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(54) **Title:** METHODS AND COMPOSITIONS FOR THE INHIBITION OF GENE EXPRESSION

(57) **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 of oncogenes involved in cancers.

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METHODS AND COMPOSITIONS FOR THE INHIBITION OF GENE EXPRESSION

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FIELD OF THE INVENTION

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 of oncogenes involved in cancers.

BACKGROUND OF THE INVENTION

Oncogenes have 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. This makes protein-based drugs ineffective since their specificity involves ligand-receptor recognition.

Antisense oligodeoxyribonucleotides (oligonucleotides) are under investigation of therapeutic compound 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.

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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 get into their target cells, and high cost due to continuous delivery of large doses. An additional problem with antisense drugs has been their nonspecific activities.

What is needed are additional non-protein based cancer therapeutics that target oncogenes. Therapeutics that are effective in low doses and that are non-toxic to the subject are particularly needed.

SUMMARY OF THE INVENTION

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 of oncogenes involved in cancers.

In one aspect the invention provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to a promoter region of a bcl-2 gene, wherein said first oligonucleotide is SEQ ID NO: 1438 and complements thereof.

In another aspect the invention provides a method, comprising

a) providing

i) a composition of the invention; and

ii) a cell capable of expressing a bcl-2 gene, wherein said cell is capable of proliferation; and

b) introducing said oligonucleotide to said cell, wherein said administering results in a reduction of proliferation of said cell.

In another aspect the invention provides a pharmaceutical composition comprising an oligonucleotide having the nucleic acid sequence of SEQ ID NO: 1438 and a liposome.

In some embodiments, the present invention provides a composition comprising a first oligonucleotide that hybridizes under physiological conditions to the promoter region of a bcl-3 gene (e.g., SEQ ID NOs: 1438, 3, 7, 8 or 9). In some embodiments, at least one of the cytosine bases in the first oligonucleotide is 5-methylcytosine. In some embodiments, all of the cytosine bases in the first oligonucleotide are 5-methylcytosine. In

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some preferred embodiments, the hybridization of the first oligonucleotide to the promoter region of a bcl-2 gene inhibits expression of the bcl-2 gene. In some embodiments, the bcl-2 gene is on a chromosome of a cell, and the hybridization of the first oligonucleotide to the promoter region of bcl-2 gene reduces proliferation of the cell. In some embodiments, the composition further comprises a second oligonucleotide. In some embodiments, the at least one (e.g. all) of the cytosines in the second oligonucleotide are 5-methylcytosine. In some embodiments, the second oligonucleotide comprises SEQ ID NOs: 3, 7, 8 or 9, and is different than the first oligonucleotide (e.g., if the second oligonucleotide has the sequence of SEQ ID NO:3, the first oligonucleotide has a sequence other than SEQ ID NO:3, etc.). In some embodiments, the second oligonucleotide hybridizes to a promoter region of a second gene, wherein the second gene is not bcl-2. In some embodiments, the second gene is an oncogene (e.g., c-ki-Ras, c-Ha-Ras, c-myc, Her-2, or TGF-α).

In other embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes to a promoter region of a bcl-2 gene at a position comprising between nucleotides 1-40 of SEQ ID NO:1, between nucleotides 161-350 of SEQ ID NO:1, between nucleotides 401-590 of SEQ ID NO:1 or between nucleotides 1002-1260 of SEQ ID NO: 1.

In yet other embodiments, the present invention provides a method, comprising: providing an oligonucleotide (e.g., SEQ ID NOs: 1438, 3, 7, 8, or 9); and a cell capable of proliferation, and comprising a bcl-2 gene capable of being expressed; and introducing the oligonucleotide to the cell. In some embodiments, the introducing results in the reduction of proliferation of the cell. In certain embodiments, the introducing results in inhibition of expression of the bcl-2 gene. In some embodiments, the cell is a cancer cell. 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 0.01 μg to 100 g, and preferably at a dosage of between 1mg to 100 mg per kg of body weight. 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 agent. In some embodiments, the cancer is pancreatic cancer, colon cancer, breast cancer, bladder cancer, lung cancer, leukemia, prostate, lymphoma, ovarian, or melanoma. In some embodiments, at least one (e.g., all) of the cytosine bases in the oligonucleotide are 5-methylcytosine.

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). 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 are present in the ratio of from 2:1 to 1:3/1 μg to 100 mg per kg body weight.

The present invention further provides a method, comprising: providing an oligonucleotide that hybridizes to the promoter region of a bcl-2 gene; and a cell comprising a bcl-2 gene; and introducing the oligonucleotide to the cell. In some embodiments, the oligonucleotide comprises at least one CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair is 5-methylcytosine. In some embodiments, the oligonucleotide is completely complementary to the promoter region of the bcl-2 gene. In other embodiments, the oligonucleotide is partially complementary to the promoter region of the bcl-2 gene. For example, in certain embodiments, the oligonucleotide contains one mismatch to the promoter region of the bcl-2 gene. In some preferred embodiments, the oligonucleotide is complementary only to the promoter region of the bcl-2 gene and is not completely complementary to other regions of the human genome. In some embodiments, the oligonucleotide is between 10 nucleotides and 60, and preferably between 15 and 35 nucleotides in length.

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). 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 are present in the ratio of from 2:1 to 1:3/1 μg to 100 mg per kg body weight.

In still further embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a bcl-2 gene. In some embodiments, the oligonucleotide comprises at least one CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair is 5-methylcytosine. In some embodiments, the oligonucleotide is completely complementary to the promoter region of the bcl-2 gene. In other embodiments, the oligonucleotide is partially complementary to the promoter region of the bcl-2 gene. For example, in certain embodiments, the oligonucleotide contains one mismatch to the promoter region of the bcl-2 gene. In some preferred embodiments, the oligonucleotide is complementary only to the promoter region of the bcl-2 gene and is not completely complementary to other regions of the human genome. In some embodiments, the oligonucleotide is between 10 nucleotides and 60, and preferably between 15 and 35 nucleotides in length.

The present invention further provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a bcl-2 gene under conditions such that expression of the bcl-2 gene is inhibited.

The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a bcl-2 gene located on a chromosome of a cell under conditions such that proliferation of the cell is reduced.

The present invention also provides a composition comprising a first oligonucleotide that hybridizes under physiological conditions to the promoter region of a bcl-2 gene, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; and a second oligonucleotide, the second oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine.

In certain embodiments, the present invention provides a kit comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a bcl-2 gene, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair is 5-methylcytosine; and instructions for using the kit for reducing proliferation of a cell comprising a bcl-2 gene on a chromosome of the cell or inhibiting gene expression. In some embodiments, the composition in the kit are 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.

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 a oncogene in the sample; and detecting the presence or absence of expression of an oncogene in the sample; introducing an oligonucleotide that hybridizes under physiological conditions to the promoter region of an oncogene expressed in the biological sample, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair is 5-methylcytosine to the subject.

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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; 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.

In yet further embodiments, the present invention provides a method of screening compounds comprising providing a cell comprising a suspected oncogene; and an oligonucleotide that hybridizes to the promoter region of the gene; and introducing the oligonucleotide to the cell; and determining if proliferation of the cell is inhibited in the presence of the oligonucleotide relative to the absence of the oligonucleotide. In some embodiments, the cell is in culture (e.g., a cancer 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.

