



Dnai for the modulation of genes

Abstract

The present invention relates to methods and compositions for the inhibition of gene expression. In particular, the present invention provides oligonucleotide-based therapeutics for the inhibition genes implicated in many diseases.

Classifications

C12N15/113 Non-coding nucleic acids modulating the expression of genes, e.g. antisense oligonucleotides; Antisense DNA or RNA; Triplex- forming oligonucleotides; Catalytic nucleic acids, e.g. ribozymes; Nucleic acids used in co-suppression or gene silencing

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Priority date : [2013-03-15](#)

Family: [US \(1\)](#) [EP \(1\)](#) [WO \(1\)](#)

Date	App/Pub Number	Status
2014-03-14	EP20140724865	Withdrawn
2016-01-20	EP2970965A2	Application

Info: [Patent citations \(2\)](#), [Non-patent citations \(1\)](#), [Legal events](#), [Similar documents](#), [Priority and Related Applications](#)

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Claims

An oligonucleotide that hybridizes to a non-coding region in or upstream of a promoter for a target gene, wherein the oligonucleotide comprises: a length of 20-34 bases; at least one CG pair; at least 40% C and G content; no more than five consecutive bases of the same nucleotide; and at least one secondary structure for said oligonucleotide.

2. The oligonucleotide of claim 1, wherein said oligonucleotide comprises a C and G content of at least 50%.
3. The oligonucleotide of claim 1, wherein said oligonucleotide comprises a C and G content from about 50 to 80%.
4. The oligonucleotide of claim 1, wherein said oligonucleotide comprises at least two CG pairs.
5. The oligonucleotide of claim 1, wherein said oligonucleotide hybridizes within a CG region, CpG island region, nuclease hypersensitive site, or CIS regulatory region.
6. The oligonucleotide of claim 1, wherein said non-coding region is located within a CG region, CpG island, nuclease hypersensitive site, or CIS regulatory region.
7. The oligonucleotide of claim 1, wherein said oligonucleotide is a reverse and full complement of a sense strand of said non-coding region of the target gene.
8. The oligonucleotide of claim 1, wherein said oligonucleotide is unique to the nucleotide sequence of the non-coding region.
9. The oligonucleotide of claim 1, wherein the nucleotide sequence of the non-coding region is not duplicated in a genome comprising the target gene.
10. The oligonucleotide of claim 1, wherein the nucleotide sequence of the non-coding region comprises less than 80% homology to other nucleotide sequences in a genome with a target gene.
11. The oligonucleotide of claim 1, wherein the nucleotide sequence of the non-coding region comprises less than 50% homology to other nucleotide sequences in a genome with a target gene.
12. The oligonucleotide of claim 1, wherein said oligonucleotide comprises at least four bases in a linear section of the secondary structure.
13. The oligonucleotide of claim 1, wherein said oligonucleotide comprises at least five bases in a linear section of the secondary structure.
14. The oligonucleotide of claim 1, wherein said oligonucleotide comprises at least one CG pair within the first 40% of the bases of said oligonucleotide.
15. The oligonucleotide of claim 1, wherein said oligonucleotide comprises at least one CG pair within the first 50% of the bases of said oligonucleotide.
16. The oligonucleotide of claim 1, wherein said oligonucleotide further comprises at least one CG pair that is prior to or in the nonlinear section of the secondary structure.
17. The oligonucleotide of claim 1, wherein said oligonucleotide comprises a linear section before a secondary structure, no oligonucleotides that extend beyond the secondary structure, and at least one CG pair within the linear section or the secondary structure.

The oligonucleotide of claim 1, wherein said oligonucleotide has a linear section before a secondary structure and no oligonucleotides that extend beyond the secondary structure

The oligonucleotide of claim 1, wherein said oligonucleotide does not comprise a single G or T base after the nonlinear section of the secondary structure.

The oligonucleotide of claim 1, wherein said secondary structure comprises at least one hairpin loop.

The oligonucleotide of claim 1, wherein said secondary structure comprises at least two hairpin loops.

The oligonucleotide of claims 19 or 20, wherein said secondary structure comprises at least three nucleotide bridges in the nonlinear section of the secondary structure.

The oligonucleotide of claim 1, wherein said oligonucleotide comprises a theoretical AG between -0.1 to -7.

The oligonucleotide of claim 23, wherein said theoretical AG is between -1 to -5.

The oligonucleotide of claim 1, wherein said oligonucleotide comprises a theoretical AT_m between 30-70 degrees Celsius.

The oligonucleotide of claim 1, wherein said oligonucleotide begins at the 5' end with the bases selected from CG, CGG, CGC, CGT, CGA, GCG, CCC, CCG, GTC, TCC, TCG, ACG, CAC, CAG, GAG, AGA, GAC, GAA, AGC, or GCC.

The oligonucleotide of claim 1, wherein said oligonucleotide ends at the 3' end with the bases selected from CG, GCG, GGC, CGG, GCC, CGC, CCG, ACG, TCG, GGG, TGC, CCC, GTG, or CTC.

28. The oligonucleotide of claim 1, wherein said non-coding region is located less than 7000 bases upstream of the coding region of the target gene.

29. The oligonucleotide of claim 1, wherein said non-coding region is located less than 5000 bases upstream of the coding region of the target gene.

30. The oligonucleotide of claim 1, wherein said non-coding region is located less than 3000 bases upstream of the coding region of the target gene.

31. The oligonucleotide of claim 1, wherein said non-coding region is located less than 1000 bases upstream of the coding region of the target gene.

32. The oligonucleotide of claim 1, wherein said non-coding region is located less than 500 bases up- or downstream of a transcription factor binding site or translocation site of target gene.

33. The oligonucleotide of claim 1, wherein said non-coding region is located less than 100 bases up- or downstream of a transcription factor binding site or translocation site of target gene.

34. The oligonucleotide of claim 1, wherein said oligonucleotide does not comprise a CpG Coley motif.

35. The oligonucleotide of claim 1, wherein said oligonucleotide does not form a triplex structure.

36. The oligonucleotide of claim 1, wherein said oligonucleotide does not form a G- quadruplex structure.

37. The oligonucleotide of claim 1, wherein said oligonucleotide is a single stranded DNA.

38. The oligonucleotide of claim 1, wherein said oligonucleotide hybridizes to an Spl motif or transcription factor binding site.

39. The oligonucleotide of claim 1, wherein said target gene is selected from Survivin, Beclin-1, STAT3, HIF1A, IL-8, KRAS, MTTP, ApoC III, ApoB, IL-17, MMP2, FAP, P-selectin, IL-6, IL-23, AKT, CRAF, Beta-catenin, PCSK9, MEK1, MEK2, CD4, WNT1, Clusterin, NRAS, EZH2, HD AC 1, PD-1, TNFa, MIF1, TTR, HBV, HAMP, ERBB2, PARP1, ITGA4, APP, FGFR1, CD68, ALK, MSI2, JAK2, CCND1, or selected from Table 2.

40. The oligonucleotide of claim 1, wherein said oligonucleotide is selected from the group consisting of any of the sequences disclosed in Table 3.

41. The oligonucleotides of claim 1, wherein said oligonucleotide hybridizes to a hot zone of a target gene.

42. The oligonucleotide of claim 1, wherein at least one of the cytosine bases in said oligonucleotide is 5-methylcytosine.

43. The oligonucleotide of claim 1, wherein at least one of the cytosine bases in said CG pair is 5-methylcytosine.

44. The oligonucleotide of claim 1, wherein all of said cytosine bases in said oligonucleotide are 5-methylcytosine.

45. The oligonucleotide of claim 1, wherein said hybridization of said oligonucleotide to the non-coding region modulates the target gene.

46. The oligonucleotide of claim 1, wherein said hybridization of said oligonucleotide to the non-coding region of the target gene modulates expression or transcription of said target gene.

47. The oligonucleotide of claim 1, wherein said hybridization of said oligonucleotide to the non-coding region of the target gene modulates a cell signaling pathway.

48. The oligonucleotide of claim 1, wherein said hybridization of said oligonucleotide to the non-coding region of said target gene produces phenotypic changes in a mammal.

49. The oligonucleotide of claim 1, wherein said hybridization of said oligonucleotide to the non-coding region of said target gene influences a non-gene target due to a chromosomal rearrangement.

50. The oligonucleotide of claim 1, wherein said target gene is on a chromosome of a cell, and wherein said hybridization of said oligonucleotide to said non-coding region reduces proliferation of said cell.

51. The oligonucleotide of claim 1, wherein said target gene is an oncogene.

52. A composition comprising an oligonucleotide according to any one of claims 1-51 and a pharmaceutically acceptable carrier.

53. The composition of claim 52, wherein the pharmaceutically acceptable carrier is a liposome.

54. The composition of claim 53, wherein the liposome is an amphoteric liposome.
55. The composition of claim 53, wherein the liposome comprises a neutral lipid.
56. The composition of claim 53, wherein the liposome comprises a mixture of neutral lipids and lipids with amphoteric properties, wherein the mixture of lipid components comprises anionic and cationic properties and at least one such component is pH responsive.
57. The composition according to any one of claims 52-56, wherein the composition further comprises an additional therapeutic agent.
58. The composition of claim 57, wherein the additional therapeutic agent is a second oligonucleotide, chemotherapeutic agent, immunotherapeutic agent, or radiotherapy.
59. The composition of claim 52, wherein said composition has two (2) therapeutic agents.
60. The composition of claim 59, wherein one therapeutic agent treats a cancer disease and the other therapeutic agent treats a non-cancer disease.
61. A method of inhibiting protein expressing in a cell with a target gene comprising introducing into said cell an oligonucleotide according to any one of claims 1-51 or composition according to any one of claims 52-60.
62. A method of mediating target-specific RNA in a mammalian cell in vitro, comprising contacting said mammalian cell in vitro with an oligonucleotide according to any one of claims 1-51 or composition according to any one of claims 52-60.
63. A method of mediating protein down regulation in a mammalian cell in vitro, comprising contacting said mammalian cell in vitro with an oligonucleotide according to any one of claims 1-51 or composition according to any one of claims 52-60.
64. A method of treating a patient having a disease characterized by the presence or undesired production of a protein implicated in said disease, comprising administering to said patient a pharmaceutically effective amount of an oligonucleotide according to any one of claims 1-51 or composition according to any one of claims 52-60.
65. A method of treating a patient having a disease characterized by the presence or undesired production of a protein implicated in said disease, comprising administering to said patient a pharmaceutically effective amount between 1
- 2 2
- mg/m and 500 mg/m of an oligonucleotide according to any one of claims 1-51 or composition according to any one of claims 52-60.
66. A method of treating a mammal having a disease characterized by the presence or undesired production of a protein implicated in disease, comprising administering to said mammal a pharmaceutically effective amount of an oligonucleotide according to any one of claims 1-51 or composition according to the description and the compositions in any of claims 52-60.
67. A method of treating a plant having a disease characterized by the presence or undesired production of a protein implicated in disease, comprising introducing to said plant an effective amount of an oligonucleotide according to any one of claims 1-51 or composition according to the description and the compositions in any of claims 52-60.
68. A method of administration of a therapeutic disclosed herein and a oligonucleotide according to any one of claims 1-51 or a composition according to any one of claim 52-60, wherein said administration is through a route selected from oral, vapor, inhalation, dermal, subdermal, subcutaneous, parental, parenterally, ear, nose, nasally, buccally, eye, otic, ophthalmically, rectal, vaginal, suppository or implant, implanted reservoir, dermal, dermal skin patch, injection, or sub-lingual.
69. A method or kit for a diagnosis and treatment of a disease comprising the steps of administering to a patient a pharmaceutically effective amount of an oligonucleotides accordingly to any one of claims 1-51 or a composition according to any one of claims 52-60, wherein the patient is characterized by the presence of, or undesired production of, a protein implicated in said disease, and the method further comprising evaluating said patient for the presence of, or undesired production of said protein.
70. An single stranded DNA oligonucleotide that hybridizes to coding or non-coding region of a target gene, wherein the oligonucleotide comprises: a length of 12-50 bases; at least 30% C and G content; and no more than seven consecutive bases of the same nucleotide.
71. The oligonucleotide of claim 70, wherein the nucleotide sequence of the non- coding region comprises less than 80% homology to other nucleotide sequences in a genome with a target gene.
72. The oligonucleotide of claim 70, wherein said oligonucleotide comprises at least one CG pair within the first 40% of the bases of said oligonucleotide.
73. The oligonucleotide of claim 70 further comprising a secondary structure.
74. The oligonucleotide of claim 70, wherein said oligonucleotide comprises a theoretical AG between -0.1 to -7.
75. The oligonucleotide of claim 70, wherein said oligonucleotide comprises a theoretical ATm between 30-70 degrees Celsius.
76. The oligonucleotide of claim 70, wherein said non-coding region is located less than 7000 bases upstream of the coding region of the target gene.
77. The oligonucleotide of claim 70, wherein said non-coding region is located less than 500 bases up- or downstream of a transcription factor binding site or translocation site of target gene.
78. The oligonucleotide of claim 70, wherein said non-coding region is located with a CG region, nuclease hypersensitive site, or CpG island of the genome comprising the target gene.
79. The oligonucleotide of claim 70, further comprises at least one CG pair and

optionally at least one of the cytosine bases in said CG pair is 5-methylcytosine.

80. The oligonucleotide of claim 70, wherein said target gene is on a chromosome of a cell, and wherein said hybridization of said oligonucleotide reduces proliferation of said cell.

81. A composition comprising an oligonucleotide according to any one of claims 70- 80 and a pharmaceutically acceptable carrier.

82. The composition of claim 81, wherein the pharmaceutically acceptable carrier is a liposome.

83. The composition according to any one of claims 81 or 82 wherein the composition further comprises an additional therapeutic agent.

84. A method of inhibiting or silencing gene transcription in a cell with a target gene comprising introducing into said cell an oligonucleotide according to any one of claims 70- 80 or composition according to any one of claims 81-83.

85. A method of mediating target-specific RNA in a mammalian cell in vitro,

comprising contacting said mammalian cell in vitro with an oligonucleotide according to any one of claims 70-80 or composition according to any one of claims 81-83.

Description

DNAi for the Modulation of Genes

FIELD OF THE INVENTION

[0001] The present invention relates to methods and compositions for the inhibition of gene expression. In particular, the present invention provides oligonucleotide-based therapeutics for the inhibition or interference of genes involved and implicated in diseases and cell systems.

PRIORITY CLAIM

[0002] This application claims priority to U.S. Provisional Patent Application Number

61/794,778 filed on March 15, 2013. The entire contents of the aforementioned application are incorporated herein by reference.

SEQUENCE LISTING

[0003] This application incorporates by reference in its entirety the Sequence Listing entitled "DNAi3728_ST25.txt" (2.90 MB), which was created March 14, 2014 and filed electronically herewith.

BACKGROUND OF THE INVENTION

[0004] The expression of gene products in cancer, e.g. oncogenes has become the central concept in understanding cancer biology and may provide valuable targets for therapeutic drugs. All oncogenes and their products operate inside the cell making protein- based drugs ineffective since their specificity involves ligand-receptor recognition.

[0005] Aside from oncogenes, proteins implicated in tumor suppression, genesis, progression, growth, proliferation, migration, cell cycle, cell signaling, metastases, invasion, transformation, differentiation, tolerance, vascular leakage, epithelial mesenchymal transition (EMT), aggregation, angiogenesis, adhesion, development of resistance, addiction to oncogenes and non-oncogenes (cytokines, chemokines, growth factors), alteration of immune surveillance or immune response, alteration of tumor stroma/local environment, endothelial activation, extracellular matrix remodeling, hypoxia and inflammation, immune activation or immune suppression, and survival and/or prevention of cell death by apoptosis, necrosis, or autophagy may be useful targets. Proteins implicated may be increased, decreased, or altered to have an impact on diseases and/or cell systems.

[0006] Similarly numerous protein products implicated (overexpressed, mutated, or suppressed) in non-cancerous diseases involving bacterial, cardiovascular (heart failure, atherosclerosis, dyslipidemia, etc.), vascular, metabolic, diabetic, dental, oral, dermatological, endocrinology, fungal, gastroenterological, bowel (e.g. Crohn's, Ulcerative Colitis, or inflammatory bowel disease, etc.) , genetic, hematological, hepatic, immunology, infections and/or infectious disease, inflammation (e.g. arthritis, etc.), musculoskeletal, nephrology, neurology (e.g. Alzheimer's, Parkinson's, Huntington's, Multiple Sclerosis, etc.), nutrition and/or weight loss, obstetrics/gynecology, ophthalmology, orthopedics, otolaryngology, pediatric/neonatology, podiatry, pulmonary/respiratory disease, rheumatology, sleep disorders, trauma, urology, stem cells, and viral (e.g. HCV, HIV, HBV, Herpes, etc.) may be useful targets.

[0007] Antisense oligonucleotides are under investigation as therapeutic compounds for specifically targeting oncogenes (Wickstrom, E. (ed). Prospects for antisense nucleic acid therapy of cancer and Aids. New York: Wiley-Liss, Inc. 1991; Murray, J. A. H. (ed).

Antisense RNA and DNA New York: Wiley-Liss, Inc. 1992). Antisense drugs are modified synthetic oligonucleotides that work by interfering with ribosomal translation of the target mRNA. The antisense drugs developed thus far destroy the targeted mRNA by binding to it and triggering ribonuclease H (RNase H) degradation of mRNA. Oligonucleotides have a half- life of about 20 minutes and they are therefore rapidly degraded in most cells (Fisher, T. L. et al, Nucleic Acids Res. 21 :3857-3865 (1993)). To increase the stability of

oligonucleotides, they are often chemically modified, e.g., they are protected by a sulfur replacing one of the phosphate oxygens in the backbone (phosphorothioate) (Milligan, J. F. et al, J. Med. Chem. 36: 1923-1937 (1993); Wagner, R. W. et al, Science 260: 1510-1513 (1993)). However, this modification can only slow the degradation of antisense and therefore large dosages of antisense drug are required to be effective.

[0008] Despite the optimism surrounding the use of antisense therapies, there are a number of serious problems with the use of antisense drugs such as difficulty in getting a sufficient amount of antisense into the cell, non-sequence-specific effects, toxicity due to the large amount of sulfur containing phosphothioates oligonucleotides and their inability to enter their target cells, and their high cost due to continuous delivery of large doses. An additional problem with antisense drugs has been their nonspecific activities. Improvements to these first generation RNA targeted nucleic acid therapeutics utilize chemical modification to prevent degradation and utilize other modifications (e.g. 2'OMe modifications, CEt, locked nucleic acids (LNA), unlocked nucleic acids, inverted bases, conformationally- restricted nucleic acids (CRN)) to enable therapeutic windows of activity to be improved. [0009] Other nucleic acid-based approaches beyond antisense also target RNA and its translational machinery rather than genomic DNA. These include double-stranded siRNA to block the translation of aberrant proteins, RNA modulation to correct gene defects by exon skipping, and double or single-stranded microRNAs that function to regulate the expression of several gene pathways through the action of miRs and anti-miRs, which replace absent sequences or antagonize sequences, respectively.

[00010] There is a need for additional non-protein based cancer therapeutics that target genes implicated in diseases. Therapeutics that are effective in low doses and that are nontoxic to the subject are particularly needed.

SUMMARY OF THE INVENTION

[00011] The present invention relates to methods and compositions for the interference (inhibition, enhancement or alteration) of gene transcription or gene expression. In particular, the present invention provides oligonucleotide-based therapeutics for the modulation of disease causing genes.

[00012] An oligonucleotide that hybridizes to a non-coding region of a target gene, wherein the oligonucleotide comprises: a length of 20-34 bases; at least one CG pairs; at least 40% C and G content; no more than five consecutive bases of the same nucleotide; and may form at least one secondary structure. This oligonucleotide can also comprise a C and G content of at least 30% and in some embodiments the oligonucleotide comprises a C and G content of from about 50 to 80%. In some embodiments the oligonucleotide comprises at least two CG pairs. In some embodiments the oligonucleotide is complementary of said non-coding region of the target gene. In some embodiments the oligonucleotide is unique to the nucleotide sequence of the non-coding region. In some embodiments the nucleotide sequence of the non-coding region is not duplicated in a genome comprising the target gene. In some embodiments the nucleotide sequence of the non-coding region comprises 60% or greater homology to other nucleotide sequences in a genome with another gene. In some other embodiments the oligonucleotide is complementary to a non-coding region of another gene that influences that target gene. In yet other embodiments the oligonucleotide is complementary to a non-coding region of another gene that influences that target gene due to a chromosomal rearrangement. In yet other embodiments the oligonucleotide is

complementary to a region upstream of the transcription start site. [00013] In some embodiments, the present invention provides a composition comprising one or more distinct oligonucleotides that hybridizes under physiological conditions to regions upstream of the transcription start site of a disease causing gene.

[00014] In some embodiments, the region or regions upstream of the start site are located in regions on, surrounding or near transcription factor binding sites. In other embodiments, the regions are located on, surrounding or near various classes of regulatory elements (promoters, proximal promoters, distal enhancers, activators/co-activators, suppressors) that serve as cis-regulatory elements involved in gene transcription.

[00015] In some embodiments, the present invention provides compositions that are complementary to residues within CG regions. In some other embodiments, the present invention provides compositions that are complementary to residues within CpG islands. In yet other embodiments, the present invention resides in areas within nuclease hypersensitive areas.

[00016] In some embodiments, the present invention provides a composition

comprising a first oligonucleotide that hybridizes under physiological conditions to the regulatory region of the target sequences. In some embodiments, at least one of the cytosine bases in the first oligonucleotide is 5-methylcytosine. In some of the embodiments, wherein at least one or all the cytosine bases in said CG pair is 5-methylcytosine. In some

embodiments, all of the cytosine bases in the first oligonucleotide are 5-methylcytosine. In yet other embodiments, some of the bases in the first oligonucleotide are modified to prevent nuclease degradation during cell culture experiments. In some preferred embodiments, the hybridization of the first oligonucleotide to the promoter region of a gene modulates expression of the target gene. In some embodiments, the target gene is on a chromosome of a cell, and the hybridization of the first oligonucleotide to the regulatory region of the gene modulates cell signaling pathways of the cell. In some embodiments, the composition further comprises a second oligonucleotide. In some embodiments, at least one (e.g. all) of the cytosines in the second oligonucleotide are 5-methylcytosine.

