogen; C1-C4 alkyl; C1-C4 alkyl substituted with C5-C6 heterocycloalkyl wherein the heteroatom is N; C6-C14 aryl; C6-C14 aryl substituted with C1-C4 alkyl or halo; C5-C6 cycloalkyl; C5-C6 heterocycloalkyl; or polycycloalkyl. In some other embodiments, R1, R2, R3, R4 are independently hydrogen; halogen; C1-C4 alkyl; C1-C4 alkoxy; or phenyl; R5 is hydrogen; C1-C4 alkyl; C1-C4 alkyl substituted with phenyl; or cyclopentyl; R6 is hydrogen; halogen; C1-C4 alkyl; C1-C4 alkyl substituted with C5-C6 heterocycloalkyl wherein the heteroatom is N; phenyl; phenyl substituted with C1-C4 alkyl or halo; C5-C6 cycloalkyl; C5-C6 heterocycloalkyl; or adamantane; and R7 is H.

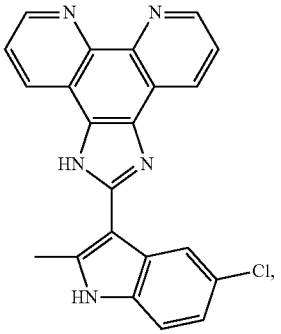
[0189] In some embodiments, the compound has the structural formula (III), or a salt thereof:

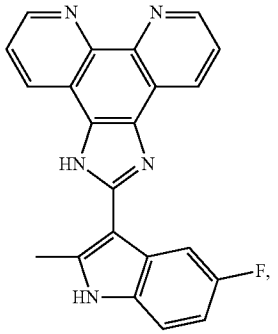
(III)



[0190] Wherein R1 is C1-C4 alkyl; and R2 is halogen. In some embodiments, R1 is methyl, isopropyl, or t-butyl.

[0191] In some embodiments, said compound has a formula selected from the group consisting of





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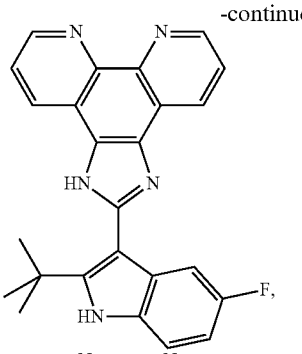
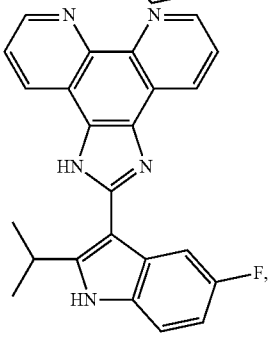
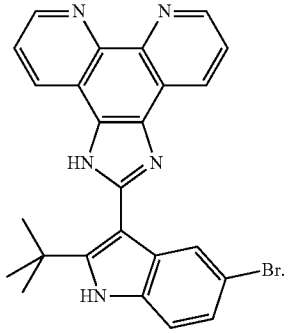




and



[0192] The active agents of the present invention are typically formulated prior to administration. The present invention thus provides pharmaceutical compositions comprising one or more active agents of the present invention. In some embodiments, the pharmaceutical compositions comprise a pharmaceutically acceptable carrier, diluent, or excipient. The pharmaceutical compositions are prepared by known procedures using well-known and readily available ingredients.

[0193] Active agents of the present invention or pharmaceutical compositions comprising the active agents may be administered via any suitable methods, including but not limited to, orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations. In some embodiments, the dosage unit formulations contain conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. In the usual course of therapy, the active agent is incorporated into an acceptable vehicle to form a composition for topical administration to the affected area, such as hydrophobic or hydrophilic creams or lotions, or into a form suitable for oral, rectal or parenteral administration, such as syrups, elixirs, tablets, troches, lozenges, hard or soft capsules, pills, suppositories, oily or aqueous suspensions, dispersible powders or granules, emulsions, injectables, or

solutions. The term parenteral as used herein includes, but are not limited to, subcutaneous injections, intradermal, intra-articular, intravenous, intramuscular, intravascular, intrastemal, intrathecal injection or infusion techniques.

[0194] The present invention also provides for pharmaceutical compositions comprising one or more of the active agents of the present invention and a vehicle, such as an artificial membrane vesicle (including a liposome, lipid micelle and the like), microparticle or microcapsule.

[0195] Compositions intended for oral use may be prepared in either solid or fluid unit dosage forms. Fluid unit dosage form can be prepared according to procedures known in the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavouring agents, colouring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. An elixir is prepared by using a hydroalcoholic (e.g., ethanol) vehicle with suitable sweeteners such as sugar and saccharin, together with an aromatic flavoring agent. Suspensions can be prepared with an aqueous vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose and the like.

[0196] Solid formulations such as tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate: granulating and disintegrating agents for example, corn starch, or alginic acid: binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc and other conventional ingredients such as dicalcium phosphate, magnesium aluminum silicate, calcium sulfate, starch, lactose, methylcellulose, and functionally similar materials. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

[0197] Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil. Soft gelatin capsules are prepared by machine encapsulation of a slurry of the compound with an acceptable vegetable oil, light liquid petrolatum or other inert oil.

[0198] Aqueous suspensions contain active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxylmethylcellulose, methyl cellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia: dispersing or wetting agents may be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example hepta-decaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl-p-hydroxy benzoate, one or more colouring agents, one or more flavouring agents or one or more sweetening agents, such as sucrose or saccharin.

[0199] Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil, for example peanut oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

[0200] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavouring and colouring agents, may also be present.

[0201] Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oil phase may be a vegetable oil, for example olive oil or peanut oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

[0202] The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or a suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Adjuvants such as local anaesthetics, preservatives and buffering agents can also be included in the injectable solution or suspension.

[0203] The pharmaceutical compositions of the present invention may be administered, together or separately, in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

[0204] Other pharmaceutical compositions and methods of preparing pharmaceutical compositions are known in the art and are described, for example, in "*Remington: The Science and Practice of Pharmacy*" (formerly "*Remingtons Pharma-*

ceutical Sciences"); Gennaro, A., Lippincott, Williams & Wilkins, Philadelphia, Pa. (2000).

[0205] The pharmaceutical compositions of the present invention may be administered to a subject by a variety of routes depending on the cancer to be treated, for example, the compositions may be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations. In one embodiment, the compounds are administered systemically to a subject, for example, by bolus injection or infusion into a subject's bloodstream or by oral administration. When used in conjunction with one or more known chemotherapeutic agents, the compounds can be administered prior to, or after, administration of the chemotherapeutic agents, or they can be administered concomitantly. The one or more chemotherapeutic may also be administered systemically, for example, by bolus injection, infusion, or oral administration.

[0206] The pharmaceutical compositions of the present invention may be used as part of a neo-adjuvant therapy (complement to primary therapy), or as part of an adjuvant therapy regimen. The present invention contemplates the use of the pharmaceutical compositions of the present invention at various stages in tumor development and progression, including, but not limited to, in the treatment of advanced and/or aggressive neoplasias (i.e. overt disease in a subject that is not amenable to cure by local modalities of treatment, such as surgery or radiotherapy), metastatic disease, locally advanced disease and/or refractory tumors (i.e. a cancer or tumor that has not responded to treatment). As used herein, the term "primary therapy" refers to a first line of treatment upon the initial diagnosis of cancer in a subject. Exemplary primary therapies may involve surgery, a wide range of chemotherapies and radiotherapy. "Adjuvant therapy" refers to a therapy that follows a primary therapy and that is administered to subjects at risk of relapsing. Adjuvant systemic therapy is usually begun soon after primary therapy to delay recurrence, prolong survival or cure a subject.

[0207] The pharmaceutical compositions of the present invention can be used alone or in combination with one or more other anti-cancer agents, such as chemotherapeutic agents as part of a primary therapy or an adjuvant therapy. Combinations of the pharmaceutical compositions of the present invention and standard chemotherapeutics may act to improve the efficacy of the chemotherapeutic and, therefore, can be used to improve standard cancer therapies. This application can be important in the treatment of drug-resistant cancers which are not responsive to standard treatment. Drug-resistant cancers can arise, for example, from heterogeneity of tumor cell populations, alterations in response to chemotherapy and increased malignant potential. Such changes are often more pronounced at advanced stages of disease.

[0208] The pharmaceutical compositions of the present invention can be used alone or in combination with radiation therapeutic. In some embodiments, the radiation therapeutic is administered at a dosage of about 40 Gy to about 80 Gy. In some embodiments the dosage is about 50 Gy to about 70 Gy, in some embodiments, the dosage is about 50 Gy to about 65 Gy. In some embodiments, the radiation therapy is administered at a dosage of about 50 Gy, about 55 Gy, about 60 Gy or about 65 Gy.

[0209] The dosage to be administered is not subject to defined limits, but it will usually be an effective amount. It will usually be the equivalent, on a molar basis of the pharmacologically active free form produced from a dosage formulation upon the metabolic release of the active free drug to achieve its desired pharmacological and physiological effects. The compositions may be formulated in a unit dosage form. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

[0210] In some embodiments, the active agents are compounds having the structure of formulas described herein. Examples of ranges for the compound(s) in each dosage unit are from about 0.05 to about 100 mg, or more usually, from about 1.0 to about 50 mg. Daily dosages of the compounds of the present invention will typically fall within the range of about 0.01 to about 100 mg/kg of body weight, or within the range of about 20 mg/m^2 to about 300 mg/m^2 in single or divided dose. However, it will be understood that the actual amount of the compound(s) to be administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms. The above dosage range is given by way of example only and is not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing harmful side effects, for example, by first dividing the larger dose into several smaller doses for administration throughout the day.

[0211] Pharmaceutical compositions comprising one or more compounds described herein in combination with one or more other anti-cancer agents can also be used.