Accordingly, in some embodiments, the present invention provides a composition comprising a first oligonucleotide that hybridizes to the promoter region of a c-ki-Ras gene under physiological conditions (e.g., SEQ ID NOs: 47, 48, 50 or 53). In certain embodiments, at least one of the cytosine bases (e.g., all) of the first oligonucleotide are 5-methylcytosine. In some embodiments, the hybridization of the first oligonucleotide to the promoter region of c-ki-Ras gene inhibits expression of the c-ki-Ras gene. In certain embodiments, the c-ki-Ras gene is on a chromosome of a cell, and the hybridization of the first oligonucleotide to the promoter region of c-ki-Ras reduces proliferation of the cell. In some embodiments, the composition further comprises a second oligonucleotide. In some embodiments, the at least one of the cytosine bases in the second oligonucleotide is 5-methylcytosine. In other embodiments, all of the cytosine bases in the second oligonucleotide are 5-methylcytosine. In some embodiments, the second oligonucleotide comprises SEQ ID NOs: 47, 48, 50 or 53, wherein the second oligonucleotide is different than the first oligonucleotide (e.g., if the second oligonucleotide has the sequence of SEQ ID NO:47, the first oligonucleotide has a sequence other than SEQ ID NO:47, etc.). In certain embodiments, the second oligonucleotide hybridizes to a promoter region of a second gene, wherein the second gene is not c-ki-Ras. In some embodiments, the second gene is an oncogene (e.g., including, but not limited to, c-Ha-Ras, c-myc, Her-2, TGF-α, and bcl-2).

In yet other embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes to a promoter region of a c-ki-Ras gene at a position comprising between nucleotides 1 and 289 of SEQ ID NO: 46 or between nucleotides 432 and 658 of SEQ ID NO: 46.

In other embodiments, the present invention provides a method, comprising providing an oligonucleotide that hybridizes to the promoter of a c-ki-Ras gene (e.g., an oligonucleotide comprising SEQ ID NOs: 47, 48, 50 or 53); and a cell comprising a c-ki-Ras gene that is capable of being expressed, and wherein the cell is capable of proliferation; and introducing the oligonucleotide to the cell. In some embodiments, 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. In other embodiments, all of the cytosine bases in the CG dinucleotide pairs of the oligonucleotide are 5-methylcytosine. In some embodiments, the oligonucleotide hybridizes to a promoter region of a c-ki-Ras gene at a position comprising between nucleotides 1 and 289 of SEQ ID NO: 46 or between nucleotides 432 and 658 of SEQ ID NO: 46. In some embodiments, the oligonucleotide is between 15 and 30 bases in length.

In some preferred embodiments, the introducing results in the reduction of proliferation of the cell. In some embodiments, the introducing results in inhibition of expression of the c-ki-Ras gene. In some embodiments, the cell is a cancer cell (e.g., including, but not limited to, pancreatic cancer, colon cancer, breast cancer, bladder cancer, lung cancer, leukemia, prostate, lymphoma, ovarian, and melanoma). In some embodiments, the cell is in a host animal. In some embodiments, the host animal is a non-human mammal. In some embodiments, the oligonucleotide is introduced to the host animal at a dosage of between 0.01 μg to 100 g, and preferably between 1mg to 100 mg per kg of body weight. 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 (e.g., for a period of between 2 hours and 2 weeks). In other embodiments, the cell is in cell culture. In certain 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 agent.

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). 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 are present in the ratio of from 2:1 to 1:3/1 μg to 100 mg per kg body weight.

In still further embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-ki-Ras gene, wherein at least one (e.g., all) of the cytosine bases in the oligonucleotide is 5-methylcytosine. In some embodiments, the oligonucleotide is completely complementary to the promoter region of the c-ki-Ras gene. In other embodiments, the oligonucleotide is partially complementary to the promoter region of the c-ki-Ras gene. Fore example, in certain embodiments, the oligonucleotide contains one mismatch to the promoter region of the c-ki-Ras gene. In some preferred embodiments, the oligonucleotide is complementary only to the promoter region of the c-ki-Ras gene and is not completely complementary to other regions of the human genome. In some embodiments, the oligonucleotide is between 10 nucleotides and 60, and preferably between 15 and 35 nucleotides in length.

The present invention further provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-ki-Ras gene under conditions such that expression of the c-ki-Ras gene is inhibited.

The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-ki-Ras gene located on a chromosome of a cell under conditions such that proliferation of the cell is reduced.

The present invention also provides a composition comprising a first oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-ki-Ras gene, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; and a second oligonucleotide, the second oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine.

In certain embodiments, the present invention provides a kit comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-ki-Ras gene, the oligonucleotide comprising at least on 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 c-ki-Ras gene on a chromosome of the cell or inhibiting gene expression. In some embodiments, the composition in the kit are 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.

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 a 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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine to the subject.

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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; 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.

In yet further embodiments, the present invention provides a method of screening compounds comprising providing a cell comprising a suspected oncogene; and an oligonucleotide that hybridizes to the promoter region of the gene; and administering the oligonucleotide to the cell; and determining if proliferation of the cell is inhibited in the presence of the oligonucleotide relative to the absence of the oligonucleotide. In some embodiments, the cell is in culture (e.g., a cancer 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.

In some embodiments, the present invention provides a composition comprising a first oligonucleotide that hybridizes to the promoter region of a c-myc gene under physiological conditions (e.g., SEQ ID NOs: 110, 111, 112, 113, 114 or 115). In some embodiments, at least one (e.g., all) of the cytosine bases in the first oligonucleotide is 5-methylcytosine. In some preferred embodiments, the hybridization of the first oligonucleotide to the promoter region of a c-myc gene inhibits expression of the c-myc gene. In some embodiments, the c-myc gene is on a chromosome of a cell, and the hybridization of the first oligonucleotide to the promoter region of the c-myc gene reduces proliferation of the cell. In some embodiments, the composition further comprises a second oligonucleotide. In some embodiments, at least one (e.g., all) of the cytosine bases in the second oligonucleotide are 5-methylcytosine. In some embodiments, the second oligonucleotide comprises SEQ ID NOs: 110, 111, 112, 113, 114 or 115, wherein the second oligonucleotide is different from the first oligonucleotide (e.g., if the second oligonucleotide has the sequence of SEQ ID NO: 110, the first oligonucleotide has a sequence other than SEQ ID NO: 110, etc.). In some embodiments, the second oligonucleotide hybridizes to a promoter region of a second gene, wherein the second gene is not c-myc. In some embodiments, the second gene is an oncogene (e.g., c-ki-Ras, c-Ha-Ras, bcl-2, Her-2, or TGF-α).

The present invention further provides a composition comprising an oligonucleotide that hybridizes to a promoter region of a c-myc gene at a position comprising between nucleotides 3-124 of SEQ ID NO:108 or between nucleotides 165-629 of SEQ ID NO:108.

The present invention further provides a method, comprising: providing an oligonucleotide that hybridizes to the promoter region of a c-myc gene under physiological conditions (e.g., SEQ ID NOs: 110, 111, 112, 113, 114 or 115); and a cell comprising a c-myc gene capable of expressing the c-myc gene, and wherein the cell is capable of proliferation; and introducing the oligonucleotide to the cell. In some embodiments, the introducing results in the reduction of proliferation of the cell. In certain embodiments, the introducing results in inhibition of expression of the c-myc gene. In some embodiments, the cell is a cancer cell. 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 0.01 μg to 100 g, and preferably at a dosage of between 1mg to 100 mg per kg of body weight. 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 agent. In some embodiments, the cancer is pancreatic cancer, colon cancer, breast cancer, bladder cancer, lung cancer, leukemia, prostate, lymphoma, ovarian, or melanoma.

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). 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 are present in the ratio of from 2:1 to 1:3/1 μg to 100 mg per kg body weight.