[00017] In yet other embodiments, the present invention provides a method,

comprising: providing an oligonucleotide; and a cell capable of transcription, and a cell capable of gene expression, and comprising a gene capable of being transcribed, and comprising a gene capable of being expressed; and introducing the oligonucleotide to the cell. In some embodiments, the introducing results in the modulation of the gene transcription. In some embodiments, the introducing results in the modulation of expression of the gene. In other embodiments, the introducing results in the modulation of proliferation of the cell. In yet other embodiments, the introducing results in the modulation of the cell phenotype. In certain embodiments, the introducing results in alteration of expression of other genes related to the target gene. In certain other embodiments, the introducing results in modulation of cell signaling pathways related to the target gene transcription. In yet other embodiments, the introducing results in an interference with the expression of other genes involved in transcription. In some embodiments, the cell is a cancer cell. In other embodiments, the cell is a prokaryote. In some other embodiments, the cell is a eukaryote. In some other

embodiments the cell is in a host plant. In other embodiments, the cell is in a host animal (e.g., a non-human mammal or a human). In some embodiments, the oligonucleotide is introduced to the host animal at a dosage of between 0.1 mg to 10 g, and preferably at a dosage of between 0.1 mg to 100 mg per kg of body weight or 1 to 500 mg per meter squared body surface area. In some embodiments, the oligonucleotide is introduced to the host animal one or more times per day. In other embodiments, the oligonucleotide is introduced to the host animal continuously. In still further embodiments, the cell is in cell culture. In some embodiments, the method further comprises the step of introducing a test compound to the cell. In some embodiments, the test compound is a known chemotherapy or therapeutic agent. In some embodiments, the cancer is pancreatic cancer, colon/gastric cancer, breast cancer, renal/bladder cancer, lung cancer, leukemia, prostate, lymphoma, ovarian, thyroid cancer, sarcoma, or melanoma. In some embodiments, the non cancer disease involves bacterial, cardiovascular (heart failure, atherosclerosis, dyslipidemia, etc.), vascular, metabolic, diabetic, dental, oral, dermatological, endocrinology, fungal,

gastroenterological, bowel (e.g. Crohn's, Ulcerative Colitis, or inflammatory bowel disease, etc.) , genetic, hematological, hepatic, immunology, infections and/or infectious disease, inflammation (e.g. arthritis, etc.), musculoskeletal, nephrology, neurology (e.g. Alzheimer's, Parkinson's, Huntington's, Multiple Sclerosis, etc.), nutrition and/or weight loss,

obstetrics/gynecology, ophthalmology, orthopedics, otolaryngology, pediatric/neonatology, podiatry, pulmonary/respiratory disease, rheumatology, sleep disorders, trauma, urology, or viral (e.g. HCV, HIV, HBV, Herpes, etc.) disease.

[00018] In some embodiments, the method further provides a drug delivery system. In some embodiments, the drug delivery system comprises a nanoparticle, nanocrystal or complex, (e.g., a liposome comprising a neutral lipid or a lipid like compound or particles comprising polymer or polymer-like compound). In some embodiments, the drug delivery system comprises a cell targeting component (e.g., a ligand or ligand like molecule for a cell surface receptor or a nuclear receptor). In yet other embodiments, the drug delivery system comprises a device to administer the test compound(s). In certain embodiments, the drug delivery system is for use in vivo, and the oligonucleotide and the liposome, nanoparticle, nanocrystal or delivery system are present in the ratio of from 1 : 1 to 1 : 1000 (weight per weight).

[00019] The present invention further provides a composition comprising an

oligonucleotide that hybridizes under physiological conditions to the coding strand of a gene under conditions such that expression of that gene is inhibited, enhanced or altered (i.e.

modulated)

[00020] The present invention further provides a composition comprising an

oligonucleotide that hybridizes under physiological conditions to the coding strand of a gene under conditions such that transcription of that gene is inhibited, enhanced or altered (i.e. modulated)

[00021] The present invention further provides a composition comprising an

oligonucleotide that hybridizes under physiological conditions to the coding strand of a gene under conditions such that cell signaling pathways related to that gene is inhibited, enhanced or altered (i.e. modulated).

[00022] The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a gene on a chromosome of a cell under conditions such that the cell phenotype is altered.

[00023] The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a gene on a chromosome of a cell under conditions such that proliferation of the cell is reduced.

[00024] The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the CG regions of a gene on a chromosome of a cell under conditions such that cell signaling pathways are modulated.

[00025] The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to CpG islands of a gene on a chromosome of a cell under conditions such that cell signaling pathways are modulated.

[00026] The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the CG regions of a gene on a chromosome of a cell under conditions such that genes related to transcription of that gene are modulated.

[00027] The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the CpG islands of a gene on a chromosome of a cell under conditions such that genes related to gene expression of that gene are modulated.

[00028] The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the CG regions of a gene on a chromosome of a cell under conditions such that genes related to cell phenotype are modulated.

[00029] The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the CpG islands of a gene on a chromosome of a cell under conditions such that genes related to cell phenotype are modulated.

[00030] The present invention additionally provides a method of inhibiting the expression of a gene in a subject (e.g., for the treatment of cancer or other

hyperproliferative/overexpressive gene disorders) comprising providing an oligonucleotide that hybridizes under physiological conditions to the coding strand of a gene involved in cancer or a hyperproliferative/overexpressive gene disorder expressed in the biological sample, the oligonucleotide comprising at least one CG dinucleotide pair; and administering the oligonucleotide to the subject under conditions such that transcription or expression of the gene is inhibited, enhanced or altered (i.e. modulated). In some embodiments, the subject is a human.

[00031] In some embodiments, the method further provides a drug delivery system. In some embodiments, the drug delivery system comprises a liposome (e.g., a liposome comprising a neutral lipid or a lipid like compound or particles comprising polymer or polymer-like compound). In some embodiments, the drug delivery system comprises a cell targeting component (e.g., a ligand or ligand like molecule for a cell surface receptor or a nuclear receptor). In certain embodiments, the drug delivery system is for use in vivo, and the oligonucleotide and the liposome, nanoparticle, nanocrystal or delivery system are present in the ratio of from 1 : 1 to 1 : 1000 (weight per weight).

[00032] The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a gene located on a chromosome of a cell under conditions such that transcription, phenotype or cell signaling pathways related to the target gene are modulated.

[00033] In certain embodiments, the present invention provides a kit comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a gene, the oligonucleotide comprising at least one CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; and instructions for using the kit for reducing proliferation of a cell comprising a gene on a chromosome of the cell or inhibiting gene expression. In some embodiments, the composition in the kit is used for treating cancer in a subject and the instructions comprise instructions for using the kit to treat cancer in the subject. In some embodiments, the instructions are instructions required by the U.S. Food and Drug Agency for labeling of pharmaceuticals.

[00034] The present invention also provides a method, comprising: providing a biological sample from a subject diagnosed with a cancer; and reagents for detecting the present or absence of expression of an oncogene in the sample; and detecting the presence or absence of expression of an oncogene in the sample; administering an oligonucleotide that hybridizes under physiological conditions to the promoter region of an oncogene expressed in the biological sample, the oligonucleotide comprising at least one CG dinucleotide pair.

[00035] The present invention additionally provides a method of inhibiting the expression of a gene in a subject (e.g., for the treatment of cancer or other hyperproliferative disorders) comprising providing an oligonucleotide that hybridizes under physiological conditions to the promoter region of a gene involved in cancer or a hyperproliferative disorder expressed in the biological sample, the oligonucleotide comprising at least one CG dinucleotide pair; and administering the oligonucleotide to the subject under conditions such that expression of the gene is inhibited. In some embodiments, the subject is a human.

[00036] The present invention additionally provides a method of modulating the transcription of a gene in a subject (e.g., for the treatment of disease) comprising an oligonucleotide that hybridizes under physiological conditions to the non-coding region of a gene involved in disease expressed in the biological sample, the oligonucleotide comprising at least one CG dinucleotide pair; and administering the oligonucleotide to the subject under conditions such that expression of the gene is inhibited. In some embodiments, the subject is a human.

[00037] In yet further embodiments, the present invention provides a method of screening compounds providing a cell comprising a suspected gene; and an oligonucleotide that hybridizes to the promoter region of the gene; and administering the oligonucleotide to the cell; and determining if the phenotype of the cell is modulated in the presence of the oligonucleotide relative to the absence of the oligonucleotide. In some embodiments, the cell is in culture (e.g., a prokaryote or eukaryote cell line). In other embodiments, the cell is in a host animal (e.g., a non-human mammal). In some embodiments, the method is a high-throughput screening method.

[00038] In other embodiments, the present invention relates to methods and

compositions for cancer therapy. In particular, the present invention provides nanoparticle, nanocrystal, liposome, or complex based cancer or non-cancer therapeutics.

[00039] Accordingly, in some embodiments, the present invention provides a pharmaceutical composition comprising (e.g., consisting of) a cationic, neutral, or anionic lipids, polymers or delivery agents in a complex or mixture with an oligonucleotide. In some preferred embodiments, the liposome is cationic, neutral, anionic or amphoteric (e.g.

SMARTICLES) in charge. In some preferred embodiments, the complex is a mixture of lipids, lipid-like, polymer or polymer-like delivery agents and a cation (e.g. lipids and calcium to form cochleates) or a mixture of lipids lipids, lipid-like, polymer or polymer-like delivery agents and an anion.

[00040] In some embodiments, the present invention provides a kit, comprising an oligonucleotide (e.g., an oligonucleotide that hybridizes to the CG regions, CpG islands or promoter region of an oncogene) and a first pharmaceutical composition comprising (e.g., consisting of) a cationic, neutral, or anionic liposome comprises an optional second pharmaceutical composition, wherein the second pharmaceutical composition comprises a known chemotherapy agent (e.g., TAXOTERE, TAXOL, or VINCRISTINE, etc.), or chemotherapy cocktail, and wherein the known chemotherapy agent is formulated separately from the first pharmaceutical composition. In some embodiments, the chemotherapy agent is present at less than one half the standard dose, more preferably less than one third, even more preferably less than one fourth and still more preferable less than one tenth, and yet more preferably less than one hundredth the standard dose.

[00041] In some embodiments, the present invention provides a kit, comprising an oligonucleotide (e.g., an oligonucleotide that hybridizes to the CG regions, CG islands, or promoter region of an oncogene) and a first pharmaceutical composition comprising (e.g., consisting of) a cationic, neutral, or anionic liposome comprises an optional second pharmaceutical composition, wherein the second pharmaceutical composition comprises a known agent (e.g., an antibiotic, an antiviral, an anti-inflammatory, etc.), or treatment cocktail, and wherein the known agent is formulated separately from the first pharmaceutical composition. In some embodiments, the agent is present at less than one half the standard dose, more preferably less than one third, even more preferably less than one fourth and still more preferable less than one tenth, and yet more preferably less than one hundredth the standard dose.

[00042] In yet other embodiments, the present invention provides a method, comprising providing a pharmaceutical composition consisting of a cationic, neutral, or anionic liposome and an oligonucleotide (e.g., an oligonucleotide that hybridizes to the promoter region of an oncogene); and exposing the pharmaceutical composition to a cancer cell. In some preferred embodiments, the liposome is a cardiolipin based cationic liposome (e.g., NEOPHECTIN). In some preferred embodiments, the charge ration of NEOPHECTIN to oligonucleotide is 6: 1. In other embodiments, the liposome comprises N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP). In some embodiments, the cancer cell is a prostate cancer cell, an ovarian cancer cell, a breast cancer cell, a leukemia cell, or lymphoma cell. In some embodiments, the cell is in a host animal (e.g., a human). In some embodiments, the pharmaceutical composition is introduced to the host animal one or more times per day (e.g., continuously). In some embodiments, the method further comprises the step of administering a known chemotherapeutic agent to the subject (e.g., TAXOTERE, TAXOL, or VINCRISTINE), wherein the known chemotherapeutic agent is formulated separately from the cationic, neutral or anionic liposome. In preferred embodiments, the known

chemotherapeutic agent is administered separately from the pharmaceutical composition. In some embodiments, the chemotherapy agent is present at less than one half the standard dose, more preferably less than one third, even more preferably less than one fourth and still more preferable less than one tenth, and yet more preferably less than one hundredth the standard dose.

DESCRIPTION OF THE FIGURES

Figure 1 demonstrates a dose-dependent response for representative oligonucleotides in MDA-MB-231 a human breast cell line.

Figure 2 demonstrates a dose-dependent response for representative oligonucleotides in A549 (human lung cell line).

Figure 3 demonstrates a dose-dependent response for representative oligonucleotides in DU145 (human prostate cell line).

Figure 4 demonstrates a dose-dependent response for representative oligonucleotides in

MCF7 (human mammary breast cell line).

Figure 5 depicts the structure of the oligonucleotide SU1.

Figure 6 depicts the structure of the oligonucleotide SU2. Figure 7 depicts the structure of the oligonucleotide SU3.

Figure 8 depicts the structure of the oligonucleotide SU1 02.

Figure 9 depicts the structure of the oligonucleotide SU1 03.

Figure 10 demonstrates target inhibition of representative oligonucleotides in DU145 (human prostate cell line).

Figure 11 demonstrates target inhibition of representative oligonucleotides in HCT-116 (human colorectal carcinoma).

Figure 12 depicts the structure of the oligonucleotide BE1.

Figure 13 depicts the structure of the oligonucleotide BE2.

Figure 14 demonstrates target inhibition of representative oligonucleotides in MDA-MB-231 a human breast cell line.

Figure 15 demonstrates target inhibition of representative oligonucleotides in DU145 (human prostate cell line).

Figure 16 depicts the structure of the oligonucleotide ST1.

Figure 17 depicts the structure of the oligonucleotide ST2.

Figure 18 demonstrates target inhibition of representative oligonucleotides in MDA-MB-231 a human breast cell line.

Figure 19 demonstrates target inhibition of representative oligonucleotides in DU145 (human prostate cell line).

Figure 20 depicts the structure of the oligonucleotide HI1.

Figure 21 depicts the structure of the oligonucleotide HI2.

Figure 22 demonstrates target inhibition of representative oligonucleotides in MDA-MB-231 a human breast cell line.

Figure 23 demonstrates target inhibition of representative oligonucleotides in DU145 (human prostate cell line).

Figure 24 depicts the structure of the oligonucleotide IL8-1.

Figure 25 depicts the structure of the olionucleotide IL8-3.

Figure 26 demonstrates target inhibition of representative olionucleotides in BxPC3 (human pancreatic cancer cell line).

Figure 27 demonstrates target inhibition of representative olionucleotides in A549 (human lung cancer cell line).

Figure 28 depicts the structure of the olionucleotide K 1.

Figure 29 depicts the structure of the olionucleotide K 2.

Figure 30 depicts the structure of the olionucleotide KR0525. Figure 31 demonstrates target inhibition of representative olionucleotides in MCF7 (human mammary breast cell line).

Figure 32 depicts the structure of the olionucleotide IL6.

Figure 33 demonstrates target inhibition of representative olionucleotides in HCT-116 (human colorectal carcinoma).

Figure 34 depicts the structure of the olionucleotide AKT4

Figure 35 demonstrates target inhibition of representative olionucleotides in MCF7 (human mammary breast cell line).

Figure 36 depicts the structure of the olionucleotide BC 1.

Figure 37 demonstrates target inhibition of representative olionucleotides in HCT-116 (human colorectal carcinoma).

Figure 38 depicts the structure of the olionucleotide MEK1 I .

Figure 39 depicts the structure of the olionucleotide MEK1 2.

Figure 40 demonstrates target inhibition of representative olionucleotides in HCT-116 (human colorectal carcinoma).

Figure 41 depicts the structure of the olionucleotide MEK2 1.

Figure 42 demonstrates target inhibition of representative olionucleotides in MCF7 (human mammary breast cell line).

Figure 43 depicts the structure of the olionucleotide WNT1 I .

Figure 44 depicts the structure of the olionucleotide WNT1 2.

Figure 45 depicts the structure of the olionucleotide WNT1 3.

Figure 46 demonstrates target inhibition of representative olionucleotides in MCF7 (human mammary breast cell line).

Figure 47 depicts the structure of the olionucleotide EZH2 2.

Figure 48 demonstrates target inhibition of representative olionucleotides in MCF7 (human mammary breast cell line).

Figure 49 depicts the structure of the olionucleotide PD1.

Figure 50 demonstrates target inhibition of representative olionucleotides in MDA-MB-231 a human breast cell line.

Figure 51 demonstrates target inhibition of representative olionucleotides in M14 (human melanoma cell line).

Figure 52 demonstrates target inhibition of representative olionucleotides in NMuMG (a normal murine mouse mammary gland cell line).

Figure 53 depicts the structure of the olionucleotide BL2. Figure 54 demonstrates target inhibition of representative olionucleotides in HCT-116 (human colorectal carcinoma).

Figure 55 demonstrates target inhibition of representative olionucleotides in MCF7 (human mammary breast cell line).

Figure 56 demonstrates target inhibition of representative olionucleotides in MDA-MB-231 a human breast cell line.

Figure 57 demonstrates target inhibition of representative olionucleotides in MCF7 (human mammary breast cell line).

Figure 58 depicts the structure of the olionucleotide CM7.

Figure 59 depicts the structure of the olionucleotide CM 12.

Figure 60 depicts the structure of the olionucleotide CM13.

Figure 61 depicts the structure of the olionucleotide CM 14.

Figure 62 demonstrates target inhibition of representative olionucleotides in MCF7 (human mammary breast cell line).

Figure 63 depicts the structure of the olionucleotide TNF1.

Figure 64 demonstrates target inhibition of representative olionucleotides in MCF7 (human mammary breast cell line).

Figure 65 depicts the structure of the olionucleotide MIFI 1 .

Figure 66 depicts the structure of the olionucleotide MIFI 2.

Figure 67 demonstrates that a representative oligonucleotide PC2 is capable of modulating target gene expression.

[00043] The figures are provided by way of example and are not intended to limit the scope of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Definitions.

[00044] To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

[00045] As used herein, the term "wherein said chemotherapy agent is present at less than one half the standard dose" refers to a dosage that is less than one half (e.g., less than 50%, preferably less than 40%, even more preferably less than 10% and still more preferably less than 1%) of the minimum value of the standard dosage range used for dosing humans. In some embodiments, the standard dosage range is the dosage range recommended by the manufacturer. In other embodiments, the standard dosage range is the range utilized by a medical doctor in the field. In still other embodiments, the standard dosage range is the range considered the normal standard of care in the field. The particular dosage within the dosage range is determined, for example by the age, weight, and health of the subject as well as the type of cancer being treated.

[00046] As used herein, the term "under conditions such that expression of said gene is modulated" refers to conditions where an oligonucleotide of the present invention hybridizes to a gene) and modulates expression of the gene by at least 10%, preferably at least 25% relative to the level of transcription in the absence of the oligonucleotide. The present invention is not limited to the modulation of expression of a particular gene. Exemplary genes include, but are not limited to Survivin, Beclin-1, STAT3, HIF1A, IL-8, KRAS, MTTP, ApoC III, ApoB, IL-17, MMP2, FAP, P-selectin, IL-6, IL-23, AKT, CRAF, Beta Catenin, PCSK9, MEK1, MEK2, CD4, WNT1, Clusterin, NRAS, EZH2, HD AC 1, and PD-1, TNFa, MIF1, TTR, HBV, HAMP, ERBB2, PARP1, ITGA4, APP, FGFR1, CD68, ALK, MSI2, JAK2, CCND1. As used herein, the term "under conditions such that transcription of said gene is modulated" refers to conditions where an oligonucleotide of the present invention hybridizes to a gene and modulates transcription of the gene by at least 10%, preferably at least 25% relative to the level of transcription in the absence of the oligonucleotide. The modulation of transcription of said gene may involve related genes. The present invention is not limited to the modulation of expression of a particular gene.

[00047] As used herein the term "expression" is the process whereby information from a gene is used in the synthesis of a functional gene product. These products may be proteins, but in non-protein coding genes such as ribosomal RNA (rRNA), transfer RNA (tRNA) or small nuclear RNA (snRNA) genes, the product is a functional RNA or transcript to generate the macromolecular machinery for gene expression. Gene expression may be modulated at several levels including transcription, RNA splicing, translation, and post-translational modification of a protein. The term may also be used against a viral gene and refer to mRNA synthesis from a RNA molecule (i.e. RNA replication). For instance, the genome of a negative-sense single-stranded RNA virus may serve as a template to translate the viral proteins for viral replication afterwards.

[00048] As used herein the term "transcription" is the first step of gene expression where a segment of DNA is copied into RNA by RNA polymerase to produce a transcript. If the gene transcribed encodes a protein, the result of transcription is messenger RNA (mRNA) and expressed to produce a protein. Alternatively, a transcribed gene may encode for non-coding RNA genes (e.g. such as microRNA etc.), ribosomal RNA, transfer RNA (tRNA), other components of the protein-assembly process, or other ribozymes.

[00049] As used herein the term "phenotype" describes the modulation of gene expression to define the properties of the expression give rise to the organism's phenotype. A phenotype is expressed by proteins that control the organism's characteristics or traits, such as its morphology, shape, development, biochemical or physiological properties, and products that act to catalyze cell signaling and metabolic pathways characterizing the organism.

[00050] As used herein the term "cell signaling" describes a complex system of signals or pathways that governs cellular activities and coordinates cell actions. A cell's ability to perceive and respond to its environment is processed through proteins involved in the cell signaling pathway.

[00051] As used herein the term "CG regions" are regions of DNA where cytosine and guanine nucleotides are enriched in the linear sequence of bases along the length of a gene. Generally CG or GC percentage that is greater than 50% with an observed-to-expected CpG ratio that is greater than 60%. CG regions of DNA are also where a cytosine nucleotide occurs next to a guanine nucleotide and may be referred to as "CpG" for "C phosphodiester bond G". Generally cytosine bases in CpGs are methylated.

[00052] As used herein the term "CpG islands" are regions of the genome that have high GC content and higher concentration of CpG sites associated with the start of the gene, promoter regions or regions 5' upstream of a gene start site. CpG islands are typically 300-3,000 base pairs in length. CpG islands are recognized to be hypomethylated. In most instances the CpG sites in the CpG islands are unmethylated and may be recognized by HpaII restriction site, CCGG.

[00053] As used herein the term "nuclease hypersensitive site" is a short region of chromatin and is detected by its super sensitivity to cleavage by DNase I and other various nucleases. The nucleosomal structure is less compact, increasing the availability of the DNA to binding by proteins, such as transcription factors and DNase I. Hypersensitive sites are found on chromatin of cells associated with genes and generally precede active promoters. When DNA is transcribed, 5' hypersensitive sites appear before transcription begins, and the DNA sequences within the hypersensitive sites are required for gene expression.