[0212] In some embodiments, the anti-cancer agents are chemotherapeutics. In some embodiments, the chemotherapeutics are selected from alkylating agents, anti-metabolites, anti-microtubule agents, Topoisomerase inhibitors, and Cytotoxic antibiotics. Examples of chemotherapeutic agents include, but are not limited to, paclitaxel (Taxol®), docetaxel (Taxotere®), cisplatin, carboplatin (Paraplatin®), gemcitabine hydrochloride (Gemzar®), doxorubicin, etoposide (Etopophos®, Vepesid®), pemetrexed (Alimta®), topotecan (Hycamtin®), vinblastine (Velbe®), Vindesine (Eldisine®), vinorelbine (Navelbine®), ifosfamide (Mitoxana®), Mitomycin, and gemcitabine. These agents may be given in combination, for example, vinorelbine and cisplatin or carboplatin; gemcitabine with cisplatin or carboplatin or paclitaxel; MIC (mitomycin, ifosfamide and cisplatin); MVP (mitomycin, vinblastine and cisplatin); and EC (etoposide and carboplatin). Examples of useful chemotherapeutic drugs include, but are not limited to, hydroxyurea, busulphan, cisplatin, carboplatin, chlorambucil, melphalan, cyclophosphamide, Ifosphamide, danorubicin, doxorubicin, epirubicin, mitoxantrone, vincristine, vinblastine, Navelbine® (vinorelbine), etoposide, teniposide, paclitaxel, docetaxel, gemcitabine, cytosine, arabinoside, bleomycin, neocarcinostatin, suramin, taxol, mitomycin C and the like.

[0213] As used herein, the term "alkylating agents" refers to agents that have the ability to alhylate molecules in a subject, including proteins, RNA and DNA. Non-limiting examples of alkylating agents include nitrogen mustards, nitrosoureas, tetrazines, aziridines, cisplatins and derivatives, and non-classical alkylating agents. Nitrogen mustards

include mechlorethamine, cyclophosphamide, melphalan, chlorambucil, ifosfamide and busulfan. Nitrosoureas include N-Nitroso-N-methylurea (MNU), carmustine (BCNU), lomustine (CCNU) and semustine (MeCCNU), fotemustine and streptozotocin. Tetrazines include dacarbazine, mitozolomide and temozolomide. Aziridines include thiotepa, mytomycin and diaziquone (AZQ). Cisplatin and derivatives include cisplatin, carboplatin and oxaliplatin. They impair cell function by forming covalent bonds with the amino, carboxyl, sulfhydryl, and phosphate groups in biologically important molecules.[20] Non-classical alkylating agents include procarbazine and hexamethylmelamine.

[0214] As used herein, the term "anti-metabolites" refers to molecule that impedes DNA, RNA, or protein synthesis. In some embodiments, anti-metabolites resemble either nucleobases or nucleosides (a nucleotide without the phosphate group), but have altered chemical groups. These drugs exert their effect by either blocking the enzymes required for DNA synthesis or becoming incorporated into DNA or RNA. By inhibiting the enzymes involved in DNA synthesis, they prevent mitosis because the DNA cannot duplicate itself. Also, after misincorporation of the molecules into DNA, DNA damage can occur and programmed cell death (apoptosis) is induced. In some embodiments, the anti-metabolites are antifolates, fluoropyrimidines, deoxynucleoside analogues and thiopurines. In some embodiments, the anti-metabolites are selected from methotrexate, pemetrexed, fluorouracil, capecitabine, cytarabine, gemcitabine, decitabine, Vidaza, fludarabine, nelarabine, cladribine, clofarabine, pentostatin, thioguanine and mercaptopurine.

[0215] As used herein, the term "anti-microtubule agents" refers to chemicals that block cell division by preventing microtubule function. Representative examples of such agents include taxanes (e.g., paclitaxel (discussed in more detail below) and docetaxel) (Schiff et al., Nature 277: 665-667, 1979; Long and Fairchild, Cancer Research 54: 4355-4361, 1994; Ringel and Horwitz, J. Natl. Cancer Inst. 83(4): 288-291, 1991; Pazdur et al, Cancer Treat. Rev. 19(4): 351-386, 1993), campothecin, mitoxantrone, eleutherobin (e.g., U.S. Pat. No. 5,473,057), sarcodictyins (including sarcodictyin A), epothilones A and B (Bollag et al., Cancer Research 55: 2325-2333, 1995), discodermolide (ter Haar et al., Biochemistry 35: 243-250, 1996), deuterium oxide (D2O) (James and Lefebvre, Genetics 130(2): 305-314, 1992; Sollott et al., J. Clin. Invest. 95: 1869-1876, 1995), hexylene glycol (2-methyl-2,4-pentanediol) (Oka et al., Cell Struct. Funct. 16(2): 125-134, 1991), tubercidin (7-deazaadenosine) (Mooberry et al., Cancer Lett. 96(2): 261-266, 1995), LY290181 (2-amino-4-(3-pyridyl)-4H-naphtho(1,2-b)pyran-3-cardonitrile) (Panda et al., J. Biol. Chem. 272(12): 7681-7687, 1997; Wood et al., Mol. Pharmacol. 52(3): 437-444, 1997), aluminum fluoride (Song et al., J. Cell. Sci. Suppl. 14: 147-150, 1991), ethylene glycol bis-(succinimidylsuccinate) (Caplow and Shanks, J. Biol. Chem. 265(15): 8935-8941, 1990), glycine ethyl ester (Mejillano et al., Biochemistry 31(13): 3478-3483, 1992), nocodazole (Ding et al., J. Exp. Med 171(3): 715-727, 1990; Dotti et al., J. Cell Sci. Suppl. 15: 75-84, 1991; Oka et al., Cell Struct. Funct. 16(2): 125-134, 1991; Weimer et al., J. Cell Biol. 136(1), 71-80, 1997), cytochalasin B (Illinger et al., Biol. Cell 73(2-3): 131-138, 1991), colchicine and CI-980 (Allen et al., Am. J. Physiol. 261(4 Pt. 1): L315-L321, 1991; Ding et al., J. Exp. Med. 171(3): 715-727, 1990; Gonzalez et al., Exp. Cell. Res. 192(1): 10-15, 1991; Stargell et al., Mol. Cell. Biol. 12(4): 1443-1450, 1992; Garcia et al., Antican. Drugs 6(4): 533-544, 1995), colcemid (Barlow et al., Cell. Motil. Cytoskeleton 19(1): 9-17, 1991; Meschini et al., J. Microsc. 176(Pt. 3): 204-210, 1994; Oka et al., Cell Struct. Funct. 16(2): 125-134, 1991), podophyllotoxin (Ding et al., J. Exp. Med. 171(3): 715-727, 1990), benomyl (Hardwick et al., J. Cell. Biol. 131(3): 709-720, 1995; Shero et al., Genes Dev. 5(4): 549-560, 1991), oryzalin (Stargell et al., Mol. Cell. Biol. 12(4): 1443-1450, 1992), majusculamide C (Moore, J. Ind. Microbiol. 16(2): 134-143, 1996), demecolcine (Van Dolah and Ramsdell, J. Cell. Physiol. 166(1): 49-56, 1996; Wiemer et al., J. Cell. Biol. 136(1): 71-80, 1997), methyl-2-benzimidazolecarbamate (MBC) (Brown et al., J. Cell. Biol. 123(2): 387-403, 1993), LY195448 (Barlow & Cabral, *Cell Motil. Cytoskel.* 19: 9-17, 1991), subtilisin (Saoudi et al., J. Cell Sci. 108: 357-367, 1995), 1069C85 (Raynaud et al., Cancer Chemother Pharmacol. 35: 169-173, 1994), steganacin (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), combretastatins (Hamel, Med Res. Rev. 16(2): 207-231, 1996), curacins (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), estradiol (Aizu-Yokata et al., Carcinogen. 15(9): 1875-1879, 1994), 2-methoxyestradiol (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), flavanols (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), rotenone (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), griseofulvin (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), vinca alkaloids, including vinblastine and vincristine (Ding et al., J. Exp. Med 171(3): 715-727, 1990; Dirk et al., Neurochem. Res. 15(11): 1135-1139, 1990; Hamel, Med. Res. Rev. 16(2): 207-231, 1996; Illinger et al., Biol. Cell 73(2-3): 131-138, 1991; Wiemer et al., J. Cell. Biol. 136(1): 71-80, 1997), maytansinoids and ansamitocins (Hamel, Med Res. Rev. 16(2): 207-231, 1996), rhizoxin (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), phomopsin A (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), ustiloxins (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), dolastatin 10 (Hamel, Med Res. Rev. 16(2): 207-231, 1996), dolastatin 15 (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), halichondrins and halistatins (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), spongistatins (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), cryptophycins (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), rhazinilam (Hamel, Med. Res. Rev. 16(2): 207-231, 1996), betaine (Hashimoto et al., Zool. Sci. 1: 195-204, 1984), taurine (Hashimoto et al., Zool. Sci. 1: 195-204, 1984), isethionate (Hashimoto et al., Zool. Sci. 1: 195-204, 1984), HO-221 (Ando et al., Cancer Chemother Pharmacol. 37: 63-69, 1995), adociasulfate-2 (Sakowicz et al., Science 280: 292-295, 1998), estramustine (Panda et al., Proc. Natl. Acad. Sci. USA 94: 10560-10564, 1997), monoclonal anti-idiotypic antibodies (Leu et al., Proc. Natl. Acad. Sci. USA 91(22): 10690-10694, 1994), microtubule assembly promoting protein (taxol-like protein, TALP) (Hwang et al., Biochem. Biophys. Res. Commun. 208(3): 1174-1180, 1995), cell swelling induced by hypotonic (190 mosmol/L) conditions, insulin (100 mmol/L) or glutamine (10 mmol/L) (Haussinger et al., Biochem. Cell. Biol. 72(1-2): 12-19, 1994), dynein binding (Ohba et al., Biochim. Biophys. Acta 1158(3): 323-332, 1993), gibberelin (Mita and Shibaoka, Protoplasma 119(1/2): 100-109, 1984), XCHOI (kinesin-like protein) (Yonetani et al., Mol. Biol. Cell 7(suppl): 211 A, 1996), lysophosphatidic acid (Cook et al., Mol. Biol. Cell 6(suppl): 260A, 1995), lithium ion (Bhattacharyya and Wolff, Biochem. Biophys. Res. Commun. 73(2): 383-390, 1976), plant cell wall components (e.g., poly-L-lysine and extensin) (Akashi et al., Planta 182(3): 363-369, 1990), glycerol buffers (Schilstra et