In some embodiments, at least one, and preferably all, of the cytosine bases in the oligonucleotide are 5-methylcytosine. In some embodiments, the oligonucleotide is between 10 nucleotides and 60, and preferably between 15 and 35 nucleotides in length. In some embodiments, the oligonucleotide hybridizes to a promoter region of a c-myc gene at a position comprising between nucleotides 3-124 of SEQ ID NO:108 or between nucleotides 165-629 of SEQ ID NO:108.

In still further embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-myc gene, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine. In some embodiments, the oligonucleotide is completely complementary to the promoter region of the c-myc gene. In other embodiments, the oligonucleotide is partially complementary to the promoter region of the c-myc gene. For example, in certain embodiments, the oligonucleotide contains one mismatch to the promoter region of the c-myc gene. In some preferred embodiments, the oligonucleotide is complementary only to the promoter region of the c-myc gene and is not completely complementary to other regions of the human genome. In some embodiments, the oligonucleotide is between 10 nucleotides and 60, and preferably between 15 and 35 nucleotides in length.

The present invention further provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-myc gene under conditions such that expression of the c-myc gene is inhibited.

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

The present invention also provides a composition comprising a first oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-myc gene, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; and a second oligonucleotide, the second oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine.

In certain embodiments, the present invention provides a kit comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-myc gene, the oligonucleotide comprising at least on 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 c-myc gene on a chromosome of the cell or inhibiting gene expression. In some embodiments, the composition in the kit are 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.

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 a 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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine to the subject.

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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; 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.

In yet further embodiments, the present invention provides a method of screening compounds comprising providing a cell comprising a suspected oncogene; and an oligonucleotide that hybridizes to the promoter region of the gene; and administering the oligonucleotide to the cell; and determining if proliferation of the cell is inhibited in the presence of the oligonucleotide relative to the absence of the oligonucleotide. In some embodiments, the cell is in culture (e.g., a cancer 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.

In some embodiments, the present invention provides a composition comprising a first oligonucleotide that hybridizes to the promoter region of a c-Ha-ras gene under physiological conditions (e.g., SEQ ID NOs: 67, 68, 69, 71, 73, 74, 76, 78, 84, 160, 161, or 162). In some embodiments, at least one of the cytosine bases in the first oligonucleotide is 5-methylcytosine. In some embodiments, all of the cytosine bases in the first oligonucleotide are 5-methylcytosine. In certain embodiments, the first oligonucleotide hybridizes under physiological conditions to the promoter region of a c-Ha-ras gene. In some preferred embodiments, the hybridization of the first oligonucleotide to the promoter region of a c-Ha-ras gene inhibits expression of the c-Ha-ras gene. In some embodiments, the c-Ha-ras gene is on a chromosome of a cell, and wherein the hybridization of the first oligonucleotide to the promoter region of c-Ha-ras gene reduces proliferation of the cell. In some embodiments, the composition further comprises a second oligonucleotide. In some embodiments, the at least one (e.g. all) of the cytosines in the second oligonucleotide are 5-methylcytosine. In some embodiments, the second oligonucleotide comprises SEQ ID NOs: 67, 68, 69, 71, 73, 74, 76, 78, 84, 160, 161, or 162, wherein the first oligonucleotide is different from the second oligonucleotide (e.g., if the second oligonucleotide has the sequence of SEQ ID NO:67, the first oligonucleotide has a sequence different than SEQ ID NO:67, etc.). In some embodiments, the second oligonucleotide hybridizes to a promoter region of a second gene, wherein the second gene is not c-Ha-ras. In some embodiments, the second gene is an oncogene (e.g., c-ki-Ras, c-myc, bcl-2, Her-2, or TGF-α).

In other embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes to a promoter region of a c-Ha-ras gene at a position comprising between nucleotides 21-220 of SEQ ID NO:66, 233-860 of SEQ ID NO:66, 1411-1530 of SEQ ID NO:66 or between nucleotides 1631-1722 of SEQ ID NO:66.

In yet other embodiments, the present invention provides a method, comprising: providing an oligonucleotide that hybridizes to the promoter region of a c-Ha-Ras gene under physiological conditions (e.g., SEQ ID NOs: 67, 68, 69, 71, 73, 74, 76, 78, 84, 160, 161, or 162); and a cell comprising a c-Ha-ras gene capable of expression, and wherein the cell is capable of proliferation; and introducing the oligonucleotide to the cell. In some embodiments, the oligonucleotide is between 15 and 30 bases in length. In some embodiments, the oligonucleotide hybridizes to the promoter region of the c-Ha-ras gene at a position comprising between nucleotides 21-220 of SEQ ID NO:66, 233-860 of SEQ ID NO:66, 1411-1530 of SEQ ID NO:66 or between nucleotides 1631-1722 of SEQ ID NO:66.

In some embodiments, the introducing results in the reduction of proliferation of the cell. In certain embodiments, the introducing results in inhibition of expression of the c-Ha-ras gene. In some embodiments, the cell is a cancer cell. In some embodiments, the cancer is pancreatic cancer, colon cancer, breast cancer, bladder cancer, lung cancer, leukemia, prostate, lymphoma, ovarian, or melanoma. 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 0.01 μg to 100 g, and preferably between 1mg to 100 mg per kg of body weight. 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 (e.g., for a period of between 2 hours and 2 weeks). In other embodiments, the cell is in cell culture. In certain 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 agent.

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). 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 are present in the ratio of from 2:1 to 1:3/1 μg to 100 mg per kg body weight.

In still further embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-Ha-ras gene. In some embodiments, at least one (e.g., all) of the cytosine bases in the oligonucleotide are 5-methylcytosine. In some embodiments, the oligonucleotide is completely complementary to the promoter region of the c-Ha-ras gene. In other embodiments, the oligonucleotide is partially complementary to the promoter region of the c-Ha-ras gene. For example, in certain embodiments, the oligonucleotide contains one mismatch to the promoter region of the c-Ha-ras gene. In some preferred embodiments, the oligonucleotide is complementary only to the promoter region of the c-Ha-ras gene and is not completely complementary to other regions of the human genome. In some embodiments, the oligonucleotide is between 10 nucleotides and 60, and preferably between 15 and 35 nucleotides in length.

The present invention further provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-Ha-ras gene under conditions such that expression of the c-Ha-ras gene is inhibited.

The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-Ha-ras gene located on a chromosome of a cell under conditions such that proliferation of the cell is reduced.

The present invention also provides a composition comprising a first oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-Ha-ras gene, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; and a second oligonucleotide, the second oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine.

In certain embodiments, the present invention provides a kit comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a c-Ha-ras gene, the oligonucleotide comprising at least on 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 c-Ha-ras gene on a chromosome of the cell or inhibiting gene expression. In some embodiments, the composition in the kit are 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.

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 a 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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine to the subject.

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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; 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.

In yet further embodiments, the present invention provides a method of screening compounds comprising providing a cell comprising a suspected oncogene; and an oligonucleotide that hybridizes to the promoter region of the gene; and administering the oligonucleotide to the cell; and determining if proliferation of the cell is inhibited in the presence of the oligonucleotide relative to the absence of the oligonucleotide. In some embodiments, the cell is in culture (e.g., a cancer 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.