Hypersensitive sites may be generated as a result of the binding of transcription factors. [00054] As used herein "cis-regulatory element" is a region of DNA or R A that regulates the expression of genes located on that same molecule of DNA A cis-regulatory element may be located upstream of the coding sequence of the gene it controls (in the promoter region or even further upstream), in an intron, or downstream of the gene's coding sequence, in either the translated or the untranscribed region. A cis-regulatory element may be located in another gene other than the target gene in instances of chromosomal rearrangements.

[00055] As used herein "non-coding" refers to a linear sequence of DNA that does not contribute to an amino acid sequence of a protein.

[00056] As used herein "Trinucleotide repeat expansion" refers to a triplet repeat expansion of DNA bases that causes any type of disorder categorized as a trinucleotide repeat disorder. Generally, the larger the expansion the more likely they are to cause disease or increase the severity of disease. Trinucleotide repeat disorders represent genetic by trinucleotide repeat expansion, a kind of mutation where trinucleotide repeats in certain genes exceed the normal, stable threshold, which differs per gene.

[00057] As used herein, the term "under conditions such that growth of said cell is reduced" refers to conditions where an oligonucleotide of the present invention, when administered to a cell (e.g., a cancer) reduces the rate of growth of the cell by at least 10%, preferably at least 25%, even more preferably at least 50%, and still more preferably at least 90% relative to the rate of growth of the cell in the absence of the oligonucleotide.

[00058] As used herein, the term "under conditions such that the expression of said target is modulated" refers to conditions where an oligonucleotide of the present invention, when administered to a cell (e.g., a cancer or non cancer or immune cell) modulates the expression of the protein by at least 10%, preferably at least 25%, relative to basal expression in the absence of the oligonucleotide.

[00059] The term "epitope" as used herein refers to that portion of an antigen that makes contact with a particular antibody.

[00060] As used herein, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[00061] As used herein, the terms "computer memory" and "computer memory device" refer to any storage media readable by a computer processor. Examples of computer memory include, but are not limited to, RAM, ROM, computer chips, digital video disc (DVDs), compact discs (CDs), hard disk drives (HDD), and magnetic tape.

[00062] As used herein, the term "computer readable medium" refers to any device or system for storing and providing information (e.g., data and instructions) to a computer processor. Examples of computer readable media include, but are not limited to, DVDs, CDs, hard disk drives, magnetic tape and servers for streaming media over networks.

[00063] As used herein, the term "Delta G" or "AG" is the change in Gibbs Free Energy (in units of kcal/mole) and is the net exchange of energy between the system and its environment and can be described by the equation $AG = \Delta H - T\Delta S$. Where ΔH (Enthalpy) represents the total energy exchange between the system and its surrounding environment (in units of kcal/mole), ΔS (Entropy) represents the energy spent by the system to organize itself (in units of cal/K*mole). Generally speaking a spontaneous system favors a more random system not an ordered system. Finally, T represents the absolute temperature of the system and is in units Kelvin (Celsius + 273.15). The change of free energy is equal to the sum of its enthalpy plus the product of the temperature and entropy of the system. A positive AG reaction is generally non-spontaneous while a negative value is spontaneous.

[00064] As used herein, the terms "processor" and "central processing unit" or "CPU" are used interchangeably and refer to a device that is able to read a program from a computer memory (e.g., ROM or other computer memory) and perform a set of steps according to the program.

[00065] As used herein, the term "non-human animals" refers to all non-human animals including, but are not limited to, vertebrates such as rodents, non-human primates, ovines, bovines, ruminants, lagomorphs, porcines, caprines, equines, canines, felines, aves, etc. and non-vertebrate animals such as drosophila and nematode. In some embodiments, "non-human animals" further refers to prokaryotes and viruses such as bacterial pathogens, fungal, viral pathogens. Non-human animals is used broadly here to also indicate plants and plant genomes, especially commercially valuable crops such as corn, soybean, cotton, the grasses and legumes including rice and alfalfa as well as commercial flowers, vegetables and trees including deciduous and evergreen.

[00066] As used herein, the term "nucleic acid molecule" refers to any nucleic acid containing molecule, including but not limited to, DNA or RNA. The term encompasses sequences that include any of the known base analogs of DNA and RNA including, but not limited to, 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine,

pseudoisocytosine, 5-(carboxyhydroxymethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine,

1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methyl guanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxoacetic acid methylester, uracil-5-oxoacetic acid, oxybutoxosine, pseudouracil, queosine,

2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxoacetic acid methylester, uracil-5-oxoacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

[00067] The term "gene" refers to a nucleic acid (e.g., DNA) sequence that comprises coding sequences necessary for the production of a polypeptide, precursor, or RNA (e.g., rRNA, tRNA). The polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or functional properties (e.g., enzymatic activity, ligand binding, signal transduction, immunogenicity, etc.) of the full-length or fragment are retained. The term also encompasses the coding region of a structural gene and the sequences located adjacent to the coding region on the 5' ends for a distance of about 1 kb or more such that the gene corresponds to the length of the full-length mRNA. Sequences located 5' of the coding region and present on the mRNA are referred to as 5' non-translated sequences. Sequences located 3' or downstream of the coding region and present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene that are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

[00068] As used herein, the term "heterologous gene" refers to a gene that is not in its natural environment. For example, a heterologous gene includes a gene from one species introduced into another species. A heterologous gene also includes a gene native to an organism that has been altered in some way (e.g., mutated, added in multiple copies, linked to non-native regulatory sequences, translocated, etc). Heterologous genes are distinguished from endogenous genes in that the heterologous gene sequences are typically joined to DNA sequences that are not found naturally associated with the gene sequences in the chromosome or are associated with portions of the chromosome not found in nature (e.g., genes expressed in loci where the gene is not normally expressed).

[00069] As used herein, the term "gene expression" refers to the process of converting genetic information encoded in a gene into RNA (e.g., mRNA, rRNA, tRNA, or snRNA) through "transcription" of the gene (i.e., via the enzymatic action of an RNA polymerase), and for protein encoding genes, into protein through "translation" of mRNA. Gene expression can be regulated at many stages in the process. "Up-regulation" or "activation" refers to regulation that increases the production of gene expression products (i.e., RNA or protein), while "down-regulation" or "repression" refers to regulation that decrease production.

"Modulation" refers to regulation that is altered. Molecules (e.g., transcription factors) that are involved in up-regulation or down-regulation are often called "activators" and "repressors or suppressors," respectively.

[00070] In addition to containing introns, genomic forms of a gene may also include sequences located on both the 5' and 3' end of the sequences that are present on the RNA transcript. These sequences are referred to as "flanking" sequences or regions (these flanking sequences are located 5' or 3' to the non-translated sequences present on the mRNA transcript). The 5' flanking region may contain regulatory sequences such as promoters and enhancers that control or influence the transcription of the gene. The 3' flanking region may contain sequences that direct the termination of transcription, post-transcriptional cleavage and polyadenylation.

[00071] The term "wild-type" refers to a gene or gene product isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene. In contrast, the term "modified" or "mutant" refers to a gene or gene product that displays modifications in sequence and/or functional properties (i.e., altered characteristics) or phenotype when compared to the wild-type gene or gene product. It is noted that naturally occurring mutants can be isolated; these are identified by the fact that they have altered characteristics

(including altered nucleic acid sequences) when compared to the wild-type gene or gene product.

[00072] As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," and "DNA encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide (protein) chain. The DNA sequence thus codes for the amino acid sequence.

[00073] As used herein, the terms "an oligonucleotide having a nucleotide sequence encoding a gene" and "polynucleotide having a nucleotide sequence encoding a gene," means a nucleic acid sequence comprising the coding region of a gene or in other words the nucleic acid sequence that encodes a gene product. The coding region may be present in a cDNA, genomic DNA or RNA form. When present in a DNA form, the oligonucleotide or polynucleotide may be single-stranded (i.e., the sense

strand) or double-stranded. Suitable control elements such as enhancers/promoters, splice junctions, polyadenylation signals, etc. may be placed in close proximity to the coding region of the gene if needed to permit proper initiation of transcription and/or correct processing of the primary RNA transcript.

Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice junctions, intervening sequences, polyadenylation signals, etc. or a combination of both endogenous and exogenous control elements.

[00074] As used herein, the term "oligonucleotide," refers to a short length of single- stranded polynucleotide chain. Oligonucleotides are typically less than 200 residues long (e.g., between 8 and 100), however, as used herein, the term is also intended to encompass longer polynucleotide chains (e.g., as large as 5000 residues). Oligonucleotides are often referred to by their length. For example a 24 residue or base oligonucleotide is referred to as a "24-mer". Oligonucleotides can form secondary and tertiary structures by self-hybridizing or by hybridizing to other polynucleotides. Such structures can include, but are not limited to, duplexes, hairpins, cruciforms, bends, and triplexes.

[00075] In some embodiments, oligonucleotides are "DNAi or DNA interference (DNAi)." As used herein, the term "DNAi" or refers to an oligonucleotide that hybridizes to region 5' upstream of the transcription start site of a gene. In some embodiments, the hybridization of the DNAi or DNAi to the promoter modulates expression of the gene.

[00076] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (i.e., a sequence of nucleotides) related by the base-pairing rules. For example, for the sequence "A-G-T," is complementary to the sequence "T-C-A."

Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" or "100 percent" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. The degree of complementarity is also defined the "native" sequence rather than having a mismatch. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

[00077] As used herein, the term "completely complementary," for example when used in reference to an oligonucleotide of the present invention refers to an oligonucleotide where all of the nucleotides are complementary to a target sequence (e.g., a gene).

[00078] As used herein, the term "partially complementary," for example when used in reference to an oligonucleotide of the present invention, refers to an oligonucleotide where at least one nucleotide is not complementary to the target sequence. Preferred partially complementary oligonucleotides are those that can still hybridize to the target sequence under physiological conditions. The term "partially complementary" refers to oligonucleotides that have regions of one or more non-complementary nucleotides both internal to the

oligonucleotide or at either end. Oligonucleotides with mismatches at the ends may still hybridize to the target sequence.

[00079] The term "homology" refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). A partially complementary sequence is a nucleic acid molecule that at least partially inhibits a completely complementary nucleic acid molecule from hybridizing to a target nucleic acid is "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution

hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous nucleic acid molecule to a target under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be tested by the use of a second target that is substantially non-complementary (e.g., less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

[00080] When used in reference to a double-stranded nucleic acid sequence such as a cDNA or genomic clone, the term "substantially homologous" refers to any probe that can hybridize to either or both strands of the double-stranded nucleic acid sequence under conditions of low stringency as described above. [00081] A gene may produce multiple RNA species that are generated by differential splicing of the primary RNA transcript. cDNAs that are splice variants of the same gene will contain regions of sequence identity or complete homology (representing the presence of the same exon or portion of the same exon on both cDNAs) and regions of complete non-identity (for example, representing the presence of exon "A" on cDNA 1 wherein cDNA 2 contains exon "B" instead). Because the two cDNAs contain regions of sequence identity they will both hybridize to a probe derived from the entire gene or portions of the gene containing sequences found on both cDNAs; the two splice variants are therefore substantially homologous to such a probe and to each other.

[00082] When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to any probe that can hybridize (i.e., it is the complement of) the single-stranded nucleic acid sequence under conditions of low stringency as described above.

[00083] As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementarity between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C or C:G ratio within the nucleic acids. An oligonucleotide is a single molecule that contains a covalent bond linking each nucleotide and often pairing of complementary nucleic acids within its structure is said to be "self- hybridized" or having secondary structure.

[00084] As used herein the term "secondary structure" means a single molecule that contains a pairing of complementary nucleic acids within its structure that contributes to a two dimensional bend in said molecule.

[00085] As used herein, the term "linear section" refers to molecules with secondary structures wherein those secondary structures have regions of DNA that are not paired in a secondary manner they only have one covalent bond to the next oligonucleotide rather than both a bond and a pairing of complementary nucleic acids as one finds in regions having secondary structure."

[00086] As used herein, the term "nuclease hypersensitive region" refers to regions of the target gene that are susceptible to oligonucleotide binding.

[00087] As used herein, the term "T_m" is used in reference to the "melting

temperature." The melting temperature is the temperature at which a population of double- stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the T_m of nucleic acids is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: T_m = 81.5 + 0.41(% G + C), when a nucleic acid is in aqueous solution at 1 M NaCl (See e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization [1985]). Other references include more sophisticated computations that take structural as well as sequence characteristics into account for the calculation of T_m. The process of hybridization and dissociation is complex and highly dynamic and at the T_m, double strands are constantly formed and broken up, resulting in multiple interactions over time. The formation of secondary structures within an oligonucleotide may influence T_m.

[00088] As used herein the term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. Under "low stringency conditions" a nucleic acid sequence of interest will hybridize to its exact complement, sequences with single base mismatches, closely related sequences (e.g., sequences with 90% or greater homology), and sequences having only partial homology (e.g., sequences with 50-90% homology). Under "medium stringency conditions," a nucleic acid sequence of interest will hybridize only to its exact complement, sequences with single base mismatches, and closely related sequences (e.g., 90% or greater homology). Under "high stringency conditions," a nucleic acid sequence of interest will hybridize only to its exact complement, and (depending on conditions such as a temperature) sequences with single base mismatches. In other words, under conditions of high stringency the temperature can be raised so as to exclude hybridization to sequences with single base mismatches.

[00089] "High stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH₂P₀₄ H₂O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

[00090] "Medium stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH₂P₀₄ H₂O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

[00091] "Low stringency conditions" comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH₂P₀₄ H₂O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5X Denhardt's reagent [50X Denhardt's contains per 500 ml: 5 g Ficoll (Type 400, Pharmacia), 5 g BSA (Fraction V; Sigma)] and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 5X SSPE, 0.1% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

[00092] The present invention is not limited to the hybridization of probes of about 500 nucleotides in length. The present invention contemplates the use of probes between approximately 8 nucleotides up to several thousand (e.g., at least 5000) nucleotides in length. One skilled in the relevant art understands that stringency conditions may be altered for probes of other sizes (See e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization [1985] and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, NY [1989]).

[00093] One skilled in the art would know numerous equivalent conditions may be employed to create low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, etc.) and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, the art knows conditions that promote hybridization under conditions of high stringency (e.g., increasing the temperature of the hybridization and/or wash steps, the use of formamide in the hybridization solution, etc.) (see definition above for "stringency").

[00094] As used herein, the term "physiological conditions" refers to specific stringency conditions that approximate or are conditions inside an animal (e.g., a human). Exemplary physiological conditions for use in vitro include, but are not limited to, 37°C, 95% air, 5% CO₂, commercial medium for culture of mammalian cells (e.g., DMEM media available from Gibco, MD), 5-10% serum (e.g., calf serum or horse serum), additional buffers, and optionally hormone (e.g., insulin and epidermal growth factor).

[00095] The term "isolated" when used in relation to a nucleic acid, as in "an isolated oligonucleotide" or "isolated polynucleotide" refers to a nucleic acid sequence that is identified and separated from at least one component or contaminant with which it is ordinarily associated in its natural source. Isolated nucleic acid is such present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acids as nucleic acids such as DNA and RNA found in the state they exist in nature. For example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs that encode a multitude of proteins. However, isolated nucleic acid encoding a given protein includes, by way of example, such nucleic acid in cells ordinarily expressing the given protein where the nucleic acid is in a chromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid, oligonucleotide, or polynucleotide may be present in single-stranded or double-stranded form. When an isolated nucleic acid, oligonucleotide or polynucleotide is to be utilized to express a protein, the oligonucleotide or polynucleotide will contain at a minimum the sense or coding strand (i.e., the oligonucleotide or

polynucleotide may be single-stranded), but may contain both the sense and anti-sense strands (i.e., the oligonucleotide or polynucleotide may be double-stranded).

[00096] As used herein, the term "purified" or "to purify" refers to the removal of components (e.g., contaminants) from a sample. For example, antibodies are purified by removal of contaminating non-immunoglobulin proteins; they are also purified by the removal of immunoglobulin that does not bind to the target molecule. The removal of non-immunoglobulin proteins and/or the removal of immunoglobulins that do not bind to the target molecule results in an increase in the percent of target-reactive immunoglobulins in the sample. In another example, recombinant polypeptides are expressed in bacterial host cells and the polypeptides are purified by the removal of host cell proteins; the percent of recombinant polypeptides is thereby increased in the sample.

[00097] "Amino acid sequence" and terms such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

[00098] The term "native protein" as used herein to indicate that a protein does not contain amino acid residues encoded by vector sequences; that is, the native protein contains only those amino acids found in the protein as it occurs in nature. A native protein may be produced by recombinant means or may be isolated from a naturally occurring source.

[00099] The term "mutant protein" as used herein to indicate that a protein containing a change in amino acid residues encoded by vector sequences that renders altered function or implicated in disease; that is, the mutant protein contains only those amino acids found in the protein as it occurs in nature. A mutant protein may be produced by recombinant means or may be isolated from a naturally occurring source.

[000100] As used herein the term "portion" when in reference to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid.

[000101] The term "Southern blot," refers to the analysis of DNA on agarose or acrylamide gels to fractionate the DNA according to size followed by transfer of the DNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized DNA is then probed with a labeled probe to detect DNA species complementary to the probe used. The DNA may be cleaved with restriction enzymes prior to electrophoresis. Following electrophoresis, the DNA may be partially depurinated and denatured prior to or during transfer to the solid support. Southern blots are a standard tool of molecular biologists (J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, NY, pp 9.31-9.58 [1989]).

[000102] The term "Northern blot," as used herein refers to the analysis of RNA by electrophoresis of RNA on agarose gels to fractionate the RNA according to size followed by transfer of the RNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized RNA is then probed with a labeled probe to detect RNA species complementary to the probe used. Northern blots are a standard tool of molecular biologists (J. Sambrook, et al, supra, pp 7.39-7.52 [1989]).

[000103] The term "Western blot" refers to the analysis of protein(s) (or polypeptides) immobilized onto a support such as nitrocellulose or a membrane. The proteins are run on acrylamide gels to separate the proteins, followed by transfer of the protein from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized proteins are then exposed to antibodies with reactivity against an antigen of interest. The binding of the antibodies may be detected by various methods, including the use of radiolabeled antibodies.

[000104] As used herein, the term "cell culture" refers to any in vitro culture of cells. Included within this term are continuous cell lines (e.g., with an immortal phenotype), primary cell cultures, transformed cell lines, finite cell lines (e.g., non-transformed cells), and any other cell population maintained in vitro.

[000105] As used, the term "eukaryote" refers to organisms distinguishable from

"prokaryotes." It is intended that the term encompass all organisms with cells that exhibit the usual characteristics of eukaryotes, such as the presence of a true nucleus bounded by a nuclear membrane, within which lie the chromosomes, the presence of membrane-bound organelles, and other characteristics commonly observed in eukaryotic organisms. Thus, the term includes, but is not limited to such organisms as fungi, protozoa, and animals (e.g., humans).

[000106] As used herein, the term "in vitro" refers to an artificial environment and to processes or reactions that occur within an artificial environment. In vitro environments can consist of, but are not limited to, test tubes and cell culture. The term "in vivo" refers to the natural environment (e.g., an animal or a cell) and to processes or reaction that occur within a natural environment.

[000107] The terms "test compound" and "candidate compound" refer to any chemical entity, pharmaceutical, drug, and the like that is a candidate for use to treat or prevent a disease, illness, sickness, disorder of bodily function (e.g., cancer or non-cancer disease) or disrupt a system (e.g. cell culture). Test compounds comprise both known and potential therapeutic compounds. A test compound can be determined to be therapeutic by screening using the screening methods of the present invention. In some embodiments of the present invention, test compounds include antisense compounds.

[000108] As used herein, the term "known chemotherapeutic agents" refers to compounds known to be useful in the treatment of disease (e.g., cancer). Exemplary chemotherapeutic agents affective against cancer include, but are not limited to,

daunorubicin, dactinomycin, doxorubicin, bleomycin, mitomycin, nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-fluorouracil (5-FU), floxuridine (5-FUdR), methotrexate (MTX), colchicine, vincristine, vinblastine, etoposide, teniposide, cisplatin, lenolamide, and diethylstilbestrol (DES).

[000109] As used herein, the term "sample" is used in its broadest sense. In one sense, it is meant to include a specimen or culture obtained from any source, as well as biological and environmental samples. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include blood products, such as plasma, serum and the like. Environmental samples include environmental material such as surface matter, soil, water, crystals and industrial samples. Such examples are not however to be construed as limiting the sample types applicable to the present invention.

[000110] "Hot Zones" in some embodiments, are regions within the promoter region of an oncogene are further defined as preferred regions for hybridization of oligonucleotides. In some embodiments, these preferred regions are referred to as "hot zones." In some preferred embodiments, hot zones are defined based on oligonucleotide compounds that are

demonstrated to be effective (see above section on oligonucleotides) and those that are contemplated to be effective based on the preferred criteria for oligonucleotides described above. Preferred hot zones encompass 20 bp upstream and downstream of each compound included in each hot zone and have at least 1 CG or more within an increment of 40 bp further upstream or downstream of each compound. In preferred embodiments, hot zones encompass a maximum of 100 bp upstream and downstream of each oligonucleotide compound included in the hot zone. In additional embodiments, hot zones are defined at beginning regions of each promoter. These hot zones are defined either based on effective sequence(s) or contemplated sequences and have a preferred maximum length of 1000 bp. Based on the above described criteria, exemplary hot zones were designed. Specific hot zones are described in the examples.

[000111] Combination and Single- Agent Therapy Using this DNAi technology. We present and define the following disease conditions as exemplary of, but not limited to, those that are potentially treatable with the DNAi therapeutic(s) described herein. Treatment of these disease entities may occur with single-agent DNAi therapy or DNAi therapy in combination with one or more therapeutics used to treat the conditions.

[000112] Cardiovascular Disease

[000113] Treating cardiovascular disease involves opening narrowed arteries, correcting abnormalities associated with irregular heartbeats and dysfunctional heart muscle or valves, reducing high blood pressure and high lipid levels, and amending imbalances in clotting that causes symptoms of pain and discomfort. Inventions may include: medical devices, dyslipidemics, antithrombotics, anticoagulants, anti-platelets, antihypertensives, antiinflammatory, antihypertrophies, diuretics, anti-anginal, channel blockers, anti-restenosis agents, anti-atherosclerotics, anti-arrhythmics, enzyme inhibitors, and complement inhibitors.