al., Biochem. J. 277(Pt. 3): 839-847, 1991; Farrell and Keates, Biochem. Cell. Biol. 68(11): 1256-1261, 1990; Lopez et al., J. Cell. Biochem. 43(3): 281-291, 1990), Triton X-100 microtubule stabilizing buffer (Brown et al., J. Cell Sci. 104(Pt. 2): 339-352, 1993; Safiejko-Mroczka and Bell, J. Histochem. Cytochem. 44(6): 641-656, 1996), microtubule associated proteins (e.g., MAP2, MAP4, tau, big tau, ensconsin, elongation factor-1-alpha (EF-1α) and E-MAP-115) (Burgess et al., Cell Motil. Cytoskeleton 20(4): 289-300, 1991; Saoudi et al., J. Cell. Sci. 108(Pt. 1): 357-367, 1995; Bulinski and Bossler, J. Cell. Sci. 107(Pt. 10): 2839-2849, 1994; Ookata et al., J. Cell Biol. 128(5): 849-862, 1995; Boyne et al., J. Comp. Neurol 358(2): 279-293, 1995; Ferreira and Caceres, J. Neurosci. 11(2): 392-400, 1991; Thurston et al., Chromosoma 105(1):20-30, 1996; Wang et al., Brain Res. Mol. Brain Res. 38(2): 200-208, 1996; Moore and Cyr, Mol. Biol. Cell 7(suppl): 221-A, 1996; Masson and Kreis, J. Cell Biol. 123 (2), 357-371, 1993), cellular entities (e.g., histone H1, myelin basic protein and kinetochores) (Saoudi et al., J. Cell. Sci. 108(Pt. 1): 357-367, 1995; Simerly et al., J. Cell Biol. 111(4): 1491-1504, 1990), endogenous microtubular structures (e.g., axonemal structures, plugs and GTP caps) (Dye et al., Cell Motil. Cytoskeleton 21(3): 171-186, 1992; Azhar and Murphy, Cell Motil. Cytoskeleton 15(3): 156-161, 1990; Walker et al., J. Cell Biol. 114(1): 73-81, 1991; Drechsel and Kirschner, Curr. Biol. 4(12): 1053-1061, 1994), stable tubule only polypeptide (e.g., STOP145 and STOP220) (Pirollet et al., Biochim. Biophys. Acta 1160(1): 113-119, 1992; Pirollet et al., Biochemistry 31(37): 8849-8855, 1992; Bosc et al., Proc. Natl. Acad. Sci. USA 93(5): 2125-2130, 1996; Margolis et al., EMBO J. 9(12): 4095-4102, 1990) and tension from mitotic forces (Nicklas and Ward, J. Cell Biol. 126(5): 1241-1253, 1994), as well as any analogues and derivatives of any of the above. Such compounds can act by either depolymerizing microtubules (e.g., colchicine and vinblastine), or by stabilizing microtubule formation (e.g., paclitaxel).

[0216] In some embodiments, an anti-tumor agent include mitotic inhibitors, for example vinca alkaloid derivatives such as vinblastine vinorelbine, vindescine and vincristine; colchines allochochine, halichondrine, N-benzoyltrimethylmethyl ether colchicinic acid, dolastatin 10, maystansine, rhizoxine, taxanes such as taxol (paclitaxel), docetaxel (Taxotere), 2'-N-[3-(dimethylamino)propyl]glutaramate (taxol derivative), thiocholchicine, trityl cysteine, teniposide, methotrexate, azathioprine, fluorouricil, cytocine arabinoside, 2'2'-difluorodeoxycytidine (gemcitabine), adriamycin and mitamycin. Alkylating agents, for example cis-platin, carboplatin oxiplatin, iproplatin, Ethyl ester of N-acetyl-DL-sarcosyl-L-leucine (Asaley or Asalex), 1,4-cyclohexadiene-1,4-dicarbamic acid, 2,5-bis(1-azirdinyl)-3,6-dioxo-, diethyl ester (diaziquone), 1,4-bis(methanesulfonyloxy)butane (bisulfan or leucosulfan) chlorozotocin, clomesone, cyanomorpholinodoxorubicin, cyclodisone, dianhydroglactitol, fluorodopan, hepsulfam, mitomycin C, hycantheonemitomycin C, mitozolamide, 1-(2-chloroethyl)-4-(3-chloropropyl)-piperazine dihydrochloride, piperazinedione, pipobroman, porfiromycin, spirohydantoin mustard, teroxirone, tetraplatin, thiotepa, triethylenemelamine, uracil nitrogen mustard, bis(3-mesyloxypropyl)amine hydrochloride, mitomycin, nitrosoureas agents such as cyclohexyl-chloroethylnitrosourea, methylcyclohexyl-chloroethylnitrosourea 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitroso-urea, bis (2-chloroethyl)nitrosourea, procarbazine, dacarbazine, nitrogen mustard-related compounds such as mechloroethamine, cyclophosphamide, ifosamide, melphalan, chlorambucil, estramustine sodium phosphate, strptozoin, and temozolamide. DNA anti-metabolites, for example 5-fluorouracil, cytosine arabinoside, hydroxyurea, 2-[(3hydroxy-2-pyrinodinyl)methylene]-hydrazinecarbothioamide, deoxyfluorouridine, 5-hydroxy-2-formylpyridine thiosemicarbazone, alpha-2'-deoxy-6-thioguanosine, aphidicolin glycinate, 5-azadeoxycytidine, beta-thioguanine deoxyriboside, cyclocytidine, guanazole, inosine glycodialdehyde, macbecin II, pyrazolimidazole, cladribine, pentostatin, thioguanine, mercaptopurine, bleomycin, 2-chlorodeoxyadenosine, inhibitors of thymidylate synthase such as raltitrexed and pemetrexed disodium, clofarabine, floxuridine and fludarabine. DNA/RNA antimetabolites, for example, L-alanosine, 5-azacytidine, acivicin, aminopterin and derivatives thereof such as N-[2-chloro-5-[[(2,4-diamino-5-methyl-6-quinazolinyl)methyl]amino]benzoyl]-L-aspartic acid, N-[4-[[(2,4-diamino-5-ethyl-6-quinazolinyl)methyl]amino]benzoyl]-L-aspartic acid, N-[2-chloro-4-[[(2,4-diaminopteridinyl)methyl]amino]benzoyl]-L-aspartic acid, soluble Baker's antifol, dichloroallyl lawsone, brequinar, ftoraf, dihydro-5-azacytidine, methotrexate, N-(phosphonoacetyl)-L-aspartic acid tetrasodium salt, pyrazofuran, trimetrexate, plicamycin, actinomycin D, cryptophycin, and analogs such as cryptophycin-52 or, for example, one of the preferred anti-metabolites disclosed in European Patent Application No. 239362 such as N-(5-[N-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-N-methylamino]-2-thenoyl)-L-glutamic acid; growth factor inhibitors; cell cycle inhibitors; intercalating antibiotics, for example adriamycin and bleomycin; proteins, for example interferon; and anti-hormones, for example anti-estrogens such as Nolvadex™ (tamoxifen) or, for example anti-androgens such as Casodex™ (4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'-(trifluoromethyl)propionanilide).

[0217] As used herein, the term "topoisomerase inhibitors" refers to agents that can modulcate the activity of topoisomerase I and/or topoisomerase II. In some embodiments, the topoisomerase inhibitor of this invention can be a topoisomerase I inhibitor, which can be, in some embodiments, a camptothecin derivative. A camptothecin derivative of this invention can be, but is not limited to Belotecan (CKD602), Camptothecin, 7-Ethyl-10-Hydroxy-CPT, 10-Hydroxy-CPT, Rubitecan (9-Nitro-CPT), 7-Ethyl-CPT, Topotecan, Irinotecan, Silatecan (DB67) and any combinations thereof. In some embodiments of this invention, the topoisomerase I inhibitor can be an indenoisoquinoline derivative, which can be but is not limited to NSC706744, NSC725776, NSC724998 and any combinations thereof. In further embodiments of this invention, the topoisomerase inhibitor is a topoisomerase II inhibitor, which in some embodiments can be an acridine derivative, which can be but is not limited to Amsacrine, in some embodiments the topoisomerase II inhibitor can be a podophyllotoxin derivative, which can be but is not limited to etoposide, and in some embodiments the topoisomerase II inhibitor can be a bisdioxopiperazine derivative, which can be but is not limited to ICRF-193, dexrazoxane (ICRF-187) and any combinations thereof. In yet further embodiments of this invention, the topoisomerase inhibitor can be Resveratrol (PMID: 20304553; PMID: 15796584)41, Epigallocatechin gallate (PMID: 18293940; PMID: 11594758; PMID: 11558576; PMID: 1313232)42,43, Genistein (PMID: 17458941)44, Daidzein (PMID: 17458941)45. Quercetin (PMID: 1313232; PMID: 16950806; PMID: 15312049),

natural flavones related to quercetin that inhibit topoisomerase, such as acacetin, apigenin, kaempferol and morin (PMID: 8567688)46-48, Luteolin (PMID: 12027807; PMID: 16950806; PMID: 15312049)46; Myricetin (PMID: 20025993)49 and any combinations thereof. In certain embodiments, the topoisomerase inhibitor can be an interfering RNA (RNAi) molecule that targets topoisomerase I, topoisomerase II or both. Nonlimiting examples of RNAi molecules include small interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA), antisense nucleic acid molecules, and the like as are well known in the art. Nonlimiting examples of siRNAs and shRNAs of this invention are provided in Table 2. In some embodiments, a zinc finger nuclease, an antibody and/or a ribozyme can be employed to inhibit topoisomerase activity in the methods of this invention.

[0218] As used herein, the term "cytotoxic antibiotics" cytotoxic antibiotics include, but are not limited to, antinomycin, bleomycin, mitomycin, plicamycin and the like. Examples of tyrosine kinase inhibitors include, but are not limited to, nilotinib, imatinib, gefitinib, erlotinib, cetuximab, panitumumab, zalutumumab, nimotuzumab, matuzuman and the like.