In some embodiments, the present invention provides a composition comprising an oligonucleotide comprising SEQ ID NOs: 31, 32, 35, 36, 37, or 38. In some embodiments, the oligonucleotide comprises at least one CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine. In some embodiments, all of the cytosine bases in all of the CG dinucleotide pairs of the oligonucleotide are 5-methylcytosine. In certain embodiments, the oligonucleotide hybridizes under physiological conditions to the promoter region of a Her-2 gene. In some preferred embodiments, the hybridization of the oligonucleotide to the promoter region of a Her-2 gene inhibits expression of the Her-2 gene. In some embodiments, the Her-2 gene is on a chromosome of a cell, and wherein the hybridization of the oligonucleotide to the promoter region of a Her-2 gene reduces proliferation of the cell. In some embodiments, the composition further comprises a second oligonucleotide. In some embodiments, the second oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine. In some embodiments, the second oligonucleotide comprises SEQ ID NOs: 31, 32, 35, 36, 37, or 38. In some embodiments, the second oligonucleotide hybridizes to a promoter region of a second gene, wherein the second gene is not Her-2. In some embodiments, the second gene is an oncogene (e.g., c-ki-Ras, c-myc, bcl-2, c-Ha-ras, or TGF-α).

In other embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes to a promoter region of a c-myc gene at a position comprising between nucleotides 205-344 of SEQ ID NO:29 or between nucleotides 382-435 of SEQ ID NO:29.

In still further embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a Her-2 gene.

In yet other embodiments, the present invention provides a method, comprising: providing an oligonucleotide (e.g., SEQ ID NOs: 31, 32, 35, 36, 37, or 38) and a cell comprising a Her-2 gene; and administering the oligonucleotide to the cell. In some embodiments, the administering results in the reduction of proliferation of the cell. In certain embodiments, the administration results in inhibition of expression of the Her-2 gene. In some embodiments, the cell is a cancer cell. 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 administered to the host animal at a dosage of between 0.01 μg to 100 g, and preferably at a dosage of between 1mg to 100 mg per kg of body weight. In some embodiments, the oligonucleotide is administered to the host animal one or more times per day. In other embodiments, the oligonucleotide is administered 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 administering a test compound to the cell. In some embodiments, the test compound is a known chemotherapy agent. In some embodiments, the cancer is pancreatic cancer, colon cancer, breast cancer, bladder cancer, lung cancer, leukemia, prostate, lymphoma, ovarian, or melanoma. In some embodiments, the oligonucleotide comprises at least one CG dinucleotide pair, wherein at least one, and preferably all, of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine.

The present invention further provides a method, comprising providing an oligonucleotide that hybridizes to the promoter region of a Her-2 gene; and a cell comprising a Her-2 gene; and administering the oligonucleotide to the cell.

In still further embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a Her-2 gene, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine. In some embodiments, the oligonucleotide is completely complementary to the promoter region of the Her-2 gene. In other embodiments, the oligonucleotide is partially complementary to the promoter region of the Her-2 gene. For example, in certain embodiments, the oligonucleotide contains one mismatch to the promoter region of the Her-2 gene. In some preferred embodiments, the oligonucleotide is complementary only to the promoter region of the Her-2 gene and is not completely complementary to other regions of the human genome. In some embodiments, the oligonucleotide is between 10 nucleotides and 60, and preferably between 15 and 35 nucleotides in length.

The present invention further provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a Her-2 gene under conditions such that expression of the Her-2 gene is inhibited.

The present invention additionally provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a Her-2 gene located on a chromosome of a cell under conditions such that proliferation of the cell is reduced.

The present invention also provides a composition comprising a first oligonucleotide that hybridizes under physiological conditions to the promoter region of a Her-2 gene, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; and a second oligonucleotide, the second oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine.

In certain embodiments, the present invention provides a kit comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a Her-2 gene, the oligonucleotide comprising at least on 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 Her-2 gene on a chromosome of the cell or inhibiting gene expression. In some embodiments, the composition in the kit are 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.

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 a 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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine to the subject.

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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; 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.

In yet further embodiments, the present invention provides a method of screening compounds comprising providing a cell comprising a suspected oncogene; and an oligonucleotide that hybridizes to the promoter region of the gene; and administering the oligonucleotide to the cell; and determining if proliferation of the cell is inhibited in the presence of the oligonucleotide relative to the absence of the oligonucleotide. In some embodiments, the cell is in culture (e.g., a cancer 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.

In some embodiments, the present invention provides a composition comprising an oligonucleotide comprising SEQ ID NOs: 134, 136, 139, 140, 141, 142, 143, or 144. In some embodiments, the oligonucleotide comprises at least one CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine. In some embodiments, all of the cytosine bases in all of the CG dinucleotide pairs of the oligonucleotide are 5-methylcytosine. In certain embodiments, the oligonucleotide hybridizes under physiological conditions to the promoter region of a TGF-α gene. In some embodiments, the hybridization of the oligonucleotide to the promoter region of a TGF-α gene inhibits expression of the TGF-α gene. In some embodiments, the TGF-α gene is on a chromosome of a cell, and wherein the hybridization of the oligonucleotide to the promoter region of a TGF-α gene reduces proliferation of the cell. In some embodiments, the composition further comprises a second oligonucleotide. In some embodiments, the second oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine. In some embodiments, the second oligonucleotide is selected from the group consisting of SEQ ID NOs: 134, 136, 139, 140, 141, 142, 143, and 144. In other embodiments, the second oligonucleotide hybridizes to a promoter region of a second gene, wherein the second gene is not TGF-α. In still further embodiments, the second gene is an oncogene (e.g., c-ki-Ras, c-Ha-Ras, bcl-2, Her-2, or c-myc).

In other embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes to a promoter region of a c-myc gene at a position comprising between nucleotides 1-90 of SEQ ID NO:131, between oligonucleotides 175-219 of SEQ ID NO:131, between nucleotides 261-367 of SEQ ID NO:131, between nucleotides 431-930 of SEQ ID NO:131, or between nucleotides 964-1237 of SEQ ID NO:131.

In yet other embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a TGF-α gene.

In still further embodiments, the present invention provides a method, comprising providing an oligonucleotide (e.g., SEQ ID NOs: 134, 136, 139, 140, 141, 142, 143, or 144); and a cell comprising a TGF-α gene; and administering the oligonucleotide to the cell. In some embodiments, the administering results in the reduction of proliferation of the cell. In certain embodiments, the administration results in inhibition of expression of the TGF-α gene. In some embodiments, the cell is a cancer cell. 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 administered to the host animal at a dosage of between 0.01 μg to 100 g, and preferably at a dosage of between 1mg to 100 mg per kg of body weight. In some embodiments, the oligonucleotide is administered to the host animal one or more times per day. In other embodiments, the oligonucleotide is administered 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 administering a test compound to the cell. In some embodiments, the test compound is a known chemotherapy agent. In some embodiments, the cancer is pancreatic cancer, colon cancer, breast cancer, bladder cancer, lung cancer, leukemia, prostate, lymphoma, ovarian, or melanoma. In some embodiments, the oligonucleotide comprises at least one CG dinucleotide pair, wherein at least one, and preferably all, of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine.

The present invention further provides a method, comprising providing an oligonucleotide that hybridizes to the promoter region of a TGF-α gene; and a cell comprising a TGF-α gene; and administering the oligonucleotide to the cell.

In still further embodiments, the present invention provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a TGF-α gene, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine. In some embodiments, the oligonucleotide is completely complementary to the promoter region of the TGF-α gene. In other embodiments, the oligonucleotide is partially complementary to the promoter region of the TGF-α gene. For example, in certain embodiments, the oligonucleotide contains one mismatch to the promoter region of the TGF-α gene. In some preferred embodiments, the oligonucleotide is complementary only to the promoter region of the TGF-α gene and is not completely complementary to other regions of the human genome. In some embodiments, the oligonucleotide is between 10 nucleotides and 60, and preferably between 15 and 35 nucleotides in length.