[000114] Antianginals

The heart muscle works continuously and requires a constant supply of nutrients and oxygen. Those nutrients and oxygen are carried to the heart muscle in the blood. The chest pain known as angina can occur when there is an insufficient supply of blood, and consequently of oxygen, to the heart muscle. There are several types of antianginal medications. These include beta blockers (acebutolol, atenolol, betaxolol, bisoprolol, labetalol, metoprolol, nadolol, pindolol, propranolol, timolol), calcium channel blockers (diltiazem, nifedipine, verapamil), and vasodilators (nitroglycerin, isosorbide dinitrate). These drugs act by increasing the amount of oxygen that reaches the heart muscle. [000115] Antiarrhythmics

[000116] Antiarrhythmics are used when the heart does not beat rhythmically or smoothly (a condition called arrhythmia), its rate of contraction must be regulated.

Antiarrhythmic drugs (disopyramide, mexiletine, procainamide, propranolol, amiodarone, tocainide) prevent or alleviate arrhythmias by altering nerve impulses in the heart.

Anticoagulants are used when clots develop on the interior wall of an artery block blood flow.

[000117] Antihyperlipidemics

[000118] Medications for treating atherosclerosis, or hardening of the arteries, act to reduce the serum levels of cholesterol and triglycerides, which form plaques on the walls of arteries. The following drug classes are used to treat high cholesterol or high lipid levels: HMG CoA reductase inhibitors (atorvastatin, simvastatin, lovastatin, and rosuvastatin, fluvastatin, pravastatin), fibrates (fenofibrate, gemfibrozil), bile acid sequestrants

(cholestyramine, colestipol, and colestesvelam), niacins (niacin, Vit B3, nicotinic acid), and cholesterol absorption inhibitors (ezetimide), or drug combinations of these classes.

[000119] Antihypertensives

[000120] High blood pressure is caused when the pressure of the blood against the walls of the blood vessels is higher than what is considered normal. High blood pressure, or hypertension, eventually causes damage to the brain, eyes, heart, or kidneys. Several different drug actions produce an antihypertensive effect. Some drugs block nerve impulses that cause arteries to constrict; others slow the heart rate and decrease its force of contraction; still others reduce the amount of a certain hormone in the blood that causes blood pressure to rise. The effect of any of these medications is to reduce blood pressure. The mainstay of antihypertensive therapy is often a diuretic, a drug that reduces body fluids. Examples of antihypertensive drugs include beta blockers, calcium channel blockers, ACE (angiotensin- converting enzyme) inhibitors (including benazepril, captopril, enalapril, lisinopril, and quinapril), and the agents valsartan, losartan, prazosin, and terazosin.

[000121] Antiplatelets

[000122] Antiplatelet drugs alter the platelet activation at the site of vascular damage crucial to the development of arterial thrombosis. Aspirin irreversibly inhibits the enzyme COX, resulting in reduced platelet production of TXA₂ (thromboxane - powerful vasoconstrictor that lowers cyclic AMP and initiates the platelet release reaction). Dipyridamole inhibits platelet phosphodiesterase, causing an increase in cyclic AMP with potentiation of the action of PGI₂ - opposes actions of TXA₂.

Clopidogrel (Plavix) affects the ADP-dependent activation of IIb/IIIa complex.

Glycoprotein IIb/IIIa receptor antagonists block a receptor on the platelet for fibrinogen and von Willebrand factor and include for example, abciximab

eptifibatid and tirofiban. Epoprostenol is a prostacyclin that is used to inhibit platelet aggregation during renal dialysis (with or without heparin) and is also used in primary pulmonary hypertension.

[000123] Antithrombotics

An antithrombotic agent is a drug that reduces thrombus formation. These include plasminogen activators: Alteplase, Reteplase, Tenecteplase, Saruplase, Urokinase,

Anistreplase, Monteplase, Streptokinase, other serine endopeptidases (Ancrod, Brinase, Fibrinolysin)

[000124] Beta Blockers

[000125] Beta-blocking medications block the response of the heart and blood vessels to nerve stimulation, thereby slowing the heart rate and lowering blood pressure. They are used in the treatment of a wide range of diseases, including angina, high blood pressure, migraine headaches, arrhythmias, and glaucoma. Metoprolol and propranolol are common beta blockers.

[000126] Calcium Channel Blockers

[000127] Calcium channel blockers (diltiazem, nifedipine, verapamil) are used for the prevention of angina (chest pain). Verapamil is also useful in correcting certain arrhythmias (heartbeat irregularities) and lowering blood pressure. This group of drugs is thought to prevent angina and arrhythmias and lower blood pressure by blocking or slowing calcium flow into muscle cells, which results in vasodilation (widening of the blood vessels) and greater oxygen delivery to the heart muscle.

[000128] Cardiac Glycosides

[000129] Cardiac glycosides include drugs that are derived from digitalis (digoxin is an example). This type of drug slows the rate of the heart but increases its force of contraction. Cardiac glycosides act as both heart depressants and stimulants: They may be used to regulate irregular heart rhythm or to increase the volume of blood pumped by the heart in heart failure.

[000130] Diuretics [000131] Diuretic drugs, such as chlorothiazide, chlorthalidone, furosemide, hydrochlorothiazide, and spironolactone, promote the loss of water and salt from the body to lower blood pressure or increase the diameter of blood vessels. Antihypertensive

medications cause the body to retain salt and water and are often used concurrently with diuretics. Most diuretics act directly on the kidneys, but there are different types of diuretics, each with different actions. This allows therapy for high blood pressure to be adjusted to meet the needs of individual patients.

[000132] Thiazide diuretics, such as chlorothiazide, chlorthalidone, and

hydrochlorothiazide, are the most commonly prescribed and generally well tolerated as once or twice a day pills. A major drawback of thiazide diuretics is that they often deplete the body of potassium and therefore compensated with potassium supplements. Loop diuretics, such as furosemide, act more vigorously than thiazide diuretics. (Loop refers to the structures in the kidneys on which these specific diuretic medications act.) Loop diuretics promote more water loss than thiazide diuretics but they also deplete more potassium from the body.

Potassium sparing diuretics are also used treat heart failure and high blood pressure and include amiloride, spironolactone, and triamterene. Generally drug combinations of amiloride and hydrochlorothiazide , spironolactone and hydrochlorothiazide, and triamterene and hydrochlorothiazide are used to enhance the antihypertensive effect and reduce potassium loss.

[000133] Vasodilators

[000134] Vasodilating medications cause the blood vessels to dilate, or widen. Some of the antihypertensive medications, such as hydralazine and prazosin, lower blood pressure by dilating the arteries or veins. Other vasodilating medicines are used in the treatment of stroke and diseases that are characterized by poor blood circulation. Ergoloid mesylates, for example, are used to reduce the symptoms of senility by increasing the flow of oxygen-rich blood to the brain.

[000135] Metabolic Disease (Diabetes)

[000136] Diabetes is usually a lifelong or chronic disease caused by high levels of sugar in the blood. Insulin is a produced by the pancreas to control blood sugar and diabetes can be caused by too little insulin, resistance to insulin, or both. There are several types of diabetes. (1) Type 1 diabetes can occur at any age, but it is most often diagnosed in children, teens, or young adults. It is caused by the destruction of islet cells in the pancreas resulting in little or no insulin thereby requiring daily injections of insulin. (2) Type 2 diabetes results from insulin resistance and relative insulin deficiency. Obesity is thought to be the primary cause of Type 2 diabetes in those genetically predisposed. (3) Gestational diabetes is high blood sugar that develops at any time during pregnancy in a woman who does not have diabetes.

[000137] The following treatments for diabetes include: insulin, biguanides (metformin), suphonylureas, nonsulfonylurea secretagogues, meglitinides/prandial glucose regulatory/glinides, alpha-glucosidase inhibitors, thiazolidineone/glitazones, glucagon-like peptide- 1 analog, amylin analogues, and dipeptidyl peptidase-4 inhibitors.

[000138] Metformin is generally recommended as a first line treatment. When metformin is not sufficient another class is added.

[000139] Sulfonylureas lower blood sugar by stimulating the pancreas to release more insulin. The first drugs of this type that were developed— Dymelor (acetohexamide), Diabinese (chlorpropamide), Orinase (tolbutamide), and Tolinase (tolazamide)— are not as widely used since they tend to be less potent and shorter-acting drugs than the newer sulfonylureas. They include Glucotrol (glipizide), Glucotrol XL (extended release), DiaBeta (glyburide), Micronase (glyburide), Glynase PresTab (glyburide), and Amaryl (glimepiride). These drugs can cause a decrease in the hemoglobin A1c (HbA1c) of up to 1%-2%.

Biguanides improve insulin's ability to move sugar into cells especially into the muscle cells and prevent the liver from releasing stored sugar. Biguanides are contraindicated in people who have kidney damage or heart failure because of the risk of precipitating a severe buildup of lactic acid (called lactic acidosis) in these patients. Biguanides can decrease the HbA1c 1%-2%. An example includes metformin (Glucophage, Glucophage XR, Riomet, Fortamet, and Glumetza).

[000140] Thiazolidinediones improve insulin's effectiveness (improving insulin resistance) in muscle and in fat tissue. They lower the amount of sugar released by the liver and make fat cells more sensitive to the effects of insulin. Actos (pioglitazone) and Avandia (rosiglitazone) are the two drugs of this class. A decrease in the HbA1c of 1%-2% can be seen with this class of oral diabetes medications. Thiazolidinediones should be used with caution in people with heart failure. Avandia is restricted for use in new patients only if they are uncontrolled on other medications and are unable to take Actos.

Alpha-glucosidase inhibitors include Precose (acarbose) and Glyset (miglitol). These drugs block enzymes that help digest starches, slowing the rise in blood sugar. These diabetes pills may cause diarrhea or gas. They can lower hemoglobin A1c by 0.5%-1%.

[000141] Meglitinides include Prandin (repaglinide) and Starlix (nateglinide). These diabetes medicines lower blood sugar by stimulating the pancreas to release more insulin. The effects of these drugs are glucose-dependent, with high blood sugar inducing insulin release, which is unlike the action of sulfonylureas which cause insulin release, regardless of glucose levels, and can lead to hypoglycemia.

[000142] Dipeptidyl peptidase IV (DPP-IV) inhibitors include Januvia (sitagliptin), Nesina (alogliptin), Onglyza (saxagliptin), Galvus (vildagliptin) and Tradjenta (linagliptin). The DPP-IV inhibitors work to lower blood sugar in patients with type 2 diabetes by increasing insulin secretion from the pancreas and reducing sugar production. These diabetes pills increase insulin secretion when blood sugars are high. They also signal the liver to stop producing excess amounts of sugar. DPP-IV inhibitors control sugar without causing weight gain. The medication may be taken alone or with other medications such as metformin.

[000143] Glucagon-like peptide analogs and agonists

[000144] Glucagon-like peptide (GLP) agonists bind to a membrane GLP receptor. As a consequence, insulin release from the pancreatic beta cells is increased.

Examples of this class include Exenatide (also Exendin-4, marketed as Byetta).

Exenatide is not an analogue of GLP but rather a GLP agonist. Typical reductions in A1C values are 0.5-1.0%. Liraglutide, a once-daily human analogue (97% homology), has been developed by Novo Nordisk under the brand name Victoza. Taspoglutide is presently in Phase III clinical trials with Hoffman-La Roche.

[000145] Alpha-glucosidase inhibitors (Acarbose, Miglitol, Voglibose), amylin analogues (Pramlintide), SGLT2 inhibitors (Canagliflozin, Dapagliflozin, Empagliflozin, Remogliflozin, Sergliflozin) and others (Benfluorex, Tolrestat)

[000146] Combination agents are the combination of two medications in one tablet and include the following examples: Glucovance, which combines glyburide (a sulfonylurea) and metformin, Metaglip, which combines glipizide (a sulfonylurea) and metformin, and

Avandamet which utilizes both metformin and rosiglitazone (Avandia). Kazano (alogliptin and metformin) and Oseni (alogliptin plus pioglitazone) are other examples.

[000147] Eye Disorders

[000148] Ocular Bacterial Infection. Antibiotics are generally used to treat, or sometimes to prevent a bacterial eye infection. Examples of common antibiotics used in the eye are sulfacetamide, erythromycin, gentamicin, tobramycin, ciprofloxacin and ofloxacin.

[000149] Ocular Inflammatory reaction. Anti-inflammatories reduce inflammation, which in the eye is usually manifest by pain, redness, light sensitivity and sometimes blurred vision. Anti-inflammatories can be either glucocorticoids/corticosteroids or NSAIDs. Corticosteroids are very effective anti-inflammatories for a wide variety of eye problems including all disorders associated with systemic inflammatory reactions (Reiter's syndrome, xerostomia, etc.). Common corticosteroids include: Prednisolone, Fluorometholone and Dexamethasone. Non-steroidal anti-inflammatories reduce the production of proinflammatory factors such as prostaglandins. Common NSAIDs include: Diclofenac, Ketorolac and Flurbiprofen.

[000150] Glaucoma. Glaucoma is a disorder of regulation of intraocular pressure.

Glaucoma medications all attempt to reduce this pressure to prevent damage to the optic nerve resulting in loss of vision. These medications may lower pressure by decreasing the amount of fluid produced in the eye, by increasing the amount of fluid exiting through the eye's natural drain, or by providing additional pathways for fluid to leave the eye. More than one glaucoma medication is used simultaneously, as these effects can combine to lower pressure further than possible with a single medication. These medications are listed by class: BETA-BLOCKERS: Timolol, Metipranolol, Carteolol, Betaxolol, Levobunolol

ALPHA AGONISTS: Brimonidine, Iopidine

PROSTAGLANDIN ANALOGUES: Latanoprost

CARBONIC ANHYDRASE INHIBITORS: Dorzolamide

CHOLINERGIC AGONISTS: Pilocarpine, Carbachol

ADENERGIC AGONISTS, Epinephrine, Dipivefrin

[000151] Ocular Viral Infection

Used primarily in treating herpes virus infections of the eye, antiviral eye medications may be used in conjunction with oral medications for elimination of the virus. The most common type of antiviral is triflurthymidine. Other topical anti-virals include adenine arabinoside and idoxuridine.

[000152] Allergic reaction

All anti-allergy topicals decrease the effects of histamine, a factor that mediates, the inflammatory reaction. Common anti-allergy medicines include levostin, patanol, Cromolyn and alomide.

[000153] Infectious Diseases

[000154] Aminoglycosides. This class of antibiotics is used to treat infections caused by Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella*, particularly *Pseudomonas aeruginosa*. This class is also effective against Aerobic bacteria (but not obligate/facultative anaerobes) and in the treatment of tularemia. The mechanism of action includes binding to the bacterial 30S ribosome/ribosomal subunit (some work by binding to the 50S subunit), inhibiting the translocation of the peptidyl-tRNA from the A-site to the P-site and also causing misreading of mRNA, leaving the bacterium unable to synthesize proteins vital to its growth. Possible toxicities include hearing loss, vertigo and nephrotoxicity. Examples of aminoglycosides include Amikacin, Gentamicin, Kanamycin, Neomycin, Netilmicin, Tobramycin, Paromomycin, Spectinomycin.

[000155] Ansamycins. Used as anti-tumor antibiotics and for treatment of traveler's diarrhea caused by *E. coli*. Examples include Geldanamycin, Herbimycin, and Rifaximin.

[000156] Carbacephem. This class prevents bacterial cell division by inhibiting cell wall synthesis. An example is Loracarbef.

[000157] Carbapenem. This class works by inhibiting cell wall synthesis. It is bactericidal for both Gram-positive and Gram-negative organisms and therefore useful for empiric broad-spectrum antibacterial coverage. (Note MRSA resistance to this class.)

Toxicity may include gastrointestinal upset and diarrhea, nausea, seizures, headache, rash and allergic reactions. Examples include Ertapenem, Doripenem, Imipenem/Cilastatin,

Meropenem.

[000158] Cephalosporins (First generation). Have the same mode of action as other beta-lactam antibiotic to disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. The class provides good coverage against Gram positive infections. Potential toxicities include gastrointestinal upset and diarrhea, nausea (if alcohol taken concurrently) and allergic reactions. Examples include Cefadroxil, Cefazolin, Cefalotin, Cefalothin, Keflin, and Cefalexin.

[000159] Cephalosporins (Second generation). This class provides less gram-positive coverage than the above with improved gram negative cover. They have the same mode of action as other beta-lactam antibiotics and disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. They may cause gastrointestinal upset and diarrhea, nausea (if alcohol taken concurrently) and allergic reactions. Examples include: Cefaclor, Cefamandole, Cefoxitin, Cefprozil and Cefuroxime.

[000160] Cephalosporins (Third generation). Same mode of action as other beta-lactam antibiotic to disrupt the synthesis of the peptidoglycan layer of bacterial cell wall. Provides improved coverage of Gram-negative organisms, except *Pseudomonas*. Has reduced Gram-positive coverage. May cause gastrointestinal upset and diarrhea, nausea (if alcohol taken concurrently) and allergic reactions. Examples include Cefixime, Cefdinir, Cefditoren, Cefoperazone, Cefotaxime, Cefpodoxime, Ceftazidime, Ceftibuten, Ceftizoxime, and Ceftriaxone.

[000161] Cephalosporins (Fourth generation). As above for mechanism and toxicity but good coverage for pseudomonal infections. Examples include Cefepime.

[000162] Cephalosporins (Fifth generation). As above for mechanism and toxicity but good coverage for Methicillin-resistant *Staphylococcus aureus*/MRSA. Examples include Ceftaroline fosamil, and Ceftobiprole.

[000163] Glycopeptides. Inhibit peptidoglycan synthesis and are active against aerobic and anaerobic Gram positive bacteria including MRSA; Vancomycin is used orally for the treatment of *C. difficile*. Examples include Teicoplanin, Vancomycin, and Telavancin

[000164] Lincosamides. Bind to 50S subunit of bacterial ribosomal RNA thereby inhibiting protein synthesis. Used to treat serious staph-, pneumo-, and streptococcal infections in penicillin-allergic patients, also anaerobic infections; clindamycin topically used for acne and possible *C. difficile*-related pseudomembranous enterocolitis, include

Clindamycin and Lincomycin.

[000165] Lipopeptides. Bind to the membrane and cause rapid depolarization, resulting in a loss of membrane potential leading to inhibition of protein, DNA and RNA synthesis Gram-positive organisms. Example is Daptomycin.

[000166] Macrolides. Are enzyme inhibitors of bacterial protein biosynthesis by binding reversibly to the subunit 50S of the bacterial ribosome, thereby inhibiting translocation of peptidyl-tRNA. Used to treat Streptococcal infections, syphilis, upper respiratory tract infections, lower respiratory tract infection, mycoplasmal infections, Lyme disease. Can cause nausea, vomiting, and diarrhea (especially at higher doses), prolonged QT interval (especially erythromycin) and Jaundice. Examples include Azithromycin,

Clarithromycin, irthromycin, Erythromycin, Roxithromycin, Troleandomycin, Telithromycin and Spiramycin.

[000167] Monobactams. Same mode of action as other beta-lactam antibiotics, to disrupt the synthesis of the peptidoglycan layer of bacterial cell walls. Example includes Aztreonam.

[000168] Nitrofurans. Are used to treat bacterial or protozoal diarrhea or enteritis. An example is Furazolidone and Nitrofurantoin to treat urinary tract infections. [000169] Oxazolidonones. Protein synthesis inhibitors, they prevent the initiation step and are used to treat vancomycin-resistant *Staphylococcus aureus*. Can cause thrombocytopenia, and peripheral neuropathy. Examples include Linezolid, Radezolid,

[000170] Penicillins. Disrupt the synthesis of the peptidoglycan layer of bacterial cell walls.

These are used to treat a wide range of infections; penicillin is used for streptococcal infections, syphilis and Lyme disease and can cause gastrointestinal upset and diarrhea, allergy with serious anaphylactic reaction, brain and kidney damage (rare). Examples include, Amoxicillin, Ampicillin, Azlocillin, Carbenicillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Mezlocillin, Methicillin, Nafcillin, Oxacillin, Penicillin G, Penicillin V, Piperacillin,

Penicillin G, Temocillin, Ticarcillin.

[000171] Penicillin combinations. The second component prevents bacterial antibiotic resistance to the first component. Examples include Augmentin, Ampicillin/sulbactam, Piperacillin/tazobactam, Ticarcillin/clavulanate.

[000172] Polypeptide_antibiotics. For treatment of eye, ear or bladder infections;

usually applied directly to the eye or inhaled into the lungs; rarely given by injection, although the use of intravenous colistin is experiencing a resurgence due to the emergence of multi drug resistant organisms. This class can cause kidney and nerve damage (when given by injection). The class inhibits isoprenyl pyrophosphate, a molecule that carries the building blocks of the peptidoglycan bacterial cell wall outside of the inner membrane. Examples include Bacitracin, Colistin, and Polymyxin B

[000173] Quinolones. For treatment of urinary tract infections, bacterial prostatitis, community-acquired pneumonia, bacterial diarrhea, mycoplasma infection, gonorrhea. Can cause nausea (rare), irreversible damage to central nervous system (uncommon), tendinitis (rare). The class works by inhibiting the bacterial DNA gyrase or the topoisomerase IV enzyme, thereby inhibiting DNA replication and transcription. Examples include,

Ciprofloxacin, Enoxacin, Gatifloxacin, Levofloxacin, Lomefloxacin, Moxifloxacin, Avelox, Nalidixic acid, Norfloxacin, Ofloxacin, Trovafloxacin, Grepafloxacin, Raxar, Sparfloxacin and Temafloxacin.

[000174] Sulfonamides. They are competitive inhibitors of the enzyme dihydropteroate synthetase, DHPS. DHPS catalyses the conversion of PABA (para-Aminobenzoic acid) to dihydropteroic acid/dihydropteroate, a key step in folate synthesis. Folate is necessary for the cell to synthesize nucleic acids (nucleic acids are essential building blocks of DNA and RNA, and in its absence cells will be unable to divide. The class is used to treat Urinary tract infections (except sulfacetamide, used for Conjunctivitis, and mafenide and silver

sulfadiazine, used topically for burns. The class can cause nausea, vomiting, and diarrhea, Allergy, including skin rashes, crystals in urine, Renal failure, decrease in white blood cell count and sensitivity to sunlight. Examples include Mafenide, Sulfacetamide, Sulfadiazine, Silver sulfadiazine, Sulfadimethoxine, Sulfamethizole, Sulfamethoxazole, Sulfanilimide, Sulfasalazine, Sulfisoxazole, and Trimethoprim-Sulfamethoxazole.