[0219] In some embodiments, the other anti-cancer agents are monoclonal antibodies, such as alemtuzumab, bevacizumab, cetuximab, gemtuzumab, rituximab, and trastuzumab; photosensitizers, such as aminolevulinic acid, methyl aminolevulinate, porfimer sodium, and verteporfin; and other agents, such as alitretinoin, altretamine, amsacrine, anagrelide, arsenic trioxide, asparaginase, bexarotene, bortezomib, celecoxib, denileukin diftitox, erlotinib, estramustine, gefitinib, hydroxycarbamide, imatinib, pentostatin, masoprocol, mitotane, pegaspargase, and tretinoin.

[0220] In some embodiments, the other anti-cancer agents are those that can be used to treat leukemia, such as drugs for Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), and Meningeal Leukemia, include but are not limited to, Abitrexate (Methotrexate), Adriamycin PFS (Doxorubicin Hydrochloride), Adriamycin RDF (Doxorubicin Hydrochloride), Arranon (Nelarabine), Asparaginase Erwinia chrysanthemi, Cerubidine (Daunorubicin Hydrochloride), Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar (Clofarabine), Cyclophosphamide, Cytarabine, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Dasatinib, Daunorubicin Hydrochloride, Doxorubicin Hydrochloride, Erwinaze (Asparaginase Erwinia Chrysanthemi), Folex (Methotrexate), Folex PFS (Methotrexate), Gleevec (Imatinib Mesylate), Iclusig (Ponatinib Hydrochloride), Imatinib Mesylate, Marqibo (Vincristine Sulfate Liposome), Mercaptopurine, Methotrexate, Methotrexate LPF (Methorexate), Mexate (Methotrexate), Mexate-AQ (Methotrexate), Nelarabine, Neosar (Cyclophosphamide), Oncaspar (Pegaspargase), Pegaspargase, Purinethol (Mercaptopurine), Rubidomycin (Daunorubicin Hydrochloride), Sprycel (Dasatinib), Tarabine PFS (Cytarabine), Vincasar PFS, incristine Sulfate), Vincristine Sulfate, Vincristine Sulfate Liposome, Hyper-CVAD, Adriamycin PFS (Doxorubicin Hydrochloride), Adriamycin RDF (Doxorubicin Hydrochloride), Arsenic Trioxide, Cerubidine (Daunorubicin Hydrochloride), Clafen (Cyclophosphamide), Cyclophosphamide, Cytarabine, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Daunorubicin Hydrochloride, Doxorubicin Hydrochloride, Neosar (Cyclophosphamide), Rubidomycin (Daunorubicin Hydrochloride), Tarabine PFS (Cytarabine), Trisenox (Arsenic Trioxide), Vincasar PFS (Vincristine Sulfate), Vincristine Sulfate, ADE, Alemtuzumab, Ambochlorin (Chlorambucil), Amboclorin (Chlorambucil), Arzerra (Ofatumumab), Bendamustine, Hydrochloride, Campath (Alemtuzumab), Chlorambucil, Clafen (Cyclophosphamide), Cyclophosphamide, Cytoxan (Cyclophosphamide), Fludara (Fludarabine Phosphate), Fludarabine Phosphate, Gazyva (Obinutuzumab), Ibrutinib, Imbruvica (Ibrutinib), Leukeran (Chlorambucil), Linfolizin (Chlorambucil), Neosar (Cyclophosphamide), Obinutuzumab, Ofatumumab, Treanda (Bendamustine Hydrochloride), CHLORAMBUCIL-PREDNISONE, CVP, Bosulif (Bosutinib), Bosutinib, Busulfan, Busulfex (Busulfan), Clafen (Cyclophosphamide), Cyclophosphamide, Cytarabine, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Dasatinib, Gleevec (Imatinib Mesylate), Iclusig (Ponatinib Hydrochloride), Imatinib Mesylate, Myleran (Busulfan), Neosar (Cyclophosphamide), Nilotinib, Omacetaxine Mepesuccinate, Ponatinib Hydrochloride, Sprycel (Dasatinib), Synribo (Omacetaxine Mepesuccinate), Tarabine PFS (Cytarabine), Tasigna (Nilotinib), Cytarabine, Cytosar-U (Cytarabine), and Tarabine PFS (Cytarabine)

[0221] In some embodiments, additional anti-cancer agents that can be used together with the compounds of the present invention include agents that can inhibit EGFR (epidermal growth factor receptor) responses, such as EGFR antibodies, EGF antibodies, and molecules that are EGFR inhibitors; VEGF (vascular endothelial growth factor) inhibitors; and erbB2 receptor inhibitors, such as organic molecules or antibodies that bind to the erbB2 receptor. EGFR inhibitors are described in, for example in WO 95/19970, WO 98/14451, WO 98/02434 and U.S. Pat. No. 5,747,498. EGFR-inhibiting agents include, but are not limited to, the monoclonal antibodies C225 and anti-EGFR 22Mab (ImClone Systems Incorporated of New York, N.Y., USA), the compounds ZD-1839 (AstraZeneca), BIBX-1382 (Boehringer Ingelheim), MDX-447 (Medarex Inc. of Annandale, N.J., USA), and OLX-103 (Merck & Co. of Whitehouse Station, N.J., USA), VRCTC-310 (Ventech Research) and EGF fusion toxin (Seragen Inc. of Hopkinton, Mass.). VEGF inhibitors, for example AG-13736 (Pfizer, Inc.), can also be combined or co-administered with the composition. VEGF inhibitors are described in, for example in WO 99/24440, WO 95/21613, WO 99/61422, U.S. Pat. No. 5,834,504, WO 98/50356 (published Nov. 12, 1998), U.S. Pat. No. 5,883,113, U.S. Pat. No. 5,886,020, U.S. Pat. No. 5,792,783, U.S. Pat. No. 6,534,524, WO 99/10349, WO 97/32856, WO 97/22596, WO 98/54093, and WO 98/02437, all of which are herein incorporated by reference in their entirety. Other examples of some specific VEGF inhibitors are IM862 (Cytran Inc. of Kirkland, Wash., USA); Avastin™ or bevacizumab, an anti-VEGF monoclonal antibody (Genentech, Inc. of South San Francisco, Calif.); and angiozyme, a synthetic ribozyme from Ribozyme (Boulder, Colo.) and Chiron (Emeryville, Calif.). ErbB2 receptor inhibitors, such as GW-282974 (Glaxo Wellcome plc), and the monoclonal antibodies AR-209 (Aronex Pharmaceuticals Inc. of The Woodlands, Tex., USA) and 2B-1 (Chiron), may be administered in combination with the composition. Such erbB2 inhibitors include those described in WO 98/02434, WO 99/35146, WO 99/35132, WO 98/02437, WO 97/13760, WO 95/19970, U.S. Pat. No. 5,587,458, and U.S. Pat. No. 5,877,305, each of which is herein incorporated by reference in its entirety.

[0222] Without wishing to be bound by any particular theory, the anti-cancer agents of the present invention can act through one or more mechanisms. Such mechanisms include, but are not limited to: (1) inhibition of CDX2 activity; (2) induction of KLF4 activity; (3) induction of p21 CDK inhibitor; (4) induction of G1/S Cell cycle arrest; (5) induction of Caspase 3 enzyme; and (6) induction of apoptosis.

[0223] The present invention provides methods of using the biomarkers and active agents of the present invention.

[0224] In some embodiments, provided are methods for determining the responsiveness of a human subject to a cancer treatment. The cancer can be Acute Myeloid leukemia (AML). The cancer also can be acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), adult T-cell leukaemia (ATLL), lymphoma, gastric cancer, multiple myeloma, myelodysplastic syndromes, or combinations thereof. In some embodiments, the treatment involves using an active agent that can modulate one or more components in the CDX2-KLF4 signaling pathway, including but not limited to, CDX2, KLF4, Caspase-3, Annexin V, p53, KDM5B, miR-2909, BAX, BCL2, BCL3, BMP, Wnt, HNF4α, Fgf, Hox, SP1, MYC, CCND1, AATF, and p21. In some embodiments, the treatment involves using an active agent that can induce G1/S cell cycle arrest. In some embodiments, the treatment involves using an active agent that can induce Capase 3 enzyme. In some embodiments, the treatment involves using an active agent that can induce apoptosis. In some embodiments, the active agent is selected from 2,4,5-trisubstituted imidazole compounds described in US 2007/0123553A1, or 2-indolyl imidazo[4,5-d]phenanthroline compounds described in U.S. Pat. No. 8,148,392, or functional derivatives thereof. In some embodiments, the active agent comprises the compound of formula I. (i.e., LOR-253).

[0225] In some embodiments, the human subject is determined to be responsive to the treatment if meeting one or more selection criteria described below:

[0226] The human subject has a genetic alteration of CDX2 locus when compared to that of a human subject or the loci of a group of human subjects who are responsive to a treatment of the present invention. Such alterations can be epigenetic and/or genetic modifications, including but not limited to those described in Costa et al. (*Oncogene,* 18(35):5010-5014, 1999), Abdel-Rahman et al. (*Oncogene,* 24:1542-1551, 2005), Yagi et al. (*Br. J. Cancer.,* 79(3-4): 440-444, 1999), Chawengsaksophak et al. (*Nature,* 386(6620):84-7, 1997), Wood et al. (*Science,* 2007; 318(5853):1108-1113), Wicking et al. (*Oncogene,* 1998; 17(5):657-659), and da Costa L T, et al. (*Oncogene,* 1999; 18(35):5010-5014), each of which is incorporated by reference in its entirety for all purposes;

[0227] The human subject has a higher level of CDX2 activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0228] The human subject has a lower level of KLF4 activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0229] The human subject has a lower level of p21 activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0230] The human subject has a higher level of p53 activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention, wherein said human has a cancer type in which KLF4 negatively regulates p53;

[0231] The human subject has a lower level of p53 activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention, wherein said human has a cancer type in which KLF4 positively regulates p53;

[0232] The human subject has a higher level of BCL3 activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0233] The human subject has a higher level of SP1 activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0234] The human subject has a higher level of MYC activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0235] The human subject has a higher level of CCND1 activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0236] The human subject has a higher level of AATF activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0237] The human subject has a lower level of Caspase 3 activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0238] The human subject has a lower level of Annexin V activity than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0239] The human subject has a higher ratio of CDX2/B activity (wherein B is KLF4, p21, Caspase 3, or Annexin V; or wherein B is p53 if the human subject has a cancer type in which KLF4 positively regulates p53) than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0240] The human subject has a lower ratio of B/CDX2 activity (wherein B is KLF4, p21, Caspase 3, or Annexin V; or wherein B is p53 if the human subject has a cancer type in which KLF4 positively regulates p53) than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0241] The human subject has a lower ratio of CDX2/C activity (wherein C is BCL3, SP1, MYC, CCND1, or AATF; or wherein C is p53 if the human subject has a cancer type in which KLF4 negatively regulates p53) than normal level, or a level similar to the level of human subjects who are responsive to a treatment of the present invention;

[0242] The human subject has a higher ratio of C/CDX2 activity (wherein C is BCL3, SP1, MYC, CCND1, or AATF; or wherein C is p53 if the human subject has a cancer type in which KLF4 negatively regulates p53) than normal level, or a level similar to the level of human

subjects who are responsive to a treatment of the present invention. Otherwise, the human subject is determined to be not responsive to the treatment.