The present invention further provides a composition comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a TGF-α gene under conditions such that expression of the TGF-α gene is inhibited.

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

The present invention also provides a composition comprising a first oligonucleotide that hybridizes under physiological conditions to the promoter region of a TGF-α gene, the oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; and a second oligonucleotide, the second oligonucleotide comprising at least on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine.

In certain embodiments, the present invention provides a kit comprising an oligonucleotide that hybridizes under physiological conditions to the promoter region of a TGF-α gene, the oligonucleotide comprising at least on 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 TGF-α gene on a chromosome of the cell or inhibiting gene expression. In some embodiments, the composition in the kit are 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.

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 a 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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine to the subject.

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 on CG dinucleotide pair, wherein at least one of the cytosine bases in the CG dinucleotide pair comprises 5-methylcytosine; 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.

In yet further embodiments, the present invention provides a method of screening compounds comprising providing a cell comprising a suspected oncogene; and an oligonucleotide that hybridizes to the promoter region of the gene; and administering the oligonucleotide to the cell; and determining if proliferation of the cell is inhibited in the presence of the oligonucleotide relative to the absence of the oligonucleotide. In some embodiments, the cell is in culture (e.g., a cancer 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.

In other embodiments, the present invention relates to methods and compositions for cancer therapy. In particular, the present invention provides liposome based cancer therapeutics.

Accordingly, in some embodiments, the present invention provides a pharmaceutical composition comprising (e.g., consisting of) a cationic, neutral, or anionic liposome and an oligonucleotide. 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 present invention provides a kit, comprising an oligonucleotide (e.g., an oligonuculeotide that hybridizes to the promoter region of an onocogene) 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), 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 forth and still more preferable less than one tenth, and yet more preferably less than one hundredth the standard dose.

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 oligonuculeotide that hybridizes to the promoter region of an onocogene); 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 forth 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 shows the nucleic acid sequence of the bcl-2 gene (SEQ ID NO:1).

Figure 2 shows the sequences of antigenes to bcl-2 used in some embodiments of the present invention. X refers to a methylated C nucleotide.

Figure 3 shows the nucleic acid sequence of the c-erbB-2 (Her-2) gene (SEQ ID NO:29).

Figure 4 shows the sequences of antigenes to c-erbB-2 used in some embodiments of the present invention. X refers to a methylated C nucleotide.

Figure 5 shows the nucleic acid sequence of the c-ki-Ras gene (SEQ ID NO:46).

Figure 6 shows the sequences of antigenes to c-ki-Ras used in some embodiments of the present invention. X refers to a methylated C nucleotide.

Figure 7 shows the nucleic acid sequence of the c-Ha-Ras gene (SEQ ID NO:66).

Figure 8 shows the sequences of antigenes to c-Ha-Ras used in some embodiments of the present invention. X refers to a methylated C nucleotide.

Figure 9 shows the nucleic acid sequence of the c-myc gene (SEQ ID NO:108).

Figure 10 shows the sequences of antigenes to c-myc used in some embodiments of the present invention. X refers to a methylated C nucleotide.

Figure 11 shows the nucleic acid sequence of the TGF-α gene (SEQ ID NO:131).

Figure 12 shows the sequences of antigenes to TGF-α used in some embodiments of the present invention. X refers to a methylated C nucleotide.

Figure 13 shows the inhibition of expression of cell growth by antigenes to c-ki-Ras used in some embodiments of the present invention

Figure 14 shows the inhibition of expression of cell growth by antigenes to bcl-2 used in some embodiments of the present invention.

Figure 15 shows the inhibition of expression of cell growth by antigenes to c-erb-2 used in some embodiments of the present invention.

Figure 16 shows the inhibition of expression of cell growth by antigenes to c-Ha-Ras used in some embodiments of the present invention.

Figure 17 shows the inhibition of expression of cell growth by antigenes to c-myc used in some embodiments of the present invention.

Figure 18 shows the inhibition of expression of cell growth by antigenes to TGF-α used in some embodiments of the present invention.

Figure 19 shows the dose response curve of inhibition of expression of cell growth by antigenes to c-ki-Ras.

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Figure 20 shows the dose response curve of inhibition of expression of cell growth of FSCCL cells (A) and MCF-7 cells (B) by antigens to bcl-2.

Figure 21 shows the dose response curve of inhibition of expression of cell growth by antigens to c-erb-2.

Figure 22 shows the dose response curve of inhibition of expression of cell growth by antigens to c-Ha-Ras used.

Figure 23 shows the dose response curve of inhibition of expression of cell growth by antigens to c-myc.

Figure 24 shows the dose response curve of inhibition of expression of cell growth of T47D cells (A) and MDA-MB-231 cells (B) by antigens to TGF-α.

Figure 25 shows exemplary variants of antigens to c-ki-Ras.

Figure 26 shows exemplary variants of antigens to bcl-2.

Figure 27 shows exemplary variants of antigens to c-erb-2.

Figure 28 shows exemplary variants of antigens to c-ha-ras.

Figure 29 shows exemplary variants of antigens to c-myc.

Figure 30 shows exemplary variants of antigens to TGF-α.

Figure 31 shows inhibition of lymphoma cells by non-methylated oligonucleotides targeted toward Bcl-2.

Figure 32 shows mean tumor volume of tumors in the PC-3 GFP prostate carcinoma subcutaneous model following treatment with compositions of the present invention.

Figure 33 shows mean body weight of tumors in the PC-3 GFP prostate carcinoma subcutaneous model following treatment with compositions of the present invention.

Figure 34 shows mean tumor volume of tumors in the PC-3 GFP prostate carcinoma subcutaneous model following treatment with compositions of the present invention.

Figure 35 shows mean final tumor volume of tumors in the PC-3 GFP prostate carcinoma subcutaneous model following treatment with compositions of the present invention.

DEFINITIONS

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

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.

As used herein, the term "under conditions such that expression of said gene is inhibited" refers to conditions where an oligonucleotide of the present invention hybridizes to a gene (*e.g.*, the promoter region of the gene) and inhibits transcription of the gene by at least 10%, preferably at least 25%, even more preferably at least 50%, and still more preferably at least 90% relative to the level of transcription in the absence of the oligonucleotide. The present invention is not limited to the inhibition of expression of a particular gene. Exemplary genes include, but are not limited to, c-ki-Ras, c-Ha-ras, c-myc, her-2, TGF-α, and bcl-2.

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.

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

When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as "antigenic determinants". An antigenic determinant may compete with the intact antigen (*i.e.*, the "immunogen" used to elicit the immune response) for binding to an antibody.

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.

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.

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.

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.

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 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, viral pathogens.

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-(carboxyhydroxylmethyl) 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-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

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 both the 5' and 3' ends for a distance of about 1 kb or more on either end 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.

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, 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).

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. Molecules (*e.g.*, transcription factors) that are involved in up-regulation or down-regulation are often called "activators" and "repressors," respectively.

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.

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) 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.

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.

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.

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 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.

In some embodiments, oligonucleotides are "antigenes." As used herein, the term "antigene" refers to an oligonucleotide that hybridizes to the promoter region of a gene. In some embodiments, the hybridization of the antigene to the promoter inhibits expression of the gene.

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" 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. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

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).

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.

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.

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.

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.

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.

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 complementary between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids. A single molecule that contains pairing of complementary nucleic acids within its structure is said to be "self-hybridized."

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.

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 relation 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 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.