[000175] Tetracyclines. Inhibit the binding of aminoacyl-tRNA to the mRNA- ribosome complex. They do so mainly by binding to the 30S ribosomal subunit in the mRNA translation complex. Can be used to treat Syphilis, Chlamydia infections, Lyme disease, mycoplasma infections, acne, rickettsial infections, and malaria caused by a parasite and not a bacterium. Toxicity includes Gastrointestinal upset, Sensitivity to sunlight, Potential toxicity to mother and fetus during pregnancy, Enamel hypoplasia (staining of teeth; potentially permanent, transient depression of bone growth. Examples include Demeclocycline,

Doxycycline, Minocycline, Oxytetracycline, and Tetracycline.

[000176] Drugs against mycobacteria include the following: Clofazimine, Dapsone, Capreomycin, Cycloserine, Ethambutol, Ethionamide, Isoniazid, Pyrazinamide, Rifampicin, Rifabutin, Rifapentine, Streptomycin, and aminoglycosides.

[000177] Other antibiotics include the following ::

[000178] Arsphenamine, Chloramphenicol, Fosfomycin, Fusidic acid, Metronidazole, Mupirocin, Platensimycin, Quinupristin/Dalfopristin, Thiamphenicol, Tigecycline,

Tinidazole, and Trimethoprim. Anti- Viral Medications by Indication

[000179] Herpes Simplex Virus (HSV), Varicella Zoster Virus (VZV) and

cytomegalovirus (CMV). Oral herpes simplex virus (HSV) causes mucous membrane lesions (i.e., cold sores), and genital HSV causes genital herpetic lesions. Treatment for HSV can also be used for the treatment of Varicella Zoster Virus (VZV) the causative agent for chicken-pox in children and shingles in adults. Typical anti-virals include Acyclovir and Valaciclovir, both inhibitors of viral DNA synthesis. Additionally, Idoxuridine and Brivudin can be incorporated into the viral DNA leading to a hindered mechanism of DNA

duplication. A third type of herpes viruses with established treatment is cytomegalovirus (CMV), particularly dangerous for unborn children, infants and immune-compromised patients. Medications used to treat CMV are Ganciclovir and Foscarnet, also indicated in some HSV infections. They act to inhibit viral DNA synthesis.

[000180] HIV. A diverse group of antiviral medications control viral load, but cannot cure HIV infections. Viral entry inhibitors such as Enfuvirtide prevent newly formed viruses from entering uninfected host cells by preventing virus-cell fusion.

[000181] Reverse transcriptase inhibitors include many drugs such as Abacavir,

Lamivudine, Zidovudine, Tenofovir, Efavirenz and Nevirapine. These drugs inhibit reverse transcriptase, an enzyme critical to the mechanism by which HIV transcribes genetic material.

Another anti-viral approach utilizes the protease inhibitors such as Atazanavir, Indinavir and Ritonavir to inhibit assembly of new viruses. Combination therapies using 2 or 3 of the aforementioned agents are very effective at reducing serum viral load to below detectable levels.

[000182] Hepatitis. One of the few anti-HBV (hepatitis B) medications is Lamivudine, a reverse transcriptase inhibitor. Additionally, adefovir and dipivoxil, medications used in the treatment of HIV can be used to inhibit transcription of viral HBV RNA into DNA.

Interferons are naturally occurring molecules that stimulate immune responses against invading species, including viral particles. Imiquimod up-regulates the natural production of interferons to boost the human immune response. Synthetically produced Alpha-interferon is also effective in treating HBV and HCV, especially in combination with other drugs.

Unfortunately, interferons are associated with a number of severe toxicities that limit their long-term usage in a number of patients.

[000183] Broad-spectrum Antiviral Medications

Ribavirin is effective in the treatment of influenza, HCV and paramyxoviruses such as measles and respiratory syncytial virus by blocking synthesis of viral RNA. A combination of Ribavirin and Alpha-interferon is proven to be effective in treatment of chronic hepatitis C infections.

[000184] Inflammation. Anti-Inflammatory medications by class

[000185] Glucocorticoids. This class of anti-inflammatory medication reduces inflammation by binding to glucocorticoid receptors (GR). The activated GR complex, in turn, up-regulates the expression of anti-inflammatory proteins in the nucleus (a process known as transactivation) and represses the expression of pro-inflammatory proteins in the cytosol by preventing the translocation of other transcription factors from the cytosol into the nucleus. These drugs are often referred to as corticosteroids. Examples include Budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone and

prednisolone. [000186] Non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs reduce inflammation by reducing the production of prostaglandins, chemicals that promote inflammation, pain, and fever. Prostaglandins also protect the lining of the stomach and intestines from the damaging effects of acid, and promote blood clotting by activating blood platelets and affect kidney function. The enzymes that produce prostaglandins are called cyclooxygenase (COX). There are two types of COX enzymes, COX-1 and COX-2. Both enzymes produce prostaglandins that promote inflammation, pain, and fever; however, only COX-1 produces prostaglandins that activate platelets and protect the stomach and intestinal lining. NSAIDs block COX enzymes and reduce production of prostaglandins. Therefore, inflammation, pain, and fever are reduced. Since the prostaglandins that protect the stomach and promote blood clotting also are reduced, NSAIDs can cause ulcers in the stomach and intestines, and increase the risk of bleeding. Aspirin is the only NSAID that inhibits the clotting of blood for a prolonged period of time, four to seven days, and is therefore effective for preventing blood clots that cause heart attacks and strokes. Ketorolac is a very potent NSAID and is used for treating severe pain that normally would be

managed with narcotics. Ketorolac causes ulcers more frequently than other NSAIDs and should not be used for more than five days. Celecoxib blocks COX-2 but has little effect on COX-1. Therefore, celecoxib is sub-classified as a selective COX-2 inhibitor, and it causes fewer ulcers and less bleeding than other NSAIDs. Commonly prescribed NSAIDs include aspirin, salsalate, celecoxib, diclofenac, etodolac, ibuprofen, indomethacin, ketoprofen, ketorolac, nabumetone, naproxen, oxaprozin, piroxicam, sulindac and tolmetin.

[000187] Neurological Diseases

Huntington's Disease and dyskinesias. Chorea is an abnormal involuntary movement disorder, one of a group of neurological disorders called dyskinesias, which are caused by overactivity of the neurotransmitter dopamine in the areas of the brain that control movement. Chorea is characterized by brief, irregular contractions that are not repetitive or rhythmic, but appear to flow from one muscle to the next. Chorea often occurs with athetosis, which adds twisting and writhing movements. Chorea is a primary feature of Huntington's disease, a progressive, hereditary movement disorder that appears in adults, but it may also occur in a variety of other conditions. Sydenham's chorea occurs in a small percentage (20 percent) of children and adolescents as a complication of rheumatic fever. Chorea can also be induced by drugs (levodopa, anti-convulsants, and anti-psychotics) metabolic and endocrine disorders, and vascular incidents. There is currently no standard course of treatment for chorea.

Treatment depends on the type of chorea and the associated disease. Treatment for Huntington's disease is supportive, while treatment for Sydenham's chorea usually involves antibiotic drugs to treat the infection, followed by drug therapy to prevent recurrence.

Adjusting medication dosages can treat drug-induced chorea. Metabolic and endocrine-related choreas are treated according to the cause(s) of symptoms.

[000188] Parkinson's Disease. Parkinson's disease (PD) belongs to a group of conditions called motor system disorders, which are the result of the loss of dopamine-producing brain cells. The four primary symptoms of PD are tremor, or trembling in hands, arms, legs, jaw, and face; rigidity, or stiffness of the limbs and trunk; bradykinesia, or slowness of movement; and postural instability, or impaired balance and coordination. PD usually affects people over the age of 50. Other symptoms may include depression and other emotional changes;

difficulty in swallowing, chewing, and speaking; urinary problems or constipation; skin problems; and sleep disruptions. There are currently no blood or laboratory tests that have been proven to help in diagnosing sporadic PD. Therefore the diagnosis is based on medical history and a neurological examination. The disease can be difficult to diagnose

accurately. There is no cure for PD, but a variety of medications are used to relieve symptoms. Patients are given levodopa combined with carbidopa. Carbidopa delays the conversion of levodopa into dopamine until it reaches the brain. Nerve cells can use levodopa to make dopamine and replenish the brain supply. Anticholinergics may help control tremor and rigidity. Other drugs, such as bromocriptine, pramipexole, and ropinirole, mimic the role of dopamine in the brain, causing the neurons to react as they would to dopamine. An antiviral drug, amantadine, also appears to reduce symptoms. Rasagiline can be used along with levodopa for patients with advanced PD or as a single-drug treatment for early PD. In some cases, surgery may be appropriate if the disease doesn't respond to drugs. A therapy called deep brain stimulation (DBS) has now been approved by the U.S. Food and Drug Administration. In DBS, electrodes are implanted into the brain and connected to a small electrical device called a pulse generator that can be externally programmed. DBS can reduce the need for levodopa and related drugs, which in turn decreases the involuntary movements called dyskinesias that are a common side effect of levodopa. It also helps to alleviate fluctuations of symptoms and to reduce tremors, slowness of movements, and gait problems. DBS requires careful programming of the stimulator device in order to work correctly.

[000189] Amyotrophic Lateral Sclerosis. Amyotrophic lateral sclerosis (ALS), sometimes called Lou Gehrig's disease or classical motor neuron disease, is a rapidly progressive, invariably fatal neurological disease that attacks the neurons responsible for controlling voluntary muscles. In ALS, both the upper motor neurons and the lower motor neurons degenerate or die, ceasing to send messages to muscles. Unable to function, the muscles gradually atrophy. Symptoms are usually first noticed in the arms and hands, legs, or swallowing muscles. Muscle weakness and atrophy occur on both sides of the

body. Individuals with ALS lose their strength and the ability to move their arms and legs, and to hold the body upright. The disease does not affect a person's ability to see, smell, taste, hear, or recognize touch. Although the disease does not usually impair a person's mind or personality, several recent studies suggest that some people with ALS may develop cognitive problems involving word fluency, decision-making, and memory. The cause of ALS is not known. No cure has yet been found for ALS. The drug riluzole prolongs life by 2-3 months but does not relieve symptoms.

[000190] Multiple Sclerosis. Multiple sclerosis (MS) is a neurologic disease that can range from benign to completely disabling. MS results from an auto-immune response to nerve-insulating myelin. Such assaults may be linked to an unknown environmental trigger, perhaps a virus.

Most people experience their first symptoms of MS between the ages of 20 and 40; the initial symptom of MS is often blurred or double vision, red-green color distortion, or even blindness in one eye. Most MS patients experience muscle weakness in their extremities and difficulty with coordination and balance. These symptoms may be severe enough to impair walking or even standing. In the worst cases, MS can produce partial or complete

paralysis. Most people with MS also exhibit paresthesias, transitory abnormal sensory feelings such as numbness, prickling, or "pins and needles" sensations. Some may also experience pain. Speech impediments, tremors, and dizziness are other frequent complaints. Occasionally, people with MS have hearing loss. Approximately half of all people with MS experience cognitive impairments such as difficulties with concentration, attention, memory, and poor judgment, but such symptoms are usually mild and are frequently

overlooked. Depression is another common feature of MS. There is as yet no cure for MS. Three forms of beta interferon (Avonex, Betaseron, and Rebif) have now been approved by the Food and Drug Administration for treatment of relapsing-remitting MS. Beta interferon has been shown to reduce the number of exacerbations and may slow the progression of physical disability. When attacks do occur, they tend to be shorter and less severe. The FDA also has approved a synthetic form of myelin basic protein, called copolymer I (Copaxone), for the treatment of relapsing-remitting MS. An immunosuppressant treatment, Novantrone (mitoxantrone), is approved by the FDA for the treatment of advanced or chronic MS. The FDA has also approved dalfampridine (Ampyra) to improve walking in individuals with MS. While steroids do not affect the course of MS over time, they can reduce the duration and severity of attacks in some patients. Spasticity, which can occur either as a sustained stiffness caused by increased muscle tone or as spasms that come and go, is usually treated with muscle relaxants and tranquilizers such as baclofen, tizanidine, diazepam, clonazepam, and dantrolene. Other drugs that may reduce fatigue in some, but not all, patients include amantadine (Symmetrel), pemoline (Cylert), and the still-experimental drug aminopyridine. Although improvement of optic symptoms usually occurs even without treatment, a short course of treatment with intravenous methylprednisolone (Solu-Medrol) followed by treatment with oral steroids is sometimes used.

[000191] Alzheimer's Disease. Alzheimer's disease is an irreversible, progressive brain disease that slowly destroys memory and thinking skills. In most people with Alzheimer's, symptoms first appear after age 60. Estimates vary, but as many as 5.1 million Americans may have Alzheimer's disease. Patients exhibit various brain abnormalities including amyloid plaques, neurofibrillary tangles, and neuronal loss. Four medications are approved by the U.S. Food and Drug Administration to treat Alzheimer's. Donepezil, rivastigmine and galantamine are used to treat mild to moderate Alzheimer's. Memantine is used to treat moderate to severe Alzheimer's. These drugs do not change the underlying disease process, are effective for some but not all people, and may help only for a limited time.

[000192] Schizophrenia. Schizophrenics display three broad categories of symptoms characterized as positive, negative and cognitive. Positive symptoms are psychotic behaviors including hallucinations, delusions, thought and movement disorders. Negative symptoms are associated with disruptions to normal behaviors. These symptoms include flat affect, lack of pleasure in everyday activities, lack of ability to begin and sustain planned activities, and speaking little, even when forced to interact as well as having neglect for basic personal hygiene. Cognitive symptoms include poor ability to understand information and use it to make decisions, trouble focusing or paying attention and problems with the ability to use information immediately after learning it. This neurologic disorder affects 1 percent of the general population, but it occurs in 10 percent of people who have a first-degree relative with the disorder. The risk is highest for an identical twin of a person with schizophrenia with a 40-65 percent chance of developing the disorder. No gene causes the disease by itself.

Aberrant dopamine and glutamate transmission is believed to play a role in schizophrenia. Treatments include antipsychotic medications and various psychosocial treatments. Older antipsychotic medications include Chlorpromazine, Haloperidol, Perphenazine, Etrafon and Fluphenazine. New antipsychotic medications include clozapine which can cause

agranulocytosis, requiring bi-weekly WBC count evaluation. Other atypical antipsychotics include Risperidone, Olanzapine, Quetiapine, Ziprasidone, Aripiprazole and Paliperidone. Side effects of many antipsychotics include drowsiness, dizziness when changing positions, blurred vision, rapid heartbeat, sensitivity to the sun, Skin rashes and menstrual problems for women. Atypical antipsychotic medications can cause major weight gain and changes in a person's metabolism. This may increase a person's risk of getting diabetes and high cholesterol. Typical antipsychotic medications can cause side effects related to physical movement, such as rigidity, persistent muscle spasms, tremors and restlessness. Long-term use of typical antipsychotic medications may lead to a condition called tardive dyskinesia (TD). TD causes uncontrolled, and in some cases permanent, involuntary muscle movements.

[000193] ADDITIONAL DESCRIPTION OF THE INVENTION

[000194] The present invention relates to methods and compositions for the treatment of any gene that is desirable to modulate expression of. This includes but is not limited to cancers. In the next sections will describe both cancer and non-cancer targets and then in the section immediately following those selected cancer and non-cancer targets we will present over 40 High Value Targets, both cancer and noncancer, with sequence information, and some of these examples will have data with detailed information about our techniques and methods as well as our surprising results.

[000195] Cancer Targets

[000196] In some embodiments, the present invention provides oligonucleotide-based therapeutics for the inhibition of oncogenes involved in a variety of cancers. The present invention is not limited to the treatment of cancer or any particular cancer. Any cancer can be targeted, including, but not limited to, breast cancers. The present invention is also not limited to the targeting of cancers or oncogenes. The methods and compositions of the present invention are suitable for use with any gene that it is desirable to inhibit the expression of (e.g., for therapeutic or research uses. Specific gene targets that have been optimally identified as susceptible to the DNAi therapeutic approach are described below.

[000197] Oncogene Targets such as,

[000198] In some embodiments, the present invention provides DNAi inhibitors of oncogenes. The present invention is not limited to the inhibition of a particular oncogene. Indeed, the present invention encompasses DNAi inhibitors to any number of oncogenes including, but not limited to, those disclosed herein.

[000199] Combination Therapies with Cancer Targets

In some embodiments, the compositions of the present invention are provided in combination with existing therapies. In other embodiments, two or more compounds of the present invention are provided in combination. In some embodiments, the compounds of the present invention are provided in combination with known cancer chemotherapy agents. The present invention is not limited to a particular chemotherapy agent.

[000200] Various classes of antineoplastic (e.g., anticancer) agents are contemplated for use in certain embodiments of the present invention. Anticancer agents suitable for use with the present invention include, but are not limited to, agents that induce apoptosis, agents that inhibit adenosine deaminase function, inhibit pyrimidine biosynthesis, inhibit purine ring biosynthesis, inhibit nucleotide interconversions, inhibit ribonucleotide reductase, inhibit thymidine monophosphate (TMP) synthesis, inhibit dihydrofolate reduction, inhibit DNA synthesis, form adducts with DNA, damage DNA, inhibit DNA repair, intercalate with DNA, deaminate asparagines, inhibit RNA synthesis, inhibit protein synthesis or stability, inhibit microtubule synthesis or function, and the like.

[000201] In some embodiments, exemplary anticancer agents suitable for use in compositions and methods of the present invention include, but are not limited to: 1) alkaloids, including microtubule inhibitors (e.g., vincristine, vinblastine, and vindesine, etc.), microtubule stabilizers (e.g., paclitaxel (TAXOL), and docetaxel, etc.), and chromatin function inhibitors, including topoisomerase inhibitors, such as epipodophyllotoxins (e.g., etoposide (VP- 16), and teniposide (VM-26), etc.), and agents that target topoisomerase I (e.g., camptothecin and irinotecan (CPT-11), etc.); 2) covalent DNA-binding agents (alkylating agents), including nitrogen mustards (e.g., mechlorethamine, chlorambucil, cyclophosphamide, ifosfamide, and busulfan (MYLERAN), etc.), nitrosoureas (e.g., carmustine, lomustine, and semustine, etc.), and other alkylating agents (e.g., dacarbazine, hydroxymethylmelamine, thiopeta, and mitomycin, etc.); 3) noncovalent DNA-binding agents (antitumor antibiotics), including nucleic acid inhibitors (e.g., dactinomycin

(actinomycin D), etc.), anthracyclines (e.g., daunorubicin (daunomycin, and cerubidine), doxorubicin (adriamycin), and idarubicin (idarubicin), etc.), anthracenediones (e.g., anthracycline analogues, such as mitoxantrone, etc.), bleomycins (BLENOXANE), etc., and plicamycin (mithramycin), etc.); 4) antimetabolites, including antifolates (e.g., methotrexate, FOLEX, and MEXATE, etc.), purine antimetabolites (e.g., 6-mercaptopurine (6-MP, PURINETHOL), 6-thioguanine (6-TG), azathioprine, acyclovir, ganciclovir, chlorodeoxyadenosine, 2-chlorodeoxyadenosine (CdA), and 2'-deoxycoformycin

(pentostatin), etc.), pyrimidine antagonists (e.g., fluoropyrimidines (e.g., 5-fluorouracil (ADRUCIL), 5-fluorodeoxyuridine (FdUrd) (floxuridine)) etc.), and cytosine arabinosides (e.g., CYTOSAR (ara-C) and fludarabine, etc.); 5) enzymes, including L-asparaginase, and hydroxyurea, etc.; 6) hormones, including glucocorticoids, antiestrogens (e.g., tamoxifen, etc.), nonsteroidal antiandrogens (e.g., flutamide, etc.), and aromatase inhibitors (e.g., anastrozole (ARIMIDEX), etc.); 7) platinum compounds (e.g., cisplatin and carboplatin, etc.); 8) monoclonal antibodies conjugated with anticancer drugs, toxins, and/or

radionuclides, etc.; 9) biological response modifiers (e.g., interferons (e.g., IFN-a, etc.) and interleukins (e.g., IL-2, etc.), etc.); 10) adoptive immunotherapy; 11) hematopoietic growth factors; 12) agents that induce tumor cell differentiation (e.g., all-trans-retinoic acid, etc.); 13) gene therapy techniques; 14) antisense therapy techniques; 15) tumor vaccines; 16) therapies directed against tumor metastases (e.g., batimastat, etc.); 17) angiogenesis inhibitors; 18) proteasome inhibitors (e.g., VELCADE); 19) inhibitors of acetylation and/or methylation (e.g., HDAC inhibitors); 20) modulators of NF kappa B; 21) inhibitors of cell cycle regulation (e.g., CDK inhibitors); 22) modulators of p53 protein function; and 23) radiation.

[000202] Any oncolytic agent that is routinely used in a cancer therapy context finds use in the compositions and methods of the present invention. For example, the U.S. Food and Drug Administration maintains a formulary of oncolytic agents approved for use in the United States. International counterpart agencies to the U.S.F.D.A.

maintain similar formularies. Table 1 provides a list of exemplary antineoplastic agents approved for use in the U.S. Those skilled in the art will appreciate that the "product labels" required on all U.S. approved chemotherapeutics describe approved indications, dosing information, toxicity data, and the like, for the exemplary agents.