[0243] In some embodiments, the normal level is the activity of a biomarker in healthy human subjects, or human subjects without or with only minor detectable symptoms of disorders.

[0244] An activity level is similar to a predetermined standard activity level (e.g., activity level of human subjects who are responsive to a treatment of the present invention) if the difference between the tested activity level and the predetermined standard activity level is statistically insignificant, or the difference is least than about 20%, about 19%, about 18%, about 17%, about 16%, about 15%, about 14%, about 13%, about 12%, about 11%, about 10%, about 9%, about 8%, about 7%, about 6% about 5%, about 4%, about 3%, about 2%, about 1%, or less of the predetermined standard activity level.

[0245] Also provided are methods for monitoring the efficacy of an active agent in the treatment of cancer. These methods include determining the activity of one or more biomarkers of the present invention in a biological sample from a patient and providing that information regarding the biomarkers to an entity that provides a determination or evaluation of the treatment or efficacy based on biomarker information. In some embodiments, the biomarker activity is determined during or after taking at least one dosage of the active agent of the present invention. In some embodiments, the entity can provide a determination that treatment with the active agent should be used or should be continued, if the human subject meets one or more selection criteria described below:

[0246] The human subject has a genetic alteration of CDX2 locus when compared to that of a human subject or the loci of a group of human subjects who are responsive to a treatment of the present invention;

[0247] The human subject has an decreased level of CDX2 activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the CDX2 activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0248] The human subject has an increased level of KLF4 activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the KLF4 activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0249] The human subject has an increased level of p21 activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the p21 activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0250] The human subject has a decreased level of p53 activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the p53 activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention, wherein said human has a cancer type in which KLF4 negatively regulates p53;

[0251] The human subject has an increased level of p53 activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the p53 activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention, wherein said human has a cancer type in which KLF4 positively regulates p53;

[0252] The human subject has a decreased level of BCL3 activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the BCL3 activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0253] The human subject has a decreased level of SP1 activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the SP1 activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0254] The human subject has a decreased level of MYC activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the MYC activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0255] The human subject has a decreased level of CCND1 activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the CCND1 activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0256] The human subject has a decreased level of AATF activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the AATF activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0257] The human subject has an increased level of Caspase 3 activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the Caspase 3 activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0258] The human subject has an increased level of Annexin V activity when compared to that of the same human subject before the treatment, and/or has a normalization or stabilization of the Annexin V activity when compared that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0259] The human subject has a decreased ratio of CDX2/B activity (wherein B is KLF4, p21, Caspase 3, or Annexin V; or wherein B is p53 if the human subject has a cancer type in which KLF4 positively regulates p53) when compared to that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0260] The human subject has an increased ratio of B/CDX2 activity (wherein B is KLF4, p21, Caspase 3, or Annexin V; or wherein B is p53 if the human subject has a cancer type in which KLF4 positively regulates

p53) when compared to that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0261] The human subject has an increased ratio of CDX2/C activity (wherein C is BCL3, SP1, MYC, CCND1, or AATF; or wherein C is p53 if the human subject has a cancer type in which KLF4 negatively regulates p53) when compared to that of a human subject or a group of human subjects who are responsive to a treatment of the present invention;

[0262] The human subject has a decreased ratio of C/CDX2 activity (wherein C is BCL3, SP1, MYC, CCND1, or AATF; or wherein C is p53 if the human subject has a cancer type in which KLF4 negatively regulates p53) when compared to that of a human subject or a group of human subjects who are responsive to a treatment of the present invention. Otherwise, the entity can provide a determination that treatment with the active agent should not be used or should be continued.

[0263] In some embodiments, the normal level of CDX2/KLF4 or KLF4/CDX2 activity ratio is used to determine if a human subject is responsive to the treatment. In some embodiments, the normal level of CDX2/KLF4 activity ratio, or CDX2/KLF4 activity ratio in human subjects responsive to the treatment is about 0.01, about 0.02, about 0.03, about 0.04, about 0.05, about 0.06, about 0.07, about 0.08, about 0.09, about 0.1, about 0.2, about 0.3, about 0.4, about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1.0, about 2.0, about 3.0, about 4.0, about 5.0, about 6.0, about 7.0, about 8.0, about 9.0, about 10.0, about 20.0, about 30.0, about 40.0, about 50.0, about 60.0, about 70.0, about 80.0, about 90.0, about 100, about 200, about 300, about 400, about 500, about 600, about 700, about 800, about 900, about 1000, or more. In some embodiments, the normal level of KLF4/CDX2 activity ratio, or KLF4/CDX2 activity ratio in human subjects responsive to the treatment is about 100, about 90, about 80, about 70, about 60, about 50, about 40, about 30, about 20, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, about 1, about 0.9, about 0.8, about 0.7, about 0.6, about 0.5, about 0.4, about 0.3, about 0.2, about 0.1, about 0.09, about 0.08, about 0.07, about 0.06, about 0.05, about 0.04, about 0.03, about 0.02, about 0.01, or less.

[0264] Also provided are methods for treating a human subject. In some embodiments, the treatment is a cancer treatment. In some embodiments, the cancer can be Acute Myeloid leukemia (AML), leukemia, ALL, pediatric T-cell ALL or pediatric B-cell ALL, CML, ATLL, lymphoma, Hodgkin lymphoma, non-Hodgkin's lymphoma (NHL), Burkitts lymphoma, or B-cell lymphoma, gastric cancer, multiple myeloma, myelodysplastic syndromes, or combinations thereof. In some embodiments, the methods comprise modulating the activity of at least one component in the CDX2-KLF4 signaling pathway. In some embodiments, the methods comprise administering an active agent to the human subject. In some embodiments, the active agent can modulate the activity of CDX2, KLF4, Caspase 3, Annexin V, p53, BCL3, SP1, MYC, CCND1, AATF, and/or p21. In some embodiments, the methods comprise administering an active agent to induce G1/S Cell cycle arrest; In some embodiments, the methods comprise administering an active agent to induce Caspase 3 enzyme. In some embodiments, the methods comprise administering an active agent to induce apoptosis. In some embodiments, the active agent is selected from 2,4,5-trisubstituted imidazole compounds described in US 2007/0123553A1, or 2-indolyl imidazo[4,5-d]phenanthroline compounds described in U.S. Pat. No. 8,148,392, or functional derivatives thereof. In some embodiments, the active agent comprises the compound of formula I. (i.e., LOR-253/APTO-253).

[0265] In some embodiments, the methods comprise administering therapeutically effective amount of an active agent of the present invention to a human subject. In some embodiments, the methods further comprise determining the activity of at least one biomarker described herein in a sample obtained from the human subject. In some embodiments, the determination can be made before, during, after the treatment. In some embodiments, the determination can be made after the human subject takes at least one, two, three, four, five, six, seven, eight, nine, ten, ore more dosages of the active agent.

[0266] Further provided are methods for providing useful information for predicting, determining, evaluating or monitoring the treatment or efficacy of treatment of cancer with the active agent of the present invention. These methods include determining activity level of one or more biomarkers described herein in a biological sample from a patient and providing that information regarding the biomarkers to an entity that provides a determination or evaluation of the treatment or efficacy based on the biomarker information. If the activity of one or more biomarkers fit the selection criteria described herein, the entity can provide a determination that treatment with an active agent treatment of the present invention should be used or should be continued. If the activity of one or more biomarkers does not fit the selection criteria described herein, the entity can provide a determination that treatment with an active agent treatment of the present invention should not be used or should be discontinued.

[0267] The following examples illustrate various aspects of the invention. The examples should, of course, be understood to be merely illustrative of only certain embodiments of the invention and not to constitute limitations upon the scope of the invention.

EXAMPLES

Example 1

In Vitro Antiproliferative Activity of LOR-253 in Leukemia and Lymphoma Cell Lines

[0268] Antiproliferative activity of LOR-253 was determined by XTT assay as follows. Adherent cells (2×10^3/well) in 100 μL of growth medium were seeded in 96-well cell culture plates and incubated overnight at 37° C. The medium was removed and replaced with a total volume of 100 μL growth medium containing LOR-253 (or 0.1% DMSO vehicle control), as described in the respective experiments. Suspension cells were plated ($4\text{-}6\times10^3$/well) in 50 μL growth medium, and 50 μL growth medium containing LOR-253 (or 0.1% DMSO vehicle control) was added to each well. After incubation of the cells at 37° C. for 5 days, cell viability was quantitated with the use of sodium 3'-[1-(phenylamino-carbonyl)-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate (XTT) colorimetric assay (Roche). XTT labeling reagent (1 mg/mL) was mixed with electron-coupling reagent, following the manufacturer's instructions, and 50 μL of the mixture was added directly to the cells. The plates were further incubated at 37° C. for 4 h, and the absorbance of each well was measured at 490 nm with a multiwell spectrophotometer (Bio-Tek Instruments Inc.). The data were adjusted relative to the blank and expressed as a percentage of cell growth compared with the vehicle control. (Ref. Huesca et al., Mol Cancer Ther. 2009. 8:2586-96; Lorus publication on LOR-133)

[0269] The in vitro assay results (FIG. **3**) indicate that leukemia/lymphoma, including AML cell lines, such as HL-60, MV 411, THP-1, HEL92.1.7, CCRF-CEM, MOLT-4, Jurkat, K-562, Ramos, and Raji, are the cell lines most sensitive to LOR-253, while other cell lines of lung cancer, bladder cancer, colon cancer, prostate cancer, melanoma, and breast cancer, are less sensitive to LOR-253. The variability in sensitivity to LOR-253 may be attributable to the extent of innate KLF4 suppression. The IC_{50} values of LOR-253 for each leukemia and lymphoma cell lines are shown below.