"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 NaH_2PO_4 H_2O 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.

"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 NaH_2PO_4 H_2O 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.

"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 NaH_2PO_4 H_2O 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, Pharamcia), 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.

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 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]).

The art knows well that numerous equivalent conditions may be employed to comprise 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").

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% CO_2, 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).

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).

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.

"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.

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.

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.

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]).

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]).

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.

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*.

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).

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.

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, or disorder of bodily function (*e.g.*, cancer). 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.

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 and diethylstilbestrol (DES).

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.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods and compositions for the treatment of cancers. In particular, 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 a 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).

I. Oncogene Targets

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

A. Ras

One gene which has captured the attention of many scientists is the human proto-oncogene, c-Ha-ras. The nucleic acid sequence of the promoter region of c-H-ras is shown in Figure 7. This gene acts as a central dispatcher, relaying chemical signals into cells and controlling cell division. Ras gene alteration may cause the gene to stay in the "on" position. The ras oncogene is believed to underlie up to 30% of cancer, including colon cancer, lung cancer, bladder and mammary carcinoma (Bos, Cancer Res. 49:4682-4689 [1989]). The ras oncogene has therefore become a target for therapeutic drugs.

There are several reports showing that oligonucleotides complementary to various sites of ras mRNA can inhibit synthesis of ras protein (p21), which decreases the cell proliferation rate in cell culture (U.S. Pat. No. 5,576,208; U.S. Pat. No. 5,582,986; Daska *et al.*, Oncogene Res. 5:267-275 [1990]; Brown *et al.*, Oncogene Res. 4:243-252 [1989]; Saison-Behmoaras *et al.*, EMBO J. 10:1111-1116 [1991)]. Oligonucleotides complementary to the 5' flanking region of the c-Ha-ras RNA transcript have shown to inhibit tumor growth in nude mice for up to 14 days (Gray *et al.*, Cancer Res. 53:577-580 [1993]). It was recently reported that an antisense oligonucleotide directed to a point mutation (G>C) in codon 12 of the c-Ha-ras mRNA inhibited cell proliferation as well as tumor growth in nude mice when it was injected subcutaneously (U.S. Pat. No. 5,576,208; U.S. Pat. No. 5,582,986; Schwab *et al.*, Proc. Natl. Acad. Sci. USA 91:10460-10464 [1994]; each of which is herein incorporated by reference). Researchers have also reported that antisense drugs shrank ovarian tumors in small clinical trials (Roush *et al.*, Science 276:1192-1194 [1997]).

B. Her-2

The HER-2 (also known as neu oncogene or erbB-2) oncogene encodes a receptor-like tyrosine kinase (RTK) that has been extensively investigated because of its role in several human carcinomas (Hynes and Stern, Biochim. et Biophy. Acta 1198:165-184 [1994]; Dougall *et al.*, Oncogene 9:2109-2123 [1994]) and in mammalian development (Lee *et al.*, Nature 378:394-398 [1995]). The nucleic acid sequence of the promoter region of Her-2 is shown in Figure 3. The sequence of the HER-2 protein was determined from a cDNA that was cloned by homology to the epidermal growth factor receptor (EGFR) mRNA from placenta (Coussens *et al.*, Science 230:1132-1139 [1985]) and from a gastric carcinoma cell line (Yamamoto *et al.*, Nature 319:230-234 [1986]). The HER-2 mRNA was shown to be about 4.5 kb (Coussens *et al.*, Science 230:1132-1139 [1985]; Yamamoto *et al.*, Nature 319:230-234 [1986]) and encodes a transmembrane glycoprotein of 185 kDa in normal and malignant human tissues (p185HER-2) (Hynes and Steen, Biochim. et Biophys. Acta 1198:165-184 [1994]; Dougall *et al.*, Oncogene 9:2109-2123 [1994]). Overexpression of HER-2 causes phenotypic transformation of cultured cells (DiFiore *et al.*, Science 237:178-182 [1987]; Hudziak *et al.*, Proc. Natl. Acad. Sci. USA 84:7159-7163 [1987]) and has been associated with aggressive clinical progression of breast and ovarian cancer (Slamon *et al.*, Science 235:177-182 [1987]; Slamon *et al.*, Science 244:707-712 [1989]).

HER-2 is one of the most frequently altered genes in cancer. It encodes a transmembrane receptor (also known as p185) with tyrosine kinase activity and is a member of the epidermal growth factor (EGF) family, and thus is related to the epidermal growth factor receptor (EGFR or HER-1). Aberrant HER-2 gene expression is present in a wide variety of cancers and are most common in breast, ovarian and gastric cancers. HER-2 is overexpressed in 25-30% of all human breast and ovarian cancers. Levels of HER-2 overexpression correlate well with clinical stage of breast cancer, prognosis and metastatic potential. Overexpression of HER-2 is associated with lower survival rates, increased relapse rates and increased metastatic potential. Tan *et al.*, (Cancer Res., 57:1199 [1997]) have shown that overexpression of the HER-2 gene increases the metastatic potential of breast cancer cells without increasing their transformation ability.

Aberrant expression of HER-2 includes both increased expression of normal HER-2 and expression of mutant HER-2. Activation of the HER-2 proto-oncogene can occur by any of three mechanisms--point mutation, gene amplification and overexpression. Gene amplification is the most common mechanism. Unlike the other EGF family members for whom ligand activation is necessary for promoting transformation, overexpression of HER-2 alone is sufficient for transformation (Cohen, *et al.*, J. Biol. Chem., 271:30897 [1996]).

Several therapeutic approaches have been used to reduce levels of the HER-2 gene product. The adenovirus type 5 gene product E1A has been studied as a potential therapeutic using a breast cancer model in nude mice. This gene product can repress HER-2/neu overexpression by repressing HER-2/neu promoter activity, and suppress the tumorigenic potential of HER-2/neu-overexpressing ovarian cancer cells. In mice bearing HER-2/neu-overexpressing breast cancer xenografts, E1A delivered either by adenovirus or liposome significantly inhibited tumor growth and prolonged mouse survival compared with the controls (Chang *et al.*, Oncogene 14:561 [1997])

Clinical trials have been conducted to evaluate a bispecific antibody which targets the extracellular domains of both the HER-2/neu protein product and Fc gamma RIII (CD16), the Fc gamma receptor expressed by human natural killer cells, neutrophils, and differentiated mononuclear phagocytes (Weiner *et al.*, J. Hematotherapy, 4:471 [1995]).

Overexpression of HER-2 has also been found to be associated with increased resistance to chemotherapy. Thus, patients with elevated levels of HER-2 respond poorly to many drugs. Methods used to inhibit HER-2 expression have been combined with commonly used chemotherapeutic agents (Ueno *et al.*, Oncogone 15:953 [1997]). Combining the adenovirus type 5 gene product, E1A, with taxol showed a synergistic effect in human breast cancer cells. Zhang *et al.*, (Oncogene, 12:571 [1996]) demonstrated that emodin, a tyrosine-specific inhibitor, sensitized non-small cell lung cancer (NSCLC) cells to a variety of chemotherapeutic drugs, including cisplatin, doxorubicin and etoposide. A HER-2 antibody was found to increase the efficacy of tamoxifen in human breast cancer cells (Witters *et al.*, Breast Cancer Res. and Treatment, 42:1 [1997]).