Table 1

Inc., San Diego CA

Allopurinol Zyloprim GlaxoSmithKline,

(1,5-dihydro-4 H -pyrazolo[3,4-d]pyrimidin-4-one Research Triangle monosodium salt) Park, NC

Altretamine Hexalen US Bioscience,

(N,N,N*,N*,N",N",- hexamethyl-1,3,5-triazine-2, 4, 6- West

triamine) Conshohocken, PA

Amifostine Ethyol US Bioscience

(ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen

phosphate (ester))

Anastrozole Arimidex AstraZeneca

(1,3-Benzenediacetonitrile, a, a, a', a'-tetramethyl-5- Pharmaceuticals, (1 H- 1 ,2,4-triazol- 1 -ylmethyl)) LP, Wilmington,

DE

Arsenic trioxide Trisenox Cell Therapeutic,

Inc., Seattle, WA

Asparaginase Elspar Merck & Co., Inc.,

(L-asparagine amidohydrolase, type EC-2) Whitehouse

Station, NJ

BCG Live TICE BCG Organon Teknika,

(lyophilized preparation of an attenuated strain of Corp., Durham, NC Mycobacterium bovis (Bacillus Calmette-Guikin

[BCG], substrain Montreal)

bexarotene capsules Targretin Ligand

(4-[-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2- Pharmaceuticals naphthalenyl) ethenyl] benzoic acid)

Bexarotene gel Targretin Ligand

Pharmaceuticals

Bleomycin Blenoxane Bristol-Myers

(cytotoxic glycopeptide antibiotics produced by Squibb Co., NY, Streptomyces verticillus; bleomycin A2 and NY

bleomycin B2) Capecitabine Xeloda Roche

(5'-deoxy-5-fluoro-N-[(pentylloxy)carbonyl]-cytidine)

Carboplatin Paraplatin Bristol-Myers

(platinum, diammine [1,1- Squibb

cyclobutanedicarboxylato(2-)-0, 0']-(SP-4-2))

Carmustine BCNU, Bristol-Myers

(1 ,3-bis(2-chloroethyl)- 1 -nitrosourea) BiCNU Squibb

Carmustine with Polifeprosan 20 Implant Gliadel Guilford

Wafer Pharmaceuticals,

Inc., Baltimore, MD

Celecoxib Celebrex Searle

(as 4-[5-(4-methylphenyl)-3- (trifluoromethyl)-1H- Pharmaceuticals, pyrazol-1-yl] benzenesulfonamide) England

Chlorambucil Leukeran Glaxo SmitfKline

(4-[bis(2chloroethyl)amino]benzenebutanoic acid)

Cisplatin Platinol Bristol-Myers (PtC12H6N2) Squibb

Cladribine Leustatin, 2- R.W. Johnson

(2-chloro-2'-deoxy-b-D-adenosine) CdA Pharmaceutical

Research Institute, NJ

Cyclophosphamide Cytoxan, Bristol-Myers

(2-[bis(2-chloroethyl)amino] tetrahydro-2H- 1,3,2- Neosar Squibb

oxazaphosphorine 2-oxide monohydrate)

Cytarabine Cytosar-U Pharmacia &

(1 -b-D-Arabinofuranosylcytosine, C₉H₁₃N₃O₅) Upjohn Company

Cytarabine liposomal DepoCyt Skye

Pharmaceuticals, Inc., San Diego, CA

Dacarbazine DTIC-Dome Bayer AG,

(5-(3,3-dimethyl-1H-tiazeno)-imidazole-4-yl)-L-phenylcarbamamide (DTIC) Leverkusen, Germany

Dactinomycin, actinomycin D Cosmegen Merck

(actinomycin produced by *Streptomyces parvullus*,

C₆₂H₈₆N₁₂O₁₆)

Darbepoetin alfa Aranesp Amgen, Inc., (recombinant peptide) Thousand Oaks,

CA

daunorubicin liposomal DanuXome Nexstar

((8S-cis)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-ribofuranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-dioxo-1,2-naphthacenedione

hydrochloride)

Daunorubicin HC1, daunomycin Cerubidine Wyeth Ayerst, ((1S,3S)-3-acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1,2-naphthacenyloxy)-L-ribofuranoside hydrochloride)

3-amino-2,3,6-trideoxy-(α)-L-ribofuranoside hydrochloride)

Denileukin diftitox Ontak Seragen, Inc., (recombinant peptide) Hopkinton, MA

Dexrazoxane Zinecard Pharmacia &

((S)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazine-dione)

Docetaxel Taxotere Aventis

((2R,3S)-N-carboxy-3-phenylisoserine, N-tert-butyl ester, 13-ester with 5b-20-epoxy-12a,4,7b,10b,13a-hydroxy-11-en-9-one 4-acetate 2-benzoate, NJ

trihydrate)

Doxorubicin HC1 Adriamycin, Pharmacia &

(8S,10S)-10-[(3-amino-2,3,6-trideoxy-α-L-ribofuranosyl)oxy]-8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride) doxorubicin Adriamycin Pharmacia &

PFS Upjohn Company

Intravenous

injection

doxorubicin liposomal Doxil Sequus

Pharmaceuticals, Inc., Menlo park, CA

dromostanolone propionate Dromostanol Eli Lilly &

(17β-Hydroxy-2α-methyl-5α-androstan-3-one) propionate Indianapolis, IN dromostanolone propionate Masterone Syntex, Corp., Palo Alto, CA

Elliott's B Solution Elliott's B Orphan Medical,

Solution Inc

Epirubicin Ellence Pharmacia &

((8S-cis)-10-[(3-amino-2,3,6-trideoxy-α-L-arabinofuranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride)

Epoetin alfa Epogen Amgen, Inc (recombinant peptide)

Estramustine Emcyt Pharmacia &

(estra- 1,3,5(10)-triene-3 , 17-diol(l 7(beta))- , 3-[bis(2- Upjohn Company chloroethyl)carbamate] 17-(dihydrogen phosphate),

disodium salt, monohydrate, or estradiol 3-[bis(2- chloroethyl)carbamate] 17-(dihydrogen phosphate),

disodium salt, monohydrate)

Etoposide phosphate Etopophos Bristol-Myers

(4'-Demethylepipodophyllotoxin 9-[4,6-0-(R)- Squibb

ethylidene-(beta)-D-glucopyranoside] , 4'- (dihydrogen phosphate)) etoposide, VP- 16 Vepesid Bristol-Myers

(4'-demethylepipodophyllotoxin 9-[4,6-0-(R)- Squibb

ethylidene-(beta)-D-glucopyranoside])

Exemestane Aromasin Pharmacia &

(6-methylenandrosta- 1 ,4-diene-3 , 17-dione) Upjohn Company

Filgrastim Neupogen Amgen, Inc (r-metHuG-CSF)

floxuridine (intraarterial) FUDR Roche

(2'-deoxy-5 -fluorouridine)

Fludarabine Fludara Berlex

(fluorinated nucleotide analog of the antiviral agent Laboratories, Inc., vidarabine, 9-b -D-arabinofuranosyladenine (ara-A)) Cedar Knolls, NJ

Fluorouracil, 5-FU Adrucil ICN

(5 -fluoro-2 ,4 (1 H, 3 H)-pyrimidinedione) Pharmaceuticals,

Inc., Humacao, Puerto Rico

Fulvestrant Faslodex IPR

(7-alpha-[9-(4,4,5,5,5-penta fluoropentylsulphonyl) Pharmaceuticals, nonyljestra- 1 ,3,5-(l 0)- triene-3, 17-beta-diol) Guayama, Puerto

Rico

Gemcitabine Gemzar Eli Lilly

(2'-deoxy-2', 2'-difluorocytidine monohydrochloride

(b-isomer))

Gemtuzumab Ozogamicin Mylotarg Wyeth Ayerst (anti-CD33 hP67.6)

Goserelin acetate Zoladex AstraZeneca

(acetate salt of [D-Ser(But)6,AzglylO]LHRH; pyro- Implant Pharmaceuticals Glu-His-Trp-Ser-Tyr-D-Ser(But)-Leu-Arg-Pro- Azgly-NH2 acetate [C59H84N18O14 (C2H4O2)x

Hydroxyurea Hydrea Bristol-Myers

Squibb Ibritumomab Tiuxetan Zevalin Biogen IDEC, Inc.,

(immunconjugate resulting from a thiourea covalent Cambridge MA bond between the monoclonal antibody Ibritumomab

and the linker-chelator tiuxetan [N-[2- bis(carboxymethyl)amino] -3 -(p- isothiocyanatophenyl)- propyl] - [N-[2- bis(carboxymethyl)amino] -2-(methyl) - ethyl] glycine)

Idarubicin Idamycin Pharmacia &

(5, 12-Naphthacenedione, 9-acetyl-7-[(3-amino- Upjohn Company 2,3,6-trideoxy-(alpha)-L- lyxo -hexopyranosyl)oxy]- 7,8,9, 10-tetrahydro-6,9, 11 -trihydroxyhydrochloride,

(7S- cis))

Ifosfamide IFEX Bristol-Myers

(3-(2-chloroethyl)-2-[(2- Squibb

chloroethyl)amino]tetrahydro-2H- 1 ,3,2- oxazaphosphorine 2-oxide)

Imatinib Mesilate Gleevec Novartis AG,

(4-[(4-Methyl- 1 -piperaziny)methyl]-N-[4-methyl-3- Basel, Switzerland [[4-(3 -pyridinyl)-2-pyrimidinyl] amino] - phenyl]benzamide methanesulfonate)

Interferon alfa-2a Roferon-A Hoffmann-La (recombinant peptide) Roche, Inc., Nutley,

NJ

Interferon alfa-2b Intron A Schering AG, (recombinant peptide) (Lyophilized Berlin, Germany

Betaseron)

Irinotecan HC1 Camptosar Pharmacia &

((4S)-4,1 l-diethyl-4-hydroxy-9-[(4- piperi- Upjohn Company dinopiperidino)carbonyloxy]-1H-pyrano[3', 4': 6,7]

indolizino[1,2-b] quinoline-3,14(4H, 12H) dione

hydrochloride trihydrate) Letrozole Femara Novartis

(4,4'-(1H- 1 ,2,4 -Triazol- 1 -ylmethylene)

dibenzonitrile)

Leucovorin Wellcovorin, Immunex, Corp.,

(L-Glutamic acid, N[4[[[(2-amino-5-formyl-4,5,6,7,8 Leucovorin Seattle, WA hexahydro4oxo6-pteridiny)]methyl] amino]benzoyl] ,

calcium salt (1 : 1))

Levamisole HC1 Ergamisol Janssen Research

((-)-(S)-2,3,5, 6-tetrahydro-6-phenylimidazo [2,1-b] Foundation, thiazole monohydrochloride Cl 1H12N2S-HC1) Titusville, NJ

Lomustine CeeNU Bristol-Myers

(1-(2-chloro-ethyl)-3-cyclohexyl-1 -nitrosourea) Squibb

Meclorothamine, nitrogen mustard Mustargen Merck

(2-chloro-N-(2-chloroethyl)-N-methylethanamine

hydrochloride)

Megestrol acetate Megace Bristol-Myers

17a(acetyloxy)- 6- methylpregna- 4,6- diene- 3,20- Squibb dione

Melphalan, L-PAM Alkeran Glaxo SmithKline

(4-[bis(2-chloroethyl) amino] -L-phenylalanine)

Mercaptopurine, 6-MP Purinethol Glaxo SmithKline

(1,7-dihydro-6 H -purine-6-thione monohydrate)

Mesna Mesnex Asta Medica

(sodium 2-mercaptoethane sulfonate)

Methotrexate Methotrexate Lederle

(N-[4-[[[(2,4-diamino-6- Laboratories pteridiny)]methyl][methylamino]benzoyl]-L-glutamic

acid)

Methoxsalen (9-methoxy-7H-furo[3,2-g] [1]- Uvadex Therakos, Inc., benzopyran-7-one) Way Exton, Pa

Mitomycin C Mutamycin Bristol-Myers

Squibb

Mitomycin C Mitozytrex SuperGen, Inc., Dublin, CA

Mitotane Lysodren Bristol-Myers

(1,1 -dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) Squibb

ethane)

Mitoxantrone Novantrone Immunex

(1,4-dihydroxy-5,8-bis[[2- [(2- Corporation hydroxyethyl)amino]ethyl]amino]-9, 10- anthracenedione dihydrochloride)

Nandrolone phenpropionate Durabolin-50 Organon, Inc., West

Orange, NJ

Nofetumomab Verluma Boehringer

Ingelheim Pharma KG, Germany

Oprelvekin Neumega Genetics Institute, (IL-11) Inc., Alexandria,

VA

Oxaliplatin Eloxatin Sanofi Synthelabo,

(cis-[(1 R,2R)- 1 ,2-cyclohexanediamine-N,N '] Inc., NY, NY

[oxalato(2-)-0,0'] platinum

Paclitaxel TAXOL Bristol-Myers

(5B, 20-Epoxy-1,2a, 4,7B, 10B, 13a-hexahydroxytax- Squibb

11-en-9-one 4,10-diacetate 2- benzoate 13-ester with

(2R, 3 S)- N-benzoyl-3-phenylisoserine)

Pamidronate Aredia Novartis

(phosphonic acid (3-amino-1-hydroxypropylidene)

bis-, disodium salt, pentahydrate, (APD))

Pegademase Adagen Enzon

((monomethoxypolyethylene glycol succinimidyl) 11 (Pegademase Pharmaceuticals, - 17 -adenosine deaminase) Bovine) Inc., Bridgewater,

NJ

Pegaspargase Oncaspar Enzon

(monomethoxypolyethylene glycol succinimidyl L- asparaginase) Pegfilgrastim Neulasta Amgen, Inc

(covalent conjugate of recombinant methionyl human

G-CSF (Filgrastim) and monomethoxypolyethylene

glycol)

Pentostatin Nipent Parke-Davis

Pharmaceutical Co., Rockville, MD

Pipobroman Vercyte Abbott

Laboratories, Abbott Park, IL

Plicamycin, Mithramycin Mithracin Pfizer, Inc., NY,

(antibiotic produced by Streptomyces plicatus) NY

Porfimer sodium Photofrin QLT

Phototherapeutics, Inc., Vancouver, Canada

Procarbazine Matulane Sigma Tau

(N-isopropyl- μ -(2-methylhydrazino)-p-toluamide Pharmaceuticals, monohydrochloride) Inc., Gaithersburg,

MD

Quinacrine Atabrine Abbott Labs

(6-chloro-9-(1-methyl-4-diethyl-amine)

butylamino-2-methoxyacridine)

Rasburicase Elitek Sanofi-Synthelabo, (recombinant peptide) Inc.,

Rituximab Rituxan Genentech, Inc.,

(recombinant anti-CD20 antibody) South San

Francisco, CA

Sargramostim Prokine Immunex Corp (recombinant peptide)

Streptozocin Zanosar Pharmacia &

(streptozocin 2'-deoxy - 2' - Upjohn Company

[[[(methylnitrosoamino)carbonyl] amino] - a(and b) - D - glucopyranose and 220 mg citric acid anhydrous)

Talc Sclerosol Bryan, Corp.,

(Mg₃Si₄O₁₀(OH)₂) Woburn, MA

Tamoxifen Nolvadex AstraZeneca

((Z)-2-[4-[(1,2-diphenyl-1-butenyl) phenoxy]-N, N- Pharmaceuticals dimethylethanamine 2-hydroxy- 1 ,2,3- propanetricarboxylate (1 : 1))

Temozolomide Temodar Schering

(3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-as- tetrazine-8-carboxamide)

Teniposide, VM-26 Vumon Bristol-Myers

(4'-demethylepipodophyllotoxin 9-[4,6-O-(R)-2-

thenylidene-(beta)-D-glucopyranoside])

Testolactone Teslac Bristol-Myers

(13 -hydroxy-3 -oxo- 13,17-secoandrosta- 1 ,4-dien- 17-

oic acid [dgr] -lactone)

Thioguanine, 6-TG Thioguanine Glaxo SmitfKline

(2-amino-l,7-dihydro-6 H - purine-6-thione)

Thiotepa Thioplex Immunex

(Aziridine, I,Γ, '-phosphinothioylidynetriss-, or Tris Corporation (1-aziridiny) phosphine sulfide)

Topotecan HC1 Hycamtin Glaxo SmitfKline

((S)-10-[(dimethylamino methyl]-4-ethyl-4,9- dihydroxy-lH-pyrano[3', 4': 6,7] indolizino [1,2-b]

quinoline-3 , 14-(4H, 12H)-dione monohydrochloride)

Toremifene Fareston Roberts

(2-(p-[(Z)-4-chloro-l,2-diphenyl-butanyl]- Pharmaceutical phenoxy)-N,N-dimethylethylamine citrate (1 : 1)) Corp., Eatontown,

NJ

Tositumomab, I 131 Tositumomab Bexxar Corixa Corp.,

(recombinant murine immunotherapeutic monoclonal Seattle, WA IgG2a lambda anti-CD20 antibody (I 131 is a

radioimmunotherapeutic antibody))

Trastuzumab Herceptin Genentech, Inc

(recombinant monoclonal IgG1 kappa anti-HER2

antibody)

Tretinoin, ATRA Vesanoid Roche

(all-trans retinoic acid)

Uracil Mustard Uracil Roberts Labs

Mustard

Capsules

Valrubicin, N-trifluoroacetyladiamycin- 14- Valstar Anthra- > Medeva valerate

((2S-cis)-2- [1,2,3,4,6,1 l-hexahydro-2,5, 12- trihydroxy-7 methoxy-6,1 l-dioxo-[[4 2,3,6-trideoxy- 3- [(trifluoroacetyl)-amino-a-L-lyxo- hexopyranosyl]oxyl]-2-naphthaceny]-2-

oxoethyl

pentanoate)

Vinblastine, Leurocristine Velban Eli Lilly

(C46H56N4O10'H2SO4)

Vincristine Oncovin Eli Lilly

(C46H56N4O10'H2SO4)

Vinorelbine Navelbine Glaxo SmithKline

(3' ,4'-didehydro-4'-deoxy-C'-norvincal leukoblastine

[R-^R*)-2,3-dihydroxybutanedioate (1 :2)(salt))

Zoledronate, Zoledronic acid Zometa Novartis

((l-Hydroxy-2-imidazol-yl-phosphonoethyl)

phosphonic acid monohydrate)

[000203]

[000204] Other identified cancer combination therapies include the following: PI3K inhibitors (CALIOL), Bruton Kinase inhibitor (PCI-32765), and BCL-6 inhibitor. This document describes the targets and associated therapy for these identified cancers as being particularly susceptible to treatment with combination therapies. Tar gets

[000205] The present invention is not limited to the cancer and non-cancer targets listed above commonly found in humans. The present invention can also be applied

both to other cancer targets (also referred to as oncogenes) (and where such cancer targets may also be involved in other disease such as inflammation, neurological,

metabolic, cardiovascular, etc.) and to non-cancer target such as Cardiovascular/Metabolic Disease, Eye Disease, Infectious Disease, Inflammation, Neurological Disease, Rare Disease, and Stem Cells. Examples of specific genes are included in Table 2, but are not limited to those described in Table.

Additional targets are not listed but can be found in the key proliferation pathways such as MAPK, PI3K, MEK, etc. The present invention can also apply to disease and growth targets for plant genome and animal genomes.

[000206] Table 2. Cancer and non-cancer targets

DNAi Disease Gene, and Cell System Targets

83 Cancer (1)33

2 Cancer 11A II

4W Cancer R.M-

Cancer 1 RK

hSS Cardiovascular- Metabolic Disease \poli-lliii

°O Infectious Disease (III 11

757 Infectious Disease PR IV

soy Inflammation (N.H RI

8 1 Inflammation SCCililAI

913 Neurological Disease R.\(il

yh Rare Disease Gill

1046 Stem Cells Cord Blood Stem Cells

[000207] Non-Cancer Targets

[000208] The present invention is not limited to the targeting of cancer genes. The methods and compositions of the present invention find use in the targeting of any gene that it is desirable to down regulate the expression of. For example, targets for immune and/or surface antigens or immune surveillance targets, angiogenic receptors, proteins and factors (kinases, heat shock, hypoxic, oxidative stress gene/protein targets), monogenic diseases, inflammation, gene transcription (transcription factors, cis regulatory elements), cell recognition receptors, cell signaling receptors, cell death (autophagy, necrosis, apoptosis), cell adhesion, survival targets (resistance), metastases targets (brain, primary to secondary tumors), chemokines/cytokines, EMT/MET, immune cell activation factors, multidrug resistance, viral proteins and viral recognition proteins, psoriasis, dermatitis and eczema

[000209] Extracellular matrix, stromal or connective tissue genes/proteins, coagulation factors and platelet aggregation or platelet overproduction, and growth factors.

[000210] For example, in some embodiments, the genes to be targeted include, but are not limited to, an immunoglobulin or antibody gene, a clotting factor gene, a protease, a pituitary hormone, a protease inhibitor, a growth factor, a somatomedin, a gonadotrophin, a chemotactin, a chemokine, a plasma protein, a plasma protease inhibitor, an interleukin, an interferon, a cytokine, a transcription factor, or a pathogen target (e.g., a viral gene, a bacterial gene, a microbial gene, a fungal gene).

[000211] In other embodiments and gene from a pathogen is targeted. Exemplary pathogens include, but are not limited to, Human Immunodeficiency virus (CD4,

APOBEC3G, Vif, LEDGF/p75), Hepatitis B virus, hepatitis C virus (SR-B1, scavenger receptor type BI; CLDN-1, claudin-1; OCLN, occluding), hepatitis A virus, respiratory syncytial virus, pathogens involved in severe acute respiratory syndrome, west nile virus, and food borne pathogens (e.g., E. coli).

[000212] The lists of Cancer and Non-Cancer targets from above is intended to be specific and accurate, but in addition to the targets above we have further found and we describe in even greater detail the targets listed below, comprising both cancer and non-cancer targets, presented in no particular order. These targets are especially well suited for DNAi targeting and therapy. The preferred list of targets is provided with the sections that follow which provided detailed descriptions of over 40 genes. These gene targets are numbered below, 1-30. Included with a description of many of these preferred targets are the background relevance of the gene, gene identification, the targeted oligonucleotide sequences, the hot zones, and the 5' upstream genetic code.

[000213] EXPERIMENTALS.

[000214] These examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

[000215] In the experimental disclosure which follows, the following abbreviations apply: N (normal); M (molar); mM (millimolar); μ M (micromolar); mol (moles); mmol (millimoles); μ mol (micromoles); nmol (nanomoles); pmol (picomoles); g (grams); mg (milligrams); μ g (micrograms); ng (nanograms); 1 or L (liters); ml (milliliters); μ l

(microliters); cm (centimeters); mm (millimeters); μ m (micrometers); nm (nanometers); and $^{\circ}$ C (degrees Centigrade).

[000216]

[000217] 1) Survivin. Survivin (BIRC5) also called bulboviral inhibitor of apoptosis repeat-containing 5 is a member of the inhibitor of apoptosis family that is expressed during mitosis in a cell cycle-dependent manner. Survivin is localized to different components of the mitotic apparatus, plays an important role in both cell division and inhibition of apoptosis. Survivin is not expressed in normal adult tissue, but is widely expressed in a majority of cancers (Fukuda and Pelus, Mol Cancer Ther 2006; 5 1087-1098), often with poor prognosis. Survivin inhibits caspase activation, the key effector enzyme in programmed cell death, and as a result there is uncontrolled growth and drug resistance. The inhibition of survivin leads to increased apoptosis and decreased tumor growth and sensitizes cells to various therapeutic interventions including chemotherapies and targeted therapies against cancer targets.