Cancer Type	Cell Line	IC_{50} (μM)
Acute myeloid leukemia	Kasumi-1	0.0069
	KG-1	0.033
	HL-60	0.046
	EOL-1	0.058
	NB4	0.06
	MV-4-11	0.069
	OCI-AML2	0.076
	THP-1	0.077
	MOLM13 (CD47+)	0.13
	NOMO-1	0.121
	SKM-1	0.145
	HEL92.1.7	0.305
Acute lymphoblastic leukemia	CCRF-CEM	0.039
	MOLT-4	0.07
	Jurkat	0.071
Chronic Myelogenous Leukemia	K562	0.25
Non-Hodgkin's Lymphoma	Ramos	0.125
	Raji	0.011
	CA46	0.19
	Toledo	0.077
	DB	0.099
	Mino	0.14
	RL	0.19
Multiple myeloma	MM.1R	0.15
	U266B1	0.18
	HuNS1	0.072

Example 2

KLF4/p21 Induction in AML Cell Lines In Vitro

[0270] To determine if KLF4 and/or p21 expressions are induced by LOR-253, AML cells (THP1, HL-60) were treated with DMSO (vehicle control) or 0.5 μM LOR-253 for 16 hours. Total RNA was extracted using the TRIzol Plus RNA Purification kit (Ambion, Life Technologies), according to the manufacturer's instructions. First strand cDNA was synthesized from 1-2 ug total RNA using random hexamer primers (Invitrogen) and the SuperScript II Reverse Transcriptase kit (Invitrogen). Quantitative RT-PCR was performed in the ABI Prism 7000 Sequence Detection System using cDNAs and human TaqMan Gene Expression Assay primer/probe sets for Krüppel-like factor 4 (KLF4), cyclin-dependent kinase inhibitor 1A (p21) and the ABI TaqMan Universal PCR master mix protocol. Gene expression was normalized with β-actin gene expression in the same sample, and fold changes in KLF4 or p21 were expressed relative to the corresponding expression level in the DMSO treated samples using the comparative CT method. Treatment of THP1 and HL-60 AML cell lines with LOR-253 results in increased expression KLF4 and p21 (See FIG. **4**).

Example 3

Treatment with LOR-253 Results in G1/S Cell Cycle Arrest in AML Cell Lines

[0271] THP1 and HL-60 AML cell lines were treated with DMSO (vehicle control) or 0.5 μM LOR-253 for 16 hours. Cells were washed once in PBS+1% FCS and fixed using ice cold 70% ethanol overnight. Fixed cells were washed twice, resuspended in PI/RNaseA solution, containing 20 μg/mL propidium iodide and 250 μg/mL RNaseA, and stained for 30 minutes at 37° C. Stained cells were analyzed using a BD FACSCalibur flow cytometer. The results indicate treatment with LOR-253 results in G1/S cell cycle arrest in AML cell lines (FIG. **5**).

Example 4

Treatment with LOR-253 Induces Apotosis in Cell Lines

[0272] THP-1 cells were treated with DMSO, 0.5 or 1 uM of LOR-253 for 16 hours. Cells were washed twice with cold PBS and resuspended Annexin V binding buffer. 1×10^5 cells in 100 μL were stained with FITC-Annexin V and propidium iodide, and incubated for 15 minutes at room temperature. After staining, 400 ul of binding buffer was added and the cells were kept on ice until analyzed on a BD FACSCanto flow cytometer. THP-1 cells treated with LOR-253 showed elevated Annexin V staining, indicating induction of apoptosis. Increasing concentration of LOR-253 used to treat the cells resulted in an increase in the early apoptotic population (Q3: Annexin V+/PI−). The results indicate treatment with LOR-253 induces apotosis in AML cell lines (FIG. **6**).

[0273] THP1 and HL-60 cells were treated with DMSO (vehicle control) or 0.5 μM LOR-253 for 48 hours. Cell lysates were collected using a 1% Triton-X100 lysis. Caspase 3 activity was measured using EnzChek Caspase-3 Assay Kit #1 (Life Technologies) with 5 μg of cell lysastes according to manufacturer's protocol. Treatment of THP1 and HL-60 cells with LOR-253 results in elevated Caspase 3 activity, indicating induction of apoptosis (FIG. **7**).

[0274] THP1 cells were treated with DMSO (vehicle control) or 0.5 μM LOR-253 for 48 hours. Total RNA was extracted using the TRIzol Plus RNA Purification kit (Ambion, Life Technologies), according to the manufacturer's instructions. First strand cDNA was synthesized from 1-2 ug total RNA using random hexamer primers (Invitrogen) and the SuperScript II Reverse Transcriptase kit (Invitrogen). Quantitative RT-PCR was performed in the ABI Prism 7000 Sequence Detection System using cDNAs and human TaqMan Gene Expression Assay primer/probe sets for BCL2-associated X protein (BAX), B-cell CLL/Lymphoma 2 (BCL2) and the ABI TaqMan Universal PCR master mix protocol. Gene expression was normalized with β-actin gene expression in the same sample, and fold changes in BAX or BCL2 were expressed relative to the corresponding expression level in the DMSO treated samples using the comparative CT method. Elevation of BAX and repression of BCL2 upon treatment of THP1 cells with LOR-253 indicates induction of apoptosis (FIG. **8**).

Example 5

In Vivo Efficacy of LOR-253 HCL in H226 Xenograft Model—Dose and Schedule

[0275] H226 model mice were treated according to several administration schedules as shown in the table below.

Group	Dose level	Administration Schedule (days)/cycle	Treatment (1 cycle = 28 days)
1	n = 8 10 mg/kg-iv	2T-12B-2T	2 cycles
2	n = 8 10 mg/kg-iv	3T-12B-3T	2 cycles

-continued

Group	Dose level	Administration Schedule (days)/cycle	Treatment (1 cycle = 28 days)
3	n = 8 10 mg/kg-iv	2T-5B-2T	2 cycles
4	n = 8 10 mg/kg-iv	3T-5B-3T	2 cycles
5	n = 8 Vehicle alone	3T-5B-3T	2 cycles

T = consecutive days treatment

B = break without treatment

[0276] The results show that groups 2, 3, and 4 are effectively treated by LOR-253 (FIG. **9**). The results indicates that schedule with one week gap is superior to two-week gap, and suggests weekly administration schedule is preferred.

Example 6

In Vivo Dose-Dependent Pharmacokinetic (PK) and Pharmacodynamic (PD) Responses in Xenograft CD-1 Nude Mice Treated by LOR-253 HCL

[0277] To study the pharmacokinetic (PK) in tumor cells treated by LOR-253, CD-1 nude mice were treated by i.v. bolus injections of LOR-253HCl at 1, 5, and 15 mg/kg. The serum level of LOR-253 was measured. The result indicates that the serum level of LOR-253 has a dose related increase (FIG. **10**).

[0278] To study the pharmacodynamic (PD) responses, mice were treated for 5 consecutive days with 1, 5, and 15 mg/kg LOR-253. Tumors were measured 16 hours after the last dose, and the KLF-4 protein level was measured. Average KLF4 protein levels increased in a dose-dependent manner, correlating with tumor biodistribution and dose-response antitumor activity (FIG. **11**).

Example 7

LOR-253 for Treating AML, Phase I Clinical Trial

[0279] Preclinical testing established a broad therapeutic index for LOR-253: efficacy of LOR-253 was demonstrated in xenograft models against multiple tumor types; extensive GLP safety and PK studies were performed in rats and dogs; no treatment-related cardiovascular effects were observed in repeat-dose toxicity study of dogs at highest dose tested (data not shown); no significant inhibition of hERG tail current density or CYP450 enzymes; and GLP blood compatibility studies confirmed the suitability of IV formulation.

[0280] Phase I: This was an open-label, phase 1 study to determine the maximum tolerated dose (MTD) or appropriate target dose if MTD not reached to identify the recommended phase 2 dose of LOR-253HCl in patients with advanced or metastatic solid tumours. In the first set of doses, LOR-253HCl was given in ascending doses until the maximum administered dose or appropriate target dose is reached. In the other set of doses, LOR-253HCl was given in ascending doses starting from 20 mg/m^2 until the maximum administered dose or appropriate target dose is reached. Patient as treated on LOR-253HCl for 2 cycles for the evaluation.

[0281] Other Name: No other names are used.

[0282] Study Type: Interventional

[0283] Study Design: Endpoint Classification: Safety Study

[0284] Intervention Model: Single Group Assignment

[0285] Masking: Open Label

[0286] Primary Purpose: Treatment

[0287] Official Title: Open-Label, Phase 1 Study of LOR-253HCl in Patients With Advanced or Metastatic Solid Tumours

Primary Outcome Measures:

[0288] To determine the maximum tolerated dose (MTD) or appropriate target dose if MTD not reached to identify the recommended phase 2 dose of LOR-253HCl in patients with advanced or metastatic solid tumours. [Time Frame: 8 weeks]

Secondary Outcome Measures:

[0289] To characterize the safety profile of LOR-253HCl when administered to patients with advanced or metastatic solid tumours. [Time Frame: 8 weeks]

Inclusion Criteria:

[0290] 1. Male or female 18 years of age or older.

[0291] 2. Histologically confirmed diagnosis of solid tumor for which no effective therapy is available or that is unresponsive to conventional therapy.