Oligonucleotides have also been used to study the function of HER-2. A triplex-forming oligonucleotide targeted to the HER-2 promoter, 42 to 69 nucleotides upstream of the mRNA transcription start site was found to inhibit HER-2 expression *in vitro* (Ebbinghaus *et al.*, J. Clin. Invest., 92:2433 [1993]). Porumb *et al.* (Cancer Res., 56:515 [1996]) also used a triplex-forming oligonucleotide targeted to the same HER-2 promoter region. Decreases in HER-2 mRNA and protein levels were seen in cultured cells. Juhl *et al.* (J. Biol. Chem., 272:29482 [1997]) used anti-HER-2 ribozymes targeted to a central region of the HER-2 RNA just downstream of the transmembrane region of the protein to demonstrate a reduction in HER-2 mRNA and protein levels in human ovarian cancer cells. A reduction in tumor growth in nude mice was also seen.

An antisense approach has been used as a potential therapeutic for HER-2 overexpressing cancers. Pegues *et al.* (Cancer Lett., 117:73 [1997]) cloned a 1.5 kb fragment of HER-2 in an antisense orientation into an expression vector; transfecting of this construct into ovarian cancer cells resulted in a reduction of anchorage-independent growth. Casalini *et al.* (Int. J. Cancer 72:631 [1997]) used several human HER-2 antisense vector constructs, containing HER-2 fragments from 151 bp to 415 bp in length, to demonstrate reduction in HER-2 protein levels and anchorage-independent growth in lung adenocarcinoma cells. Colomer *et al.* (Br. J. Cancer, 70:819 [1994]) showed that phosphodiester antisense oligonucleotides targeted at or immediately downstream of, the translation initiation codon inhibited proliferation of human breast cancer cells by up to 60%. Wiechen *et al.* (Int. J. Cancer 63:604 [1995]) demonstrated that an 18-nucleotide phosphorothioate oligonucleotide targeted to the coding region, 33 nucleotides downstream of the translation initiation codon, of HER-2 reduced anchorage-independent growth of ovarian cancer cells. Bertram *et al.* (Biochem. Biophys. Res. Commun., 200:661 [1994]) used antisense phosphorothioate oligonucleotides targeted to the translation initiation region and a sequence at the 3' part of the translated region of the mRNA which has high homology to a tyrosine kinase consensus sequence, and demonstrated a 75% reduction in HER-2 protein levels in human breast cancer cells. Liu *et al.*, (Antisense and Nucleic Acid Drug Develop., 6:9 [1996]) used antisense phosphorothioate oligonucleotides targeted to the 5' cap site and coding region. The most effective oligonucleotide, targeted to the 5' cap site, reduced HER-2 protein expression by 90%. Cell proliferation was also reduced by a comparable amount. Vaughn *et al.* (Nuc. Acids. Res., 24:4558 [1996]) used phosphorothioate, phosphorodithioate and chimeric antisense oligonucleotides targeted at or adjacent to (either side) the translation initiation region of HER-2. An alternating dithioate/diester oligonucleotide targeted to the translation initiation region worked slightly better than an all phosphorothioate oligonucleotide. Brysch *et al.* (Cancer Gene Ther., 1: 99

[1994]) used chemically modified antisense oligonucleotides targeted to the translation initiation codon of HER-2 to reduce protein levels and cause growth arrest of human breast cancer cell line.

C. C-Myc

The c-myc gene product is encoded by an immediate early response gene, the expression of which can be induced by various mitogens. The nucleic acid sequence of the promoter region of the c-myc gene is shown in Figure 9. C-myc expression is involved in the signal transduction pathways leading to cell division. Studies have demonstrated that proliferating cells have higher levels of c-myc mRNA and c-myc protein than do quiescent cells. Antibodies directed against the human c-myc protein are known to inhibit DNA synthesis in nuclei isolated from human cells. Conversely, constitutive expression of c-myc produced by gene transfer inhibits induced differentiation of several cell lines. Constitutive expression of c-myc predisposes transgenic mice to the development of tumors.

Some studies have suggested that the c-myc gene product may play a proliferative role in SMCs. Balloon de-endothelialization and injury of rat aortas is known to increase c-myc mRNA expression of vascular SMC prior to their subsequent proliferation and migration. Also, SMCs in culture proliferate when exposed to several mitogens, including PDGF, FGF, EGF, IGF-1 and to serum. Each of these mitogens has been found to be capable of increasing the expression in other cell lines of either c-myc protein, c-myc mRNA, or both. Additionally, blood serum has been found to increase c-myc mRNA levels in SMCs.

Harel-Bellan *et al.* (J. Immun. 140; 2431-2435 (1988)) demonstrated that antisense oligonucleotides complementary to c-myc mRNA effectively inhibited the translation thereof in human T cells. These T cells were prevented from entering the S phase of cell division. c-myc proto-oncogene sequences are described in Marcu *et al.*, Ann. Rev. Biochem., 61:809-860 [1992]; Watt *et al.*, Nature, 303:725-728 [1983)]; Battey *et al.*, Cell, 34:779-787 (1983); and Epstein et al, NTIS publication PB93-100576

D. Bcl2

In many types of human tumors, including lymphomas and leukemias, the human bcl-2 gene is overexpressed, and may be associated with tumorigenicity (Tsujimoto *et al.*, Science 228:1440-1443 [1985]). The nucleic acid sequence of the promoter region of bcl-2 is shown in Figure 1. High levels of expression of the human bcl-2 gene have been found in all lymphomas with t (14; 18) chromosomal translocations including most follicular B cell lymphomas and many large cell non-Hodgkin's lymphomas. High levels of expression of the bcl-2 gene have also been found in certain leukemias that do not have a t(14; 18) chromosomal translation, including most cases of chronic lymphocytic leukemia acute, many lymphocytic leukemias of the pre-B cell type, neuroblastomas, nasophryngeal carcinomas, and many adenocarcinomas of the prostate, breast, and colon. (Reed *et al.*, Cancer Res. 51:6529 [1991]; Yunis *et al.*, New England J. Med. 320:1047; Campos *et al.*, Blood 81:3091-3096 [1993]; McDonnell *et al.*, Cancer Res. 52:6940-6944 [1992); Lu *et al.*, Int. J Cancer 53:29-35 [1993]; Bonner *et al.*, Lab Invest. 68:43A [1993]).

E. TGF-α

Transforming Growth Factor Alpha (TGF-α) is a polypeptide of 50 amino acids. The nucleic acid sequence of the TGF-α promoter is shown in Figure 11. It was first isolated from a retrovirus-transformed mouse cell line and subsequently was identified in human tumor cells, in early rat embryo cells and in cell cultures from the human pituitary gland. TGF-α is closely related to Epidermal Growth Factor (EGF), both structurally and functionally, and both bind to the same receptor, i.e., Epidermal Growth Factor Receptor (EGFR).

The sequence and three dimensional structure of both EGF and TGF-α have been determined (Campbell *et al.*, Prog. Growth Factor Res. 1:13 [1989]). TGF-α is a 50 amino acid polypeptide having about 40% homology of residues with EGF. Both peptides are characterized by three well defined loops (denoted A, B and C) and have three intramolecular disulphide bonds.

Several growth factors, including TGF-α and EGF, are believed to exert their biological effects via interaction with the Epidermal Growth Factor Receptor (EGF Receptor). The EGF Receptor is a Type 1 receptor tyrosine kinase. The EGF Receptor and its ligands are of interest for their roles in normal physiological processes as well as in hyperproliferative and neoplastic diseases.

The *in vivo* precursor of TGF-α is a 160 amino acid residue membrane-bound protein (pro-TGF-.alpha.) that is cleaved to yield a soluble compound (Massague, J. Biol. Chem., 265:21393-21396 [1990]). This cleavage removes an extracellular portion comprised of 50 amino acids with a molecular weight of 6 Kd and is considered to be an important regulatory event (Pandiella *et al.*, Proc. Natl. Acad. Sci. USA, 88:1726-1730

[1990]) that can be stimulated by phorbol esters acting via protein kinase C (Pandiella *et al.*, J. Biol. Chem., 266:5769-5773 [1991]).