Survivin expression is increased in tumors and regulated by the cell cycle (expressed in mitosis in a cell cycle dependent manner); expression is also linked to p53 and is targeted by the WNT1 pathway and is upregulated by β -catenin. A review of approaches targeted against survivin may be found in "Targeting surviving in cancer: a patent review" (Expert Opinion on Therapeutic Patents, December 2010, Vol. 20, No. 12 : Pages 1723-1737).

[000218] An antisense therapeutic being developed (LY2181308) downregulates survivin expression in human cancer cells derived from lung, colon, pancreas, liver, breast, prostate, ovary, cervix, skin, and brain as measured by quantitative RT-PCR and

immunoblotting analysis (Carrasco et al., Mol Cancer Ther 2011; 10(2); 221-32). Specific inhibition of survivin expression in multiple cancer cell lines induced caspase-3-dependent apoptosis, cell cycle arrest in the G2-M phase, and multinucleated cells and sensitized tumor cells to chemotherapeutic-induced apoptosis. In an in vivo human xenograft tumor model, LY2181308 produced significant antitumor activity as compared with saline or its sequence-specific control oligonucleotide and sensitized to gemcitabine, paclitaxel, and docetaxel with inhibition of surviving expression in xenograft tumors. LY2181308 is being evaluated in a clinical setting (Phase II) in combination with docetaxel for the treatment of prostate cancer.

[000219] Protein: Survivin Gene: BIRC5 (Homo sapiens, chromosome 17, 76210277 - 76221716 [NCBI Reference Sequence: NC_000017.10] ; start site location: 76210398; strand: positive)

Targeted Sequences

Relative upstream

Sequence Design

Sequence (5' - 3') location to gene start ID No: ID

site

1 SU1 GAGCGCACGCCCTCTTAGGCGG 73

75 SU2 CACCCCGAGGTACGATCAGTGCGTACC 2990

105 SU3 GACATCGCTGTCCCGGCGAGTACATCGTT 665

155 SU1_02 GAGCGCACGCCCTCTTAGGCG 73

229 SU1_03 GAGCGCACGCCCTCTTAGGCGGTCCA 73

303 GTCGCCCTGGGTCTGCTGATTGGC 1918

322 CAGCGAGCCTGGGCCCATCGGCACATCT 2905

357 CCCGCGCCTTCTGGGAGTAGAGGC 102

431 TCCCGGCGAGTACATCGTTGACTGCACG 675

481 AACCTCCTCCCGCCACGGGTT 1229

CGCGGCCTTCTGGGAGTAGA 108

GCGGCCTTCTGGGAGTAGAG 109

CGGCCTTCTGGGAGTAGAGG 110

GGAGCGCACGCCCTCTTAGG 76

GGGAGCGCACGCCCTCTTAG 75

CGGGAGCGCACGCCCTCTTA 74

TCGGGAGCGCACGCCCTTT 73

GTCGGGAGCGCACGCCCTCT 72

TGTCGGGAGCGCACGCCCTC 71

ATGTCGGGAGCGCACGCCCT 70

CATGTCGGGAGCGCACGCC 69

GCATGTCGGGAGCGCACGCC 68

GGCATGTCGGGAGCGCACGC 67

GGGCATGTCGGGAGCGCACG 66

GGGCATGTCGGGAGCGCAC 65

CGGGCATGTCGGGAGCGCA 64

GCGGGCATGTCGGGAGCGC 63

CGCGGGCATGTCGGGAGCG 62

CCGCGGGCATGTCGGGAGC 61

GCCCGGGCATGTCGGGAG 60

CGCCCGGGCATGTCGGGA 59

GCGCCCGGGCATGTCGGG 58

CGCGCCCGGGCATGTCGG 57

AGGGGCCCTGCCTGGCTCGGGGGAGGCAGGGCTGAAGTACCACATTAGGGCATG
TTCCGGGGAAGTAGATTCTCTGAATAACTTGGATGGCTCCCTGGAGCATTTAGGA
CAGAAGCCACCTGGAAAATAGAGATGGTCACCCACGTCAGCCTTGACAGTGCC
CAGAAAGTCTTGTCACTTGGTAAATGTTAACAGCTATGATCCGTTCTTTAAGACC
CTGGGGAGTTTTAAGTTTTACCCACCAGACCTGAGAAGGGTAAAGGGCTGCAG
ATTCTGTTCTTTAACTGGGGCCAGTGTGAGCCATCTTTGACTCAGTGTGCAAT
AGACCTTGATTCTGCAGTGGGACCTCCCAGGCCCTTGCCCCCGCAACTTCTG
GACTCATATCCGTCAGACATCACTTGTACCTTCCAGCATCAGGAGAAGTGGAT
CCCTCCTGGCTCCACACTCTTAGGCTCTTTGTAAGTAGCTGGTGAGGGTTTTCTTC
TCTCTGCAAGGGAGGCTGGTAGAACTATGGATGTGATTCGTACAATTTTAGAGAC
AAAAAGAAAGTACCAGGAGTCAATTTATTTTCAGCTGCTTATTGCATAGGTCCG
GGAGTTGAGCATGGAGTCCAGCAGCTACTAACTAGTTATCTCTGTACCTGGCTTC
CATTTACTGGTCTTAGCTTGTCCGTGATTCTTATTGCCCTTATTTCTACCCAG
AGGGACTGTTGGCCCTAGATGGAGTGGTCTTTTTAAATTTTTTTTTAAATTTT
TTGAGACAGAGTCTCACTCTGTACCTAGGCTGTAGTGCAGTGTGCGATCTCGG
CTCACTGCAACCTCCGCTCCTGAGTCAAGCAATCTCCTGTCTCAGCCTCCTGA
GTAGCTGGGATTACAGGTGTGTACCACTATGCCAGCTAATTTTTGTATTTTAGT AGAGATGGGATTTCACCATATTGGCCAGGTTGGTCTGAACTCCTGACCTCAAAT
GATCTGCCACCTTAGCCTCCCGAAGTGTGGGATTGCAGGTGTGAGCCACCGCA
CCTGGCCTGGGCAGAGTGAAGTCTTATGCTGGGGAGCCATCAGCATGCTCAAAC
CTCCTGCAATTGTAGCACACTTTGTAAGTCTTTCCACAAAAGGGCAGAAGTAA
TTTGGGACTTTCATGAGACCATTCACTTTGTAGCACATACTACTTTGAAGTTTATA
CCTTGAAAACCTCATGATGGTATTCCCAGGCTTGCACGTAATCTGCACTCAAAA
CATAGCTGTAGAATTGAACTAAAGCATCCCTCTGTCCAATTAAGACCTATAACCT
CTCTTTTGTAGACAGAACTCGCTCTGTACCCAGGTTGGAGTGCAGTGGTGCAA
TCTCAGCTCACTGCATCCTTCGCCTCCTGGATTCAAGCGATTCTCTTGCCTTAGCC
TCCGAAGTAACTGGGACTACAGGTGCCGCCACCACGCCTGGGTAATTTTTGTAT
TTTTAGTAGAGACGGGTTTCGCCATGGCCAGGCTGGTCTCAAACCTCTGCCTC
AAGTGATCCTCCCGCTCAGCCTCCCAAAGTGTGGGATTACAGGGTGCACCACC
ACACCCAGCCAGGACCTATGATCTAATTCATTGTTGGGTAGCTTCACAATTTTC
TTCTGGACGCCTTAGTAAGTCCACACTTAAAGCAGCCACCACATGGCATACTTTA
CCTTCTGTTTTCTTTCCCTCCCTACCTAGACCCTCCTAACTTTTGGGGTTTTT
TTCCTTTCCTCAGGGTCAGTTTAAAAGGAGGATCGAGCTCACTGTGGAGTATCC
ATGGAGATGTGGAGCCTTGTACCAACCTCTAACTGCAGAAGTGGGATG

[000497] III. DNA Methylation

[000498] In some embodiments, the present invention provides using oligonucleotide that are methylated at specific sites for screening purposes. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that one mechanism for the regulation of gene activity is methylation of cytosine residues in DNA. 5- methylcytosine (5-MeC) is the only naturally occurring modified base detected in DNA (Ehrlick et al, Science 212: 1350-1357 (1981)). Although not all genes are regulated by methylation, hypomethylation at specific sites or in specific regions in a number of genes is correlated with active transcription (Doerfler, Annu. Rev. Biochem. 52:93-124 [1984];

Christman, Curr. Top. Microbiol. Immunol. 108:49-78 [1988]; Cedar, Cell 34:5503-5513 [1988]). DNA methylation in vitro can prevent efficient transcription of genes in a cell-free system or transient expression of transfected genes. Methylation of C residues in some specific cis-regulatory regions can also block or enhance binding of transcriptional factors or repressors (Doerfler, supra; Christman, supra; Cedar, Cell 34:5503-5513 (1988); Tate et al, Curr. Opin. Genet. Dev. 3:225-231 [1993]; Christman et al, Virus Strategies, eds. Doerfler, W. & Bohm, P. (VCH, Weinheim, N.Y.) pp. 319-333 [1993]).

[000499] Disruption of normal patterns of DNA methylation has been linked to the development of cancer (Christman et al, Proc. Natl. Acad. Sci. USA 92:7347-7351 [1995]). The 5-MeC content of DNA from tumors and tumor derived cell lines is generally lower than normal tissues (Jones et al, Adv. Cancer Res 40: 1-30 [1983]). Hypomethylation of specific oncogenes such as c-myc, c-Ki-ras and c-Ha-ras has been detected in a variety of human and animal tumors (Nambu et al., Jpn. J. Cancer (Gann) 78:696-704 [1987]; Feinberg et al., Biochem. Biophys. Res. Commun. 111 :47-54 [1983]; Cheah et al, JNCI73: 1057- 1063

[1984]; Bhave et al, Carcinogenesis (Lond) 9:343-348 [1988]. In one of the best studied examples of human tumor progression, it has been shown that hypomethylation of DNA is an early event in development of colon cancer (Goetz et al., Science 228: 187-290 [1985]).

Interference with methylation in vivo can lead to tumor formation. Feeding of methylation inhibitors such as L-methionine or 5-azacytidine or severe deficiency of 5-adenosine methionine through feeding of a diet depleted of lipotropes has been reported to induce formation of liver tumors in rats (Wainfan et al., Cancer Res. 52:2071s – 2077s [1992]).

Studies show that extreme lipotrope deficient diets can cause loss of methyl groups at specific sites in genes such as c-myc, ras and c-fos (Dizik et al, Carcinogenesis 12: 1307-1312

[1991]). Hypomethylation occurs despite the presence of elevated levels of DNA MTase activity (Wainfan et al, Cancer Res. 49:4094-4097 [1989]). Genes required for sustained active proliferation become inactive as methylated during differentiation and tissue specific genes become hypomethylated and are active. Hypomethylation can then shift the balance between the two states. In some embodiment, the present invention thus takes advantage of this naturally occurring phenomena, to provide compositions and methods for site specific methylation of specific gene promoters, thereby preventing transcription and hence translation of certain genes. In other embodiments, the present invention provides methods and compositions for upregulating the expression of a gene of interest (e.g., a tumor suppressor gene) by altering the gene's methylation patterns.

[000500] The present invention describes the use of unmodified completely

complementary DNA oligonucleotide sequences to inhibit gene expression. The present invention is not limited to the use of methylated oligonucleotides or modified oligonucleotides to identify therapeutic sequences. We describe the use of non-methylated oligonucleotides for the inhibition of gene expression and we prove this system works by providing the results of experiments conducted during the course of development of the present invention. For example we demonstrate that an unmethylated oligonucleotide targeted toward Bcl-2 inhibited the growth of lymphoma cells to a level that was comparable to that of a methylated oligonucleotide.

[000501] IV. Oligonucleotides

[000502] The term "oligonucleotide," refers to a short length of single-stranded polynucleotide chain. Oligonucleotides are typically less than 200 residues long (e.g., between 8 and 100), however, as used herein, the term is also intended to encompass longer polynucleotide chains (e.g., as large as 5000 residues). Oligonucleotides are often referred to by their length. For example a 24 residue or base oligonucleotide is referred to as a "24-mer". Oligonucleotides can form secondary and tertiary structures by self-hybridizing or by hybridizing to other polynucleotides. Such structures can include, but are not limited to, duplexes, hairpins, cruciforms, bends, and triplexes.

[000503] In some embodiments, the present invention provides DNai oligonucleotides for inhibiting the expression of oncogenes. Exemplary design and production strategies for DNAs are described below. The below description is not intended to limit the scope of DNai compounds suitable for use in the present invention. One skilled in the relevant recognizes that additional DNAs are within the scope of the present invention.

A. Oligonucleotide Design

[000504] In some embodiments, oligonucleotides are designed based on preferred design criteria. Such oligonucleotides can then be tested for efficacy using the methods disclosed herein. For example, in some embodiments, the oligonucleotides are methylated at least one, preferably at least two, and even more preferably, all of the CpG islands. In other

embodiments, the oligonucleotides contain no methylation. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that preferred oligonucleotides are those that have at least a 40% CG content and at least 1 CG

dinucleotides. In some embodiments, oligonucleotides are designed with at least 1 A or T to minimize self hybridization. In some embodiments, commercially available computer programs are used to survey oligonucleotides for the ability to self hybridize. Preferred oligonucleotides are at least 10, and preferably at least 15 nucleotides and no more than 100 nucleotides in length. Particularly preferred oligonucleotides are 20-34 nucleotides in length. In some embodiments, oligonucleotides comprise the universal protein binding sequences CCGCCC and CGCG or the complements thereof. In some embodiments, oligonucleotides comprise the universal protein binding sequences (G/T)CCGCCC(G) and the complements thereof. It is also preferred that the oligonucleotide hybridize to a promoter region of a gene upstream from the TATA box of the promoter. It is also preferred that oligonucleotide compounds are not completely homologous to other regions of the human genome. The homology of the oligonucleotide compounds of the present invention to other regions of the genome can be determined using available search tools (e.g., BLAST, available at the Internet site of NCBI). [000505] In some embodiments, oligonucleotides are designed to hybridize to regions of the promoter region of an oncogene known to be bound by proteins (e.g., transcription factors). Exemplary oligonucleotide compounds of the present invention are shown in Table 3. The present invention is not limited to the oligonucleotides described herein. Other suitable oligonucleotides may be identified (e.g., using the criteria described above). Exemplary oligonucleotide variants of the disclosed oligonucleotides can include smaller oligonucleotide sequences of 20-mer or can be right or left shifted 20 base pairs. Candidate oligonucleotides may be tested for efficacy using any suitable method, including, but not limited to, those described in the illustrative examples below. Using the in vitro assay described below in the material and methods and Figures, candidate oligonucleotides can be evaluated for their ability to prevent cell proliferation or target inhibition at a variety of concentrations.

Particularly preferred oligonucleotides are those that inhibit gene expression of target proteins as a low concentration (e.g., less than 20 μ M, and preferably, less than or equal to 10 μ M in the in vitro assays disclosed herein).

B. Materials and Methods

Oligonucleotide Preparation (Figures: 1-25, 27-30, 31-49, 54-67)

[000506] All oligonucleotides were synthesized utilizing cyanoethyl phosphoramidite chemistry, purified by reverse phase high-performance liquid chromatography (RP-HPLC), and lyophilized by The Midland Certified Reagent Company (Midland, TX). Methylated oligonucleotides were methylated at all CpG sites.

Cell Culture (Figures: 1-25, 27-30, 31-49, 54-67)

[000507] Human lung carcinoma cells (A549; ATCC) were cultivated in DMEM medium (ATCC) containing 10% fetal bovine serum (FBS; Invitrogen) and maintained under a humidified atmosphere of 5% CO₂ at 37°C. Cells were split 1 : 8 at 90% confluence and used for experiments between passages 12 and 20 (2,500 cells per well were plated 12-24 hours prior to adding oligonucleotides).

[000508] Human breast carcinoma cells (MDA-MB-231 ; ATCC) were cultivated in Leibovitz's L-15 medium (ATCC) containing 10% fetal bovine serum (FBS; Invitrogen) and maintained under a humidified atmosphere at 37°C. Cells were split 1 : 6 at 90% confluence and used for experiments between passages 15 and 22 (2,500 cells per well were plated 12-24 hours prior to adding oligonucleotides).

[000509] Human prostate carcinoma cells (DU145; ATCC) were cultivated in EMEM medium (ATCC) containing 10% fetal bovine serum (FBS; Invitrogen) and maintained under a humidified atmosphere of 5% CO₂ at 37°C. Cells are split 1 :8 at 90% confluence and used for experiments between passages 10 and 16 (2,500 cells per well were plated 12-24 hours prior to adding oligonucleotides).

[000510] Human breast carcinoma cells (MCF-7; ATCC) were cultivated in 50:50 RPMI/DMEM medium (ATCC) containing 10% fetal bovine serum (FBS; Corning),

0.01mg/mL insulin (Sigma- Aldrich) and maintained under a humidified atmosphere at 37°C at 5% CO₂. Cells were split 1 :6 at 90% confluence and used for experiments between passages 15 and 18 (2,500 cells per well were plated 12-24 hours prior to adding

oligonucleotides).

[000511] Human colorectal carcinoma cells (HCT-116; ATCC) were cultivated in McCoy's 5A medium (Corning) containing 10% fetal bovine serum (FBS; Corning) and maintained under a humidified atmosphere at 37°C at 5% CO₂. Cells were split 1 :6 at 90% confluence and used for experiments between passages 4 and 7 (2,500 cells per well were plated 12-24 hours prior to adding oligonucleotides).

[000512] HepG2 cells were plated using 5,000 cells per well in 96 well plate (for both qPCR experiment and cell count experiments). Cells were incubated for 24 hours prior to treatment with DNAi oligonucleotides. Twenty-four hours after plating DNAi oligonucleotides were added to the cells at final concentration of 15uM. At each timepoint (24, 72, and 144 hours) cells from 96 well plate were washed with 1xPBS once and total RNA isolated using MagMax-96 Total RNA isolation kit (Lifetech, cat#AM1830). At 72 hour timepoint cells were over 90% confluent, therefore cells were washed with 1xPBS twice, trypsinized with 0.05% Trypsin-EDTA and transferred from each individual well (96- well plate) into 24-well plate. STAT3 DNAi oligonucleotides were added to the cells in 24- well plate at final concentration of 15uM.

[000513] HepG2 cells were trypsinized (as described above) and cells from each well (96-well plate) were diluted in 1 mL of complete growth medium prior to cell counting performed using Guava PCA-96 flow cytometry system. HepG2 cell culture work was performed at Altogen Labs (Austin, TX).

mRNA Expression Analysis and RNA Isolation (Figure 67)

[000514] All RNA was isolated using the MAGMAX96 Total RNA Isolation kit (cat#AM1830; Lifetech). The manufacturer's protocol was followed, including a final elution of 50 elution solution. RNA was stored at -20°C for later use.

Reverse Transcription (RT) (Figure 67)

[000515] Isolated RNA was reverse transcribed into cDNA in a single reaction containing RNase Inhibitor Protein (15518; Lifetech) and MMLV-Reverse Transcriptase (18057; Lifetech). RNA input into the RT reaction was based on a 7.5 µl, input per 20 µl, reaction size for all samples.

qPCR (Figure 67)

[000516] Fluorescence based, real-time reverse transcription-PCR (qRT-PCR) is a standard tool used for quantification of mRNA levels. This technique has high throughput capabilities with both high sensitivity and specificity for the target of interest. The amplification reaction consisted of dNTPs (PCR grade; Roche) and Platinum Taq Polymerase (10966; Lifetech). Cycling conditions were as follows: 95°C for 1 minute; then 50 cycles of 95°C for 5 seconds and 60°C for 20 seconds. Results were determined by real-time PCR on the ABI Prism 7900 SDS real-time PCR machine (Applied Biosystems, Foster City, CA). All qPCR work was performed at Altogen Labs (Austin, TX).

[000517] As shown in Figure 67, PC2 (206; exposed at 15µM), a PCSK9 targeted oligonucleotide, demonstrated an approximate 40% decrease of PCSK9 mRNA at 72 hours post-exposure compared to control PCSK9 mRNA levels in HepG2 cells. While PC2 (206) decreased PCSK9 mRNA expression, it was not cytotoxic to cells at either 24 or 72 hours post-exposure in the same experiment. This demonstrates that an oligonucleotide is capable of modulating target gene expression with expected phenotypic changes.

[000518] Altogen Labs (Austin, TX) performed the cell culture work for A549, MDA- MB-231, DU145 and START Preclinical (San Antonio, TX) performed the cell culture work for MCF-7 and HCT-116.

Cell Growth Inhibition Assay (Figures: 1-25, 27-30, 31-49, 54-66)

[000519] Cells were harvested from T-75 flask by a single wash with 1xPBS and incubation with 2 ml of 0.05% Trypsin-EDTA (Invitrogen) for 7 minutes at 37°C. Trypsin was inactivated by addition of 8 ml of complete medium (total volume of 10 ml). Cells were counted using hemocytometer and cell count confirmed by Guava PCA flow cytometry. Cells were then plated and assayed. Cell growth inhibition was assessed using a Vybrant MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Cell Proliferation Assay (cat#V13154) purchased from Life Technologies (Carlsbad, CA). For each cell line 2,500 cells per well were plated 12 hours prior to adding oligonucleotides. Absorbance measurements at 570 nm were made using a Molecular Devices Spectramax Plus (Sunnyvale, CA) microplate reader. Each treatment was run in quadruplicate. Altogen Labs (Austin, TX) and START Preclinical (San Antonio, TX) performed the cell growth inhibition assay.

Included in Tables 4 and 5 are the sequences for the control and negative control

oligonucleotides used in the experiments. Oligonucleotide Preparation (Figures: 26, 50-53; descriptions referenced in U.S. Patent No.: 7,524,827)

[000520] All oligonucleotides were synthesized, gel purified and lyophilized by

BIOSYNTHESIS (Lewisville, Texas) or Qiagen (Valencia, CA). Methylated

oligonucleotides were methylated at all CpG sites. Methylated Oligonucleotides were dissolved in pure sterile water (Gibco, Invitrogen Corporation) and used to treat cells in culture.

Cell Culture (Figures: 26, 50-53; descriptions referenced in U.S. Patent No.: 7,524,827)

[000521] Human breast cancer cells, MCF7 and MDA-MB-231, were obtained from Karmanos Cancer Institute. All cells were cultured in DMEM/F12 media (Gibco, MD) supplemented with 10 mM HEPES, 29 mM sodium bicarbonate, penicillin (100 units/ml) and streptomycin (100 µg/ml). In addition, 10% calf serum, 10 µg/ml insulin (Sigma Chemical, St Louis, MO), and 0.5 nM estradiol was used in MCF7 media and 10% fetal calf serum was used for MDA-MB 231. All flasks and plates were incubated in a humidified atmosphere of 95% air and 5% CO₂ at 37°C.