[0292] 3. Meet laboratory parameter requirements at study entry.

Exclusion Criteria:

[0293] 1. Chemotherapy, radiotherapy, biologic therapy, immunotherapy or any other investigational drugs within 21 days of beginning study treatment with LOR-253HCl.

[0294] 2. A hematologic malignancy.

[0295] 3. A history of brain or other central nervous system metastases.

[0296] 4. Have a presence of a significant infection.

[0297] 5. Clinically significant autoimmune disease.

[0298] 6. Uncontrolled intercurrent illness.

[0299] 7. With iron or copper overload syndromes.

[0300] 8. Pregnancy or breast feeding.

[0301] Safety and Antitumor Activity Demonstrated in Phase I Trial:

[0302] Dose-Escalating Study in Patients with Advanced Solid Tumors

[0303] Excellent Safety Profile (N=27 Patients) across 7 dose levels

[0304] Most common AE and SAE: Fatigue and 1 case of reversible hypophosphatemia

[0305] Stable Disease achieved in 41% of evaluable patients; No RECIST PR

[0306] Tumor shrinkage (FIG. **12**) in patient with NSCLC (poorly differentiated adenocarcinoma) and extensive metastases refractory to prior standard and investigational therapies

[0307] The phase I trial results demonstrated that LOR-253 is a safe and active drug for treating solid tumors.

Example 8

Absolute Quantification of CDX2 and KLF4

[0308] Absolute quantitation was performed using serially diluted standards of known concentrations of CDX2 and KLF4 genes to generate a standard curve. Plasmids containing CDX2 or KLF4 were prepared at 3×10^2-3×10^6 copies (FIG. **13**). Total RNA was extracted from cells using the

TRIzol Plus RNA Purification kit (Ambion, Life Technologies), according to the manufacturer's instructions. First strand cDNA was synthesized from 1-2 ug total RNA using random hexamer primers (Invitrogen) and the SuperScript II Reverse Transcriptase kit (Invitrogen). Quantitative RT-PCR was performed in the ABI Prism 7000 Sequence Detection System using cDNAs and human TaqMan Gene Expression Assay primer/probe sets for Krüppel-like factor 4 (KLF4), caudal type homeobox 2 (CDX2), and the ABI TaqMan Universal PCR master mix protocol. KLF4 and CDX2 expression levels in MM.1R, Eol-1, Mino, HEL92.1.7, CA46, RL, THP1, and Toledo cell lines were determined as copy numbers using the standard curves (FIG. **14** and FIG. **15**). Ratios of CDX2/KLF4 and KLF4/CDX2 copy numbers were calculated and plotted on a logarithmic scale against IC_{50} values. The results indicate that CDX2/KLF4 ratios inversely correlate with IC_{50} values in AML cell lines i.e. cells with higher CDX2/KLF4 ratios have lower IC_{50} values. Conversely, KLF4/CDX2 ratios directly correlate with IC50 i.e. cells with low KLF4/CDX2 ratios have low IC_{50} values. See table below and FIG. **16** and FIG. **17**.

CDX2 and KLF4 expression levels and ratios in heme cancers

Cell line	CDX2	KLF4	CDX2/KLF4	KLF4/CDX2
MM.1R	3.99	537.74	0.0074	134.77
EOL-1	21729.06	2104.58	10.32	0.0968
Mino	1.84	11.12	0.165	6.04
THP-1	216.68	8730.42	0.025	40.3
Toledo	10.01	8.87	1.13	0.886
RL	11.56	UD	—	—
CA46	33.18	UD	—	—
HEL92.1.7	4.56	UD	—	—

UD, undetectable

[0309] Additional experiments of absolute quantitation were performed using the same method on more cell lines including MM.1R, Eol-1, Mino, HEL92.1.7, CA46, RL, THP1, Toledo, U266B1, MV411, DB, HL60, CD47 LOW (or MOLM13 CD47−), CD47 HIGH (or MOLM13 CD47+), KG-1, and SKM-1 cell lines. The results are shown in FIG. **27** and FIG. **28**, which indicate the same findings as described above.

Example 9

In Vivo Efficacy of LOR-253 HCL in a Kasumi-1 AML Xenograft Model

[0310] The anti-tumor activity of LOR-253HCl was evaluated in another in vivo animal model of human AML. The human AML cell line Kasumi-1 was implanted subcutaneously into athymic nude mice. Some tumor-bearing mice were treated with LOR-253HCl at 30 mg/kg (15 mg/kg twice per day) for two consecutive days per week for four weeks (the "LOR-253 treatment group"). LOR-253 was administered by intravenous (i.v.) bolus injection as the hydrochloric salt form (LOR-253-HCl) formulated in 20% Polyethylene Glycol 400, 10% Propylene Glycol, and 10% Solutol HS in 60% water. Other tumor-bearing mice were treated with a formulation that did not have LOR-253 (the "vehicle control group").

[0311] The major endpoint was to observe for tumor growth inhibition after treatment with LOR-253HCl and compare the anti-tumor effects of different dosing schedules. Tumor sizes were measured three times per week from day 10 after the tumor cell inoculation. Tumors were measured in three dimensions using calipers and the volume was expressed in millimeters cubed using the formula: V=0.5 a×b×c, where a, b, and c are the length, width, and height of the tumor, respectively. Mean tumor volumes+/−standard error (SE) were calculated from each measurement and then plotted in a standard graph to compare the anti-tumor efficacy of drug treatment to that of control (FIG. **20**). Toxicity was assessed by clinical observations and by measurement of mouse body weight in grams twice per week over the course of the study. Mean body weights were calculated from each measurement and then plotted to compare body weight changes in the drug treatment group to that of control (FIG. **21**).

[0312] The tumor inhibition results as shown in FIG. **20** demonstrate that the LOR-253 treatment group produced a statistically significantly increased inhibitory effect in this model compared to the vehicle control group (p=0.028 by Student's t-test). In addition, the toxicity results as shown in FIG. **21** demonstrate that the mice in the treatment group did not show body weight loss. The mice in the treatment group also did not show other overt signs of toxicity. These results indicate that this treatment schedule was well tolerated. LOR-253HCl showed significant tumor growth inhibition as a single agent at twice per week dosing without obvious signs of toxicity, suggesting that LOR-253HCl has a sufficient therapeutic window and that this agent is a potential chemotherapeutic agent for the treatment of AML.

Example 10

In Vivo Efficacy of LOR-253 HCL in an HL-60 AML Xenograft Model

[0313] The anti-tumor activity of LOR-253HCl, as a single agent and in combination with azacitidine, was evaluated in another in vivo animal model of human AML. The human AML cell line HL-60 was implanted subcutaneously into athymic nude mice. Tumor-bearing mice were treated with LOR-253HCl alone or in combination with azacitidine, with azacitidine alone, or with a negative control vehicle. The detailed treatment conditions are as follows.

[0314] Group 1: Negative control group. LOR-253HCl control vehicle, 2×/day, 3 cycles of 2 consecutive days, 5 days between cycles, i.v., plus 1% D-mannitol every four days by s.c. injection

[0315] Group 2: Azacitidine (in 1% D-mannitol) at 10 mg/kg 1× on days 1, 4, 8, 11, 15 and 18 by subcutaneous (s.c.) injection

[0316] Group 3: LOR-253HCl at 15 mg/kg bid for 3 cycles, each cycle is 2 consecutive days of dosing per week with 5 days of non-dosing, i.v., n=9 (2T-5B)

[0317] Group 4: LOR-253HCl at 15 mg/kg 2×/day (bid) for 3 cycles, each cycle is 1 day of dosing per week with 6 days of non-dosing, i.v., n=9 (1T-6B)

[0318] Group 5: Combination of LOR-253 (2T-5B) and azacitidine. LOR-253HCl at 15 mg/kg bid for 3 cycles, each cycle is 2 consecutive days of dosing per week with 5 days of non-dosing, i.v., n=9 (2T-5B) plus azacitidine at 10 mg/kg 1× every four days by s.c. injection

[0319] Group 6: Combination of LOR-253 (1T-6B) and azacitidine. LOR-253HCl at 15 mg/kg bid for 3 cycles, each cycle is 1 day of dosing per week with 6 days of non-dosing, i.v., n=9 (1T-6B) plus azacitidine at 10 mg/kg 1× every four days by s.c. injection

[0320] The major endpoint was to observe for tumor growth inhibition after treatment with LOR-253HCl in combination with azacitidine. Tumor sizes were measured three times per week. Tumors were measured in three dimensions using calipers and the volume was expressed in millimeters cubed using the formula: V=0.5 a×b×c, where a, b, and c are the length, width, and height of the tumor, respectively. Mean tumor volumes+/−standard error (SE) were calculated from each measurement and then plotted in a standard graph to compare the anti-tumor efficacy of drug treatment to that of control (FIG. **22**).

[0321] The tumor inhibition results as shown in FIG. **22** demonstrate that LOR-253HCl administered alone at 15 mg/kg twice per day for either one (Group 4) or two (Group 3) consecutive days per week inhibited growth of HL-60 tumors to approximately the same extent as or slightly more than azacitidine alone. Both once and twice weekly dosing of LOR-253HCl in combination with azacitadine (Group 6 and Group 5, respectively) resulted in significantly higher levels of tumor growth inhibition compared to either single agent alone ($p=0.0002$ and $p=0.0006$ for 1× and 2×LOR-253HCl treatment, respectively; compared to control, as determined by Student's t-test). FIG. **23** and FIG. **24** show the tumor size data from individual animals at the beginning (Day 1) and end (Day 19) of this study.

[0322] Because LOR-253HCl in combination with azacitadine resulted in even higher levels of tumor growth inhibition than either single agent alone, LOR-253HCl may also provide additive anticancer efficacy to a standard of care chemotherapeutic for hematological malignancies.

Example 11

In Vivo Efficacy of LOR-253 HCL in a KG-1 AML Xenograft Model

[0323] The anti-tumor activity of LOR-253HCl was evaluated in yet another in vivo animal model of human AML. Xenograft model mice for AML cell line KG-1 was generated with the same method as in Examples 9 and 10, and were treated with LOR-253HCl or control according to the following regime.

[0324] Control-IV-Day 1

[0325] LOR-253-15 mg/kg-iv, bid, 2T/wk-Day 1

[0326] Control-IV-Day 8

[0327] LOR-253-15 mg/kg-iv, bid, 2T/wk-Day 8

[0328] Control-IV-Day 13

[0329] LOR-253-15 mg/kg-iv, bid, 2T/wk-Day 13

[0330] Control-IV-Day 16

[0331] LOR-253-15 mg/kg-iv, bid, 2T/wk-Day 16

[0332] Control-IV-Day 20

[0333] LOR-253-15 mg/kg-iv, bid, 2T/wk-Day 20

[0334] Control-IV-Day 26

[0335] LOR-253-15 mg/kg-iv, bid, 2T/wk-Day 26

[0336] Tumor sizes were measure as described in Examples 9 and 10, and the results are shown in FIG. **25**. LOR-253HCl showed significant tumor growth inhibition as a single agent in this AML animal model as well.

Example 12

In Vivo Efficacy of LOR-253 HCL in a THP-1 AML Xenograft Model

[0337] The anti-tumor activity of LOR-253HCl, as a single agent and in combination with azacitidine, was evaluated in yet another in vivo animal model of human AML. Xenograft model mice for AML cell line THP-1 was generated with the same method as in Examples 9 and 10, and were treated with LOR-253HCl alone or in combination with azacitidine, with azacitidine alone, or with a negative control vehicle, and tumor sizes were measured as described in Examples 9 and 10. The detailed treatment conditions are shown as follows, and the results are shown in FIG. **26**.

[0338] Group 1 Control: received i.v. treatments with LOR-253 control vehicle (CV) on Days 1, 2, 8, 9, 15 &16; received subcutaneous (SC) treatments with Azacitidine control vehicle on Days 1, 4, 8, 11, 15, 18, 22, 25, 29 &32; and received intraperitoneal (IP) treatments with LOR-253-CV on Days 22, 23, 24, 25, 29&30.

[0339] Group-2-LOR-253: received i.v. treatments on Days 1, 2, 8, 9, 15 &16; received IP treatments on Days 22, 23, 24, 25, 29&30

[0340] Group-3-Azacitidine: received SC treatments on Days 1, 4, 8, 11, 15, 18, 22, 25, 29 &32.

[0341] Group-4-Combination: received i.v. treatments with LOR-253 on Days 1, 2, 8, 9, 15 &16; received IP treatments with LOR-253 on Days 22, 23, 24 &25; and received SC-treatment with Azacitidine on Days 1, 4, 8, 11, 15, 18, 22 &25

[0342] The tumor inhibition results of this Example demonstrate that LOR-253HCl administered alone inhibited growth of THP-1 tumors to about the same extent as or slightly more than azacitidine alone. When used in combination with azacitadine, LOR-253HCl again resulted in significantly higher levels of tumor growth inhibition.

[0343] The disclosures, including the claims, figures and/or drawings, of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entireties.

[0344] Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes.

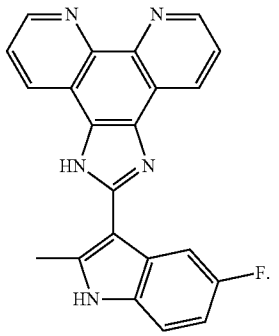
[0345] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

[0346] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

What is claimed:

1. A method for determining the responsiveness of a human subject comprising determining the level of activity of KLF4 in a biological sample from a human subject, wherein a lower than normal level of KLF4 activity is indicative that the human subject is responsive to the compound of formula I:

(I)



2. The method of claim 1, wherein a lower than normal level of KLF4 activity is indicative that the human subject is responsive to the compound of formula I for treatment of acute myelogenous leukemia (AML).

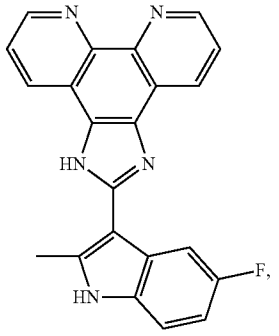
3. The method of claim 1, wherein a lower than normal level of KLF4 activity is indicative that the human subject is responsive to the compound of formula I for treatment of myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), adult T-cell leukaemia (ATLL), lymphoma, gastric cancer, or multiple myeloma.

4. The method of claim 1, wherein the human subject has AML.

5. The method of claim 1, wherein the human subject has MDS, ALL, CML, ATLL, lymphoma, gastric cancer, or multiple myeloma.

6. A method for monitoring the efficacy of the compound of formula I comprising determining the level of activity of KLF4 in a human subject under treatment with the compound of formula I:

(I)



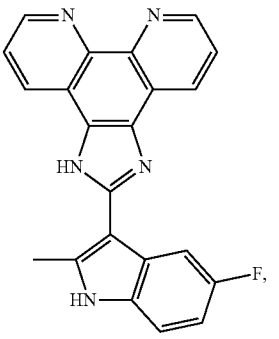
wherein an increase of the level of activity of KLF4 directly related to the treatment with the compound of formula I indicates the efficacy of the compound of formula I and wherein a decrease or unchanged level of activity of KLF4 during the treatment with the compound of formula I indicates the lack of efficacy of the compound of formula I.

7. The method of claim 6, wherein the human subject is treated for AML.

8. The method of claim 6, wherein the human subject is treated for MDS, ALL, CML, ATLL, lymphoma, gastric cancer, or multiple myeloma.

9. A method for treating a human subject comprising determining the level of activity of KLF4 in a human subject and administering to the human subject an effective amount of a compound or a pharmacologically acceptable salt or solvate thereof, the compound having formula I:

(I)



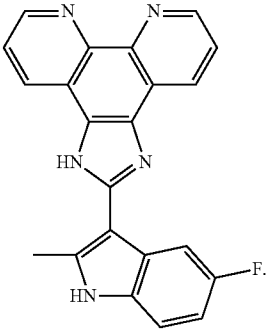
if the level of KLF4 activity of the human subject is lower than normal.

10. The method of claim 9, wherein the human subject has AML.

11. The method of claim 9, wherein the human subject has MDS, ALL, CML, ATLL, lymphoma, gastric cancer, or multiple myeloma.

12. A method for determining the responsiveness of a human subject comprising assaying for a genetic alteration of CDX2 or determining the level of activity of CDX2 in a biological sample from a human subject, wherein the presence of a genetic alteration of CDX2 or an abnormal level of CDX2 activity is indicative that the human subject is responsive to the compound of formula I:

(I)



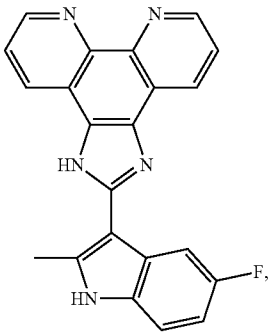
13. The method of claim 12, wherein the presence of a genetic alteration of CDX2 or an abnormal level of CDX2 activity is indicative that the human subject is responsive to the compound of formula I for treatment of AML.

14. The method of claim 12, wherein the presence of a genetic alteration of CDX2 or an abnormal level of CDX2 activity is indicative that the human subject is responsive to the compound of formula I for treatment of MDS, ALL, CML, ATLL, lymphoma, gastric cancer, colorectal cancer or multiple myeloma.

15. The method of claim 12, wherein the human subject has AML.

16. The method of claim **1**, wherein the human subject has MDS, ALL, CML, ATLL, lymphoma, gastric cancer, colorectal cancer or multiple myeloma.

17. A method for treating a human subject comprising assaying for a genetic alteration of CDX2 or determining the level of activity of CDX2 in a biological sample from a human subject and administering to the human subject an effective amount of a compound or a pharmacologically acceptable salt or solvate thereof, the compound having formula I:



(I)

if the level of activity of CDX2 of the human subject is higher than normal or in the presence of a genetic alteration of CDX2 in the human subject.

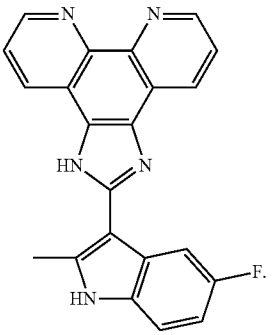
18. The method of claim **17**, wherein the human subject has AML.

19. The method of claim **17**, wherein the human subject has MDS, ALL, CML, ATLL, lymphoma, gastric cancer, colorectal cancer or multiple myeloma.

20. The method of claim **1**, further comprising assaying for a genetic alteration of CDX2 or determining the level of activity of CDX2 in the biological sample from the human subject, wherein a lower than normal level of KLF4 activity in combination with the presence of a genetic alteration of CDX2 or a higher than normal level of CDX2 activity is indicative that the human subject is responsive to the compound of formula I.

21. The method of claim **9**, further comprising assaying for a genetic alteration of CDX2 or determining the level of activity of CDX2 in the biological sample from the human subject, and administering to the human subject an effective amount of the compound of formula I, or the pharmacologically acceptable salt or solvate thereof, if the level of KLF4 activity of the human subject is lower than normal in combination with the presence of a genetic alteration of CDX2 or a higher than normal level of CDX2 activity.

22. A method for determining the responsiveness of a human subject comprising determining the level of activity of at least two biomarkers in a biological sample from a human subject, wherein at least one biomarker is CDX2, and at least one other biomarker is KLF4, wherein an abnormally high ratio of CDX2/KLF4 activity and/or an abnormally low ratio of KLF4/CDX2 is indicative that the human subject is responsive to the compound of formula I:



(I)

23. The method of claim **22**, wherein the activity is gene copy number.

24. The method of claim **22**, wherein an abnormally high ratio of CDX2/KLF4 activity and/or an abnormally low ratio of KLF4/CDX2 is indicative that the human subject is responsive to the compound of formula I for treatment of AML.

25. The method of claim **22**, wherein an abnormally high ratio of CDX2/KLF4 activity and/or an abnormally low ratio of KLF4/CDX2 is indicative that the human subject is responsive to the compound of formula I for treatment of MDS, ALL, CML, ATLL, lymphoma, gastric cancer, colorectal cancer or multiple myeloma.

* * * * *