[000522] BxPC-3 pancreatic carcinoma cell line was cultured in RPMI 1640 with 10% FBS.

[000523] NMuMG (normal mouse mammary gland cells) cell line was grown in DMEM media with 4.5 g/l glucose, 10 µg/ml insulin and 10% FBS.

[000524] All the above cells were seeded at 2,500 to 5,000 cells/well in 96 well plates. The cells were treated with oligonucleotide compounds in fresh media (100 μ l total volume) 24 hours after seeding. The media was replaced with fresh media without oligonucleotides 24 hours after treatment and every 48 hours for 6 to 7 days or until the control cells were 80 to 100% confluent. The inhibitory effect of oligonucleotide was evaluated using an MTT staining technique.

Cell Growth Inhibition Assay (Figures: 26, 50-53; descriptions referenced in U.S. Patent No.: 7,524,827)

[000525] Cell growth inhibition was assessed using 3-[4,5-Dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) purchased from Sigma Chemical (St. Louis, MO). Cells were resuspended in culture media at 50,000 cells/ml and 100 μ l was distributed into each well of a 96-well, flat bottomed plate (Costar Corning, NY, USA) and incubated for 24 hours. Media was changed to 100 μ l fresh media containing the desired concentration of oligonucleotides and incubated for 24 hours. Controls had media with pure sterile water equal to the volume of oligonucleotide solution. The media was changed without further addition of oligonucleotides every 24 hours until the control cultures were confluent (6 to 7 days).

Thereafter the media was removed and plates were washed two times with phosphate-buffered saline (PBS) and 100 μ l of serum free media containing 0.5 mg/ml MTT dye was added into each well and incubated for 1 hour at 37°C. The media with dye was removed, washed with PBS and 100 μ l of dimethyl sulfoxide (DMSO) was added to solubilize the reactive dye. The absorbance values were read using an automatic multiwell

spectrophotometer (Bio-Tek Microplate Autoreader, Winooski, VT, USA). Each treatment was repeated at least 3 times with 8 independent wells each time. Included in Tables 4 and 5 are the sequences for the control and negative control oligonucleotides used in the experiments.

C. Preparation and Formulation of Oligonucleotides

[000526] Any of the known methods of oligonucleotide synthesis can be used to prepare the modified oligonucleotides of the present invention. In some embodiments utilizing methylated oligonucleotides the nucleotide, dC is replaced by 5-methyl-dC where appropriate, as taught by the present invention. The modified or unmodified oligonucleotides of the present invention are most conveniently prepared by using any of the commercially available automated nucleic acid synthesizers. They can also be obtained from commercial sources that synthesize custom oligonucleotides pursuant to customer specifications.

[000527] While oligonucleotides are a preferred form of compound, the present invention comprehends other oligomeric oligonucleotide compounds, including but not limited to oligonucleotide mimetics such as are described below. The oligonucleotide compounds in accordance with this invention preferably comprise from about 20 to about 34 nucleobases (i.e., from about 20 to about 34 linked bases), although both longer and shorter sequences may find use with the present invention.

[000528] Specific examples of preferred compounds useful with the present invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

[000529] Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates,

thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

[000530] Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

[000531] In other preferred oligonucleotide mimetics, both the sugar and the

internucleoside linkage (i.e., the backbone) of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos.: 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al, Science 254: 1497 (1991).

[000532] In some embodiments, oligonucleotides of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular -CH₂, -NH-0-CH₂-, -CH₂-N(CH₃)-0-CH₂- [known as a methylene (methylimino) or MMI backbone], -CH₂-0-N(CH₃)-CH₂-, -CH₂-N(CH₃)-N(CH₃)-CH₂-, and -O-N(CH₃)-CH₂-CH₂- [wherein the native phosphodiester backbone is represented as -O-P-O-CH₂-] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

[000533] Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C1 to C10 alkyl or C2 to C10 alkenyl and alkynyl. Particularly preferred are O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃,

O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: Cl to C10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the

pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also known as 2*-O-(2-methoxyethyl) or 2*-MOE) (Martin et al, Helv. Chim. Acta 78:486 [1995]) i.e., an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminoethoxy (i.e., a O(CH₂)₂ON(CH₃)₂ group), also known as 2'-DMAOE, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O-CH₂-O-CH₂-N(CH₃)₂.

[000534] Other preferred modifications include 2'-methoxy(2'-O~CH₃), 2'-aminopropoxy(2'-OCH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

[000535] Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methyl cytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine

substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

[000536] Another modification of the oligonucleotides of the present invention involves chemically linking to the oligonucleotide one or more moieties or conjugates that enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, (e.g., hexyl-S-tritylthiol), a thiocholesterol, an aliphatic chain, (e.g., dodecandiol or undecyl residues), a phospholipid, (e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate), a polyamine or a polyethylene glycol chain or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxcholesterol moiety.

[000537] One skilled in the relevant art knows well how to generate oligonucleotides containing the above-described modifications. The present invention is not limited to the antisense oligonucleotides described above. Any suitable modification or substitution may be utilized.

[000538] It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention also includes pharmaceutical compositions and formulations that include the antisense compounds of the present invention as described below.

D. Cocktails [000539] In some embodiments, the present invention provides cocktails comprising two or more oligonucleotides directed towards promoter regions of genes (e.g., oncogenes). In some embodiments, the two oligonucleotides hybridize to different regions of the promoter of the same gene. In other embodiments, the two or more oligonucleotides hybridize to promoters of two different genes. The present invention is not limited to a particular mechanism. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is contemplated that the combination of two or more compounds of the present invention provides an inhibition of cancer cell growth that is greater than the additive inhibition of each of the compounds administered separately.

V. Research Uses

[000540] The present invention is not limited to therapeutic applications. For example, in some embodiments, the present invention provides compositions and methods for the use of oligonucleotides as a research tool.

[000541] A. Kits

For example, in some embodiments, the present invention provides kits comprising oligonucleotides specific for inhibition of a gene of interest, and optionally cell lines (e.g., cancer cells lines) known to express the gene. Such kits find use, for example, in the identification of metabolic pathways or the involvement of genes in disease (e.g., cancer), as well as in diagnostic applications. In some embodiments, the kits further comprise buffer and other necessary reagents, as well as instructions for using the kits.

B. Target validation

[000542] In some embodiments, the present invention provides methods and

compositions for use in the validation of gene targets (e.g., genes suspected of being involved in disease). For example, in some embodiments, the expression of genes identified in broad screening applications (e.g., gene expression arrays) as being involved in disease is downregulated using the methods and compositions of the present invention. The methods and compositions of the present invention are suitable for use in vitro and in vivo (e.g., in a non-human animal) for the purpose of target validation. In other embodiments, the compounds of the present invention find use in transplantation research (e.g., HLA

inhibition).

C. Drug Screening

[000543] In other embodiments, the methods and compositions of the present invention are used in drug screening applications. For example, in some embodiments, oligonucleotides of the present invention are administered to a cell (e.g., in culture or in a non-human animal) in order to inhibit the expression of a gene of interest. In some embodiments, the inhibition of the gene of interest mimics a physiological or disease condition. In other embodiments, an oncogene or disease causing gene is inhibited. Test compounds (e.g., small molecule drugs or oligonucleotide mimetics) are then administered to the test cell and the effect of the test compounds is assayed.

[000544] The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone, which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, e.g., Zuckermann et al, J. Med. Chem. 37: 2678-85

[1994]); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the One-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are preferred for use with peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) Anticancer Drug Des. 12: 145).

[000545] Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al, Proc. Natl. Acad. Sci. U.S.A. 90:6909 [1993]; Erb et al, Proc. Nad. Acad. Sci. USA 91 : 11422 [1994]; Zuckermann et al, J. Med. Chem. 37:2678 [1994]; Cho et al, Science 261 : 1303 [1993]; Carrell et al, Angew. Chem. Int. Ed. Engl. 33:2059 [1994]; Carell et al, Angew. Chem. Int. Ed. Engl. 33:2061 [1994]; and Gallop et al, J. Med. Chem. 37: 1233 [1994].

[000546] Libraries of compounds may be presented in solution (e.g., Houghten,

Biotechniques 13:412-421 [1992]), or on beads (Lam, Nature 354:82-84 [1991]), chips (Fodor, Nature 364:555-556 [1993]), bacteria or spores (U.S. Patent No. 5,223,409; herein incorporated by reference), plasmids (Cull et al, Proc. Natl. Acad. Sci. USA 89: 18651869 [1992]) or on phage (Scott and Smith, Science 249:386-390 [1990]; Devlin Science 249:404-406 [1990]; Cwirla et al, Proc. Natl. Acad. Sci. 87:6378-6382 [1990]; Felici, J. Mol. Biol. 222:301 [1991]).

VI. Compositions and Delivery

[000547] In some embodiments, the oligonucleotide compounds of the present invention are formulated as pharmaceutical compositions for delivery to a subject as a pharmaceutical. The novel antigen compounds of the present invention find use in the treatment of a variety of disease states and conditions in which it is desirable to inhibit the expression of a gene or the growth of a cell. In some preferred embodiments, the compounds are used to treat disease states resulting from uncontrolled cell growth, for example including, but not limited to, cancer. The present invention is not limited to the treatment of a particular cancer. The oligonucleotide compounds of the present invention are suitable for the treatment of a variety of cancers including, but not limited to, breast, colon, lung, stomach, pancreatic, bladder, leukemia, and lymphoma. In other preferred embodiments, the compounds are used to treat disease states resulting from gene expression, for example including, but not limited to, non cancer diseases. The below discussion provides exemplary, non-limiting examples of formulations and dosages.

A. Pharmaceutical Compositions

[000548] The present invention further provides pharmaceutical compositions (e.g., comprising the oligonucleotide compounds described above). The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated.

Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary (e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer); intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration.

[000549] Pharmaceutical compositions and formulations for topical administration may include transdermal patches, needleless injectors, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

[000550] Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

[000551] Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[000552] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, nanoparticle, nanocrystal, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[000553] The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[000554] The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[000555] In one embodiment of the present invention the pharmaceutical compositions may be formulated and used as foams. Pharmaceutical foams include formulations such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product.

[000556] Agents that enhance uptake of oligonucleotides at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (WO 97/30731), cochleates (Patent application numbers 20080242625 and 20120294901) also enhance the cellular uptake of oligonucleotides.

[000557] The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or antiinflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[000558] Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or nonaqueous media, capsules, gel capsules, sachets, tablets or minitables. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which oligonucleotides of the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts include chenodeoxycholic acid (CDC A) and

ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, gluchoic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid,

taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate, sodium glycodihydrofusidate. Preferred fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprinate, monoolein, dilaurin, glyceryl 1-monocaprinate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a

pharmaceutically acceptable salt thereof (e.g. sodium). Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20- cetyl ether. Oligonucleotides of the invention may be delivered orally in granular form including sprayed dried particles, or complexed to form micro or nanoparticles or

nanocrystals. Oligonucleotide complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches;

polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and starches. Particularly preferred complexing agents include chitosan, N-trimethylchitosan, poly-L- lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylamino-methylethylene P(TDAE), polyaminostyrene (e.g. p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate),

poly(isobutylcyanoacrylate), poly(isohexylcyanoacrylate), DEAE-methacrylate, DEAE- hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, phosphatidylserine, calcium, and polyethyleneglycol (PEG).

[000559] Certain embodiments of the invention provide pharmaceutical compositions containing (a) one or more oligonucleotide compounds and (b) one or more other chemotherapeutic agents that function by a non-oligonucleotide mechanism. Examples of such chemotherapeutic agents include, but are not limited to, cytotoxic agents, small molecule protein inhibitors, antibodies, and anti-sense anticancer drugs such as daunorubicin, dactinomycin, doxorubicin, bleomycin, mitomycin, nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5- fluorouracil (5-FU), floxuridine (5-FuDR), methotrexate (MTX), colchicine, vincristine, vinblastine, etoposide, teniposide, cisplatin, lenalomid, and diethylstilbestrol (DES). Antiinflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Other non-oligonucleotide chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

B. Delivery

[000560] The oligonucleotide compounds of the present invention may be delivered using any suitable method. In some embodiments, naked DNA is administered. In other embodiments, lipofection is utilized for the delivery of nucleic acids to a subject. In still further embodiments, oligonucleotides are modified with phosphothioates for delivery (See e.g., U.S. Patent 6,169,177, herein incorporated by reference).

[000561] In some embodiments, nucleic acids for delivery are compacted to aid in their uptake (See e.g., U.S. Patents 6,008,366, 6,383,811 herein incorporated by reference). In some embodiment, compacted nucleic acids are targeted to a particular cell type (e.g., cancer cell) via a target cell binding moiety (See e.g., U.S. Patents 5,844,107, 6,077,835, each of which is herein incorporated by reference).

[000562] In some embodiments, oligonucleotides are conjugated to other compounds to aid in their delivery. For example, in some embodiments, nucleic acids are conjugated to polyethylene glycol to aid in delivery (See e.g., U.S. Patents 6,177,274, 6,287,591, 6,447,752, 6,447,753, and 6,440,743, each of which is herein incorporated by reference). In yet other embodiments, oligonucleotides are conjugated to protected graft copolymers, which are chargeable" drug nano-carriers (Pharmaln). In still further embodiments, the transport of oligonucleotides into cells is facilitated by conjugation to vitamins (Endocyte, Inc, West Lafayette, IN; See e.g., U.S. Patents 5,108,921, 5,416,016, 5,635,382, 6,291,673 and WO 02/085908; each of which is herein incorporated by reference). In other embodiments, oligonucleotides are conjugated to nanoparticles (e.g., NanoMed Pharmaceuticals;

Kalamazoo, MI).

[000563] In preferred embodiments, oligonucleotides are enclosed in lipids (e.g., liposomes or micelles) to aid in delivery (See e.g., U.S. Patents 6,458,382, 6,429,200; each of which is herein incorporated by reference). Preferred liposomes include, but are not limited to amphoteric liposomes (e.g., SMARTICLES).. In still further embodiments, oligonucleotides are complexed with additional polymers to aid in delivery (See e.g., U.S. Patents 6,379,966, 6,339,067, 5,744,335; each of which is herein incorporated by reference and Intradigm Corp., Rockville, MD). Cochleates see e.g. Patent application number: 20080242625 and

20120294901.

[000564] In still further embodiments, the controlled high pressure delivery system developed by Minis (Madison, WI) is utilized for delivery of oligonucleotides.

C. Dosages

[000565] Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. In some embodiments, the oligonucleotide is introduced to the host animal at a dosage of between 0.1 mg to 10 g, and preferably at a dosage of between 00.1 mg to 100 mg per kg of body weight or 1 to 300 mg per meter squared body surface area. The administering physician can determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and the delivery means, and can generally be estimated based on EC50s found to be effective in in vitro and in vivo animal models or based on the examples described herein. In general, dosage is from 10 mg to 10 g per kg of body weight , and may be given once or more daily, weekly, monthly or yearly. In some embodiments, dosage is continuous (e.g., intravenously) for a period of from several minutes to several days or weeks. In some embodiments, treatment is given for a defined period followed by a treatment free period. In some embodiments, the pattern of continuous dosing followed by a treatment free period is repeated several times (e.g., until the disease state is diminished).

[000566] The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues.

Following successful treatment, it may be desirable to have the subject undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 10 mg to 10 g, preferably from 1mg to 5 mg, and even more preferably from 0.1 mg to 30 mg per kg of body weight or 0.1 mg/m to 200 mg/m , once or more daily, to once every 20 years.

VII. Customized Patient Care

[000567] In some embodiments, the present invention provides customized patient care.

[000568] The compositions of the present invention are targeted to specific genes unique to a patient's disease (e.g., cancer). For example, in some embodiments, a sample of the patient's cancer or other affected tissue (e.g., a biopsy) is first obtained. The biopsy is analyzed for the presence of expression of a particular gene (e.g., oncogene). In some preferred embodiments, the level of expression of an gene in a patient is analyzed. Expression may be detected by monitoring for the presence of RNA or DNA corresponding to a particular oncogene. Any suitable detection method may be utilized, including, but not limited to, those disclosed below. 5 10 15 20

[000569] Following the characterization of the gene expression pattern of a patient's gene of interest, a customized therapy is generated for each patient. In preferred embodiments, oligonucleotide compounds specific for genes that are aberrantly expressed in the patient (e.g., in a tumor) are combined in a treatment cocktail. In some embodiments, the treatment cocktail further includes additional chemotherapeutic agents (e.g., those described above). The cocktail is then administered to the patient as described above.

[000570] In some embodiments, the analysis of cancer samples and the selection of oligonucleotides for a treatment compound is automated. For example, in some embodiments, a software program that analyses the expression levels of a series of oncogenes to arrive at the optimum selection and concentration of oligonucleotides

Parent Citations (2)

Publication number	Priority date	Publication date	Assignee	Title
FR2790757B1 *	1999-03-09	2003-08-01	Bioalliance	Antisense oligonucleotides containing a sequence stabilized by a secondary structure and mRNA in a tissue sample (e.g., cancer tissue or other biopsy). In other embodiments, expression of a protein is measured in bodily fluids, including, but not limited to, blood, plasma, lymph, serum, mucus, and urine. In some preferred embodiments, the level of mRNA expression is measured quantitatively. RNA expression may be measured by any suitable method, including but not limited to, those disclosed below.
US2004016104A1	2004-06-10	2004-06-26	Weza Sheikhnejad	Methods and compositions for the inhibition of gene expression

[000571] In some embodiments, RNA is detected by Northern blot analysis. Northern blot analysis involves the separation of RNA and hybridization of a complementary labeled probe. In other embodiments, RNA expression is detected by enzymatic cleavage of specific structures (INVADER assay, Third Wave Technologies; See e.g., U.S. Patent Nos.

Non-Parent Citations (1)

5,667; 5,985,557; and 5,994,069; each of which is herein incorporated by reference). The INVADER assay detects specific nucleic acid (e.g., RNA) sequences by using structure-specific enzymes to cleave a complex formed by the

Title
hybridization of overlapping oligonucleotide probes.

[000572] In still further embodiments, RNA (or corresponding cDNA) is detected by hybridization to a oligonucleotide probe). A variety of hybridization assays using a variety of technologies for hybridization and detection are available. For example, in some

embodiments, TaqMan assay (PE Biosystems, Foster City, CA; See e.g., U.S. Patent Nos. 5,962,233 and 5,538,848, each of which is herein incorporated by reference) is utilized. The assay is performed during a PCR reaction. The TaqMan assay exploits the 5'-3' exonuclease activity of the AMP LIT AQ GOLD DNA polymerase. A probe consisting of an

Similar Documents

Publication number	Priority date	Publication date	Title
US2004016104A1	2004-06-10	2004-06-26	Methods and compositions for the inhibition of gene expression
US2004016104A1	2004-06-10	2004-06-26	Methods and compositions for the inhibition of gene expression

[000573] In yet other embodiments, reverse-transcription PCR (RT-PCR) is used to detect the expression of RNA. In RT-PCR, RNA is first reverse-transcribed to complementary DNA or "cDNA" using a reverse-transcriptase enzyme. The cDNA is then used as a template for a PCR reaction. PCR products can be detected by any suitable method, including but not limited to, gel electrophoresis and staining with a DNA specific stain or hybridization to a labeled probe. In some embodiments, the quantitative reverse transcriptase PCR with standardized mixtures of non-competitive templates method described in U.S. Patents 5,639,606, 5,643,765, and 5,876,978 (each of which is herein incorporated by reference) is utilized.

Publication number	Priority date	Publication date	Title
US2004016104A1	2004-06-10	2004-06-26	Methods and compositions for the inhibition of gene expression
US2004016104A1	2004-06-10	2004-06-26	Methods and compositions for the inhibition of gene expression

[000574] In yet other embodiments, mRNA or protein expression is measured using branched DNA technology (e.g. QuantiGene). Branched DNA (bdNA) quantitatively measures gene expression by a sandwich nucleic acid hybridization method that uses bdNA probes specific to the target RNA. The signal from captured target RNA is amplified and enhances assay sensitivity. Recognition of the probe is amplified by rolling circle amplification (RCA) based detection techniques. In the branched DNA negative-assays measure RNA directly from the sample surface, without RNA purification or enzymatic manipulation, potentially avoiding inefficiencies and variability introduced by errors inherent to these processes.

Publication number	Priority date	Publication date	Title
US2004016104A1	2004-06-10	2004-06-26	Methods and compositions for the inhibition of gene expression

B. Detection of Protein
 [000575] In other embodiments, gene expression of oncogenes is detected by measuring the expression of the corresponding protein or polypeptide. In some embodiments, protein expression is detected in a tissue sample or other bodily fluids, protein expression is detected in bodily fluids. In some embodiments, the level of protein expression is quantitated. Protein expression may be detected by any suitable method. In some embodiments, proteins are detected by their binding to an antibody raised against the protein. The generation of antibodies is well known to those skilled in the art.

Publication number	Priority date	Publication date	Title
US2004016104A1	2004-06-10	2004-06-26	Methods and compositions for the inhibition of gene expression

[000576] In one embodiment, antibody binding is detected by detecting a label of the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many methods are known in the art for detecting antibody binding. In one embodiment, the detection of antibody binding is detected by detecting a label of the primary antibody.

Publication number	Priority date	Publication date	Title
US2004016104A1	2004-06-10	2004-06-26	Methods and compositions for the inhibition of gene expression

[000577] In some embodiments, automated detection assays are utilized. Methods for the automation of immunoassays include those described in U.S. Patents 5,885,530, 4,981,785, 6,159,750, and 5,358,691, each of which is herein incorporated by reference. In some embodiments, the analysis and presentation of results is also automated. For example, in some embodiments, TLR stimulation in human mesothelial cells leads to the induction of each of a series of proteins in the absence of type I oncogenes is utilized.

Publication number	Priority date	Publication date	Title
US2004016104A1	2004-06-10	2004-06-26	Methods and compositions for the inhibition of gene expression

[000578] In other embodiments, the immunoassay described in U.S. Patents 5,999,077 and 5,972,280, each of which is herein incorporated by reference.

Publication number	Priority date	Publication date	Title
US2004016104A1	2004-06-10	2004-06-26	Methods and compositions for the inhibition of gene expression