Cultured human prostatic tumor lines contain elevated levels of TGF-α mRNA and proliferate in response to TGF-α (Wilding *et al.*, The Prostate, 15:1-12 [1989]). TGF-α appears to have both autocrine and paracrine function, stimulating physiologic activities such as cell division and angiogenesis. When induced in transgenic mice, TGF-α produced epithelial hyperplasia and focal dysplastic changes that resembled carcinoma in situ (Sandgren *et al.*, Cell, 61:1121-1135 [1990]).

F. c-ki-RAS

The c-Ki-RAS (KRAS) oncogene is expressed ubiquitously. KRAS, with a length of more than 30 kb, is much larger than HRAS or NRAS. The sequence of the promoter region of c-ki-ras is shown in Figure 5. Although the 3 ras genes, HRAS, KRAS, and NRAS, have different genetic structures, all code for proteins of 189 amino acid residues, generically designated p21. These genes acquire malignant properties by single point mutations that affect the incorporation of the 12th or 61st amino acid residue of their respective p21. KRAS is involved in malignancy much more often than is HRAS. In a study of 96 human tumors or tumor cell lines in the NIH 3T3 transforming system, (Pulciani *et al.*, *Nature* 300: 539 (1982) found a mutated HRAS locus only in T24 bladder cancer cells, whereas transforming KRAS genes were identified in 8 different carcinomas and sarcomas.

In a serous cystadenocarcinoma of the ovary, Feig *et al.* (*Science* 223: 698 (1984)) showed the presence of an activated KRAS oncogene not activated in normal cells of the same patient. The transforming gene product displayed an electrophoretic mobility in SDS-polyacrylamide gels that differed from the mobility of KRAS transforming proteins in other tumors. Thus, a previously undescribed mutation was responsible for activation of KRAS in this ovarian carcinoma. To study the role of oncogenes in lung cancer, Rodenhuis *et al.* (*New Eng. J. Med.* 317: 929 (1987)) used an assay based on oligonucleotide hybridization following an *in vitro* amplification step. Genomic DNA was examined from 39 tumor specimens obtained at thoracotomy. The KRAS gene was found to be activated by point mutations in codon 12 in 5 of 10 adenocarcinomas. Two of these tumors were less than 2 cm in size and had not metastasized. No HRAS, KRAS, or NRAS mutations were observed in 15 squamous cell carcinomas, 10 large cell carcinomas, 1 carcinoid, 2 metastatic adenocarcinomas from primary tumors outside the lung, and 1 small cell carcinoma. An approximately 20-fold amplification of the unmutated KRAS gene was observed in a tumor that proved to be a solitary lung metastasis of a rectal carcinoma. Yanez *et al.* (*Oncogene* 1:315 (1987)) found mutations in codon 12 of the KRAS gene in 4 of 16 colon cancers, 2 of 27 lung cancers, and 1 of 8 breast cancers; no mutations were found at position 61. Of the 6 possible amino acid replacements in codon 12, all but one were represented in the 7 mutations identified.

G. Other Oncogene Targets

The present invention is not limited to the oncogenes described above. The methods of the present invention are suitable for use with any oncogene with a known promoter region. Exemplary oncogenes included, but are not limited to, BCR/ABL, ABL1/BCR, ABL, BCL1, CD24, CDK4, EGFR/ERBB-1, HSTF1, INT1/WNT1, INT2, MDM2, MET, MYB, MYC, MYCN, MYCL1, RAF1, NRAS, REL, AKT2, APC, BCL2-ALPHA, BCL2-BETA, BCL3, BCR, BRCA1, BRCA2, CBL, CCND1, CDKN1A, CDKN1C, CDKN2A, CDKN2B, CRK, CRK-II, CSF1R/FMS, DBL, DDOST, DCC, DPC4/SMAD4, E-CAD, E2F1/RBAP, ELK1, ELK3, EPH, EPHA1, E2F1, EPHA3, ERG, ETS1, ETS2, FER, FGR, FLI1/ERGB2, FOS, FPS/FES, FRA1, FRA2, FYN, HCK, HEK, HER3/ERBB-2, ERBB-3, HER4/ERBB-4, HST2, INK4A, INK4B, JUN, JUNB, JUND, KIP2, KIT, KRAS2A, KRAS2B, LCK, LYN, MAS, MAX, MCC, MLH1, MOS, MSH2, MYBA, MYBB, NF1, NF2, P53, PDGFB, PIM1, PTC, RB1, RET, ROS1, SKI, SRC1, TAL1, TGFBR2, THRA1, THRB, TIAM1, TRK, VAV, VHL, WAF1, WNT2, WT1, YES1, ALK/NPM1, AMI1, AXL, FMS, GIP, GLI, GSP, HOX11, HST, IL3, INT2, KS3, K-SAM, LBC, LMO-1, LMO-2, L-MYC, LYL1, LYT-10, MDM-2, MLH1, MLL, MLM, N-MYC, OST, PAX-5, PMS-1, PMS-2, PRAD-1, RAF, RHOM-1, RHOM-2, SIS, TAL2, TAN1, TIAM1, TSC2, TRK, TSC1, STK11, PTCH, MEN1, MEN2, P57/KIP2, PTEN, HPC1, ATM, XPA/XPG, BCL6, DEK, AKAP13, CDH1, BLM, EWSR1/FLI1, FES, FGF3, FGF4, FGF6, FANCA, FLI1/ERGB2, FOSL1, FOSL2, GLI, HRAS1, HRX/MLLT1, HRX/MLLT2, KRAS2, MADH4, MAS1, MCF2, MLLT1/MLL, MLLT2/HRX, MTG8/RUNX1, MYCLK1, MYH11/CBFB, NFKB2, NOTCH1, NPM1/ALK, NRG/REL, NTRK1, PBX1/TCF3, PML/RARA, PRCA1, RUNX1, RUNX1/CBFA2T1, SET, TCF3/PBX1, TGFB1, TLX1, P53, WNT1, WNT2, WT1, αv-β3, PKCα, TNFα, Clusterin, Surviving, TGFβ, c-fos, c-SRC, and INT-1.

II. Non-Oncogene Targets

The present invention is not limited to the targeting of oncogenes. 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, 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 somatomedian, 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).

Examples of specific genes include, but are not limited to, ADAMTS4, ADAMTS5, APOA1, APOE, APP, B2M, COX2, CRP, DDX25, DMC1, FKBP8, GH1, GHR, IAPP, IFNA1, IFNG, IL1, Il10, IL12, IL13, IL2, IL4, IL7, IL8, IPW, MAPK14, Mei1, MMP13, MYD88, NDN, PACE4, PRNP, PSEN1, PSEN2, RAD51, RAD51C, SAP, SNRPN, TLR4, TLR9, TTR, UBE3A, VLA-4, and PTP-1B, c-RAF, m-TOR, LDL, VLDL, ApoB-100, HDL, VEGF, rhPDGF-BB, NADs, ICAM-1, MUC1, 2-dG, CTL, PSGL-1, E2F, NF-kB, HIF, and GCPRs.

In other embodiments and gene from a pathogen is targeted. Exemplary pathogens include, but are not limited to, Human Immunodeficiency virus, Hepatitis B virus, hepatitis C virus, hepatitis A virus, respiratory syncytial virus, pathogens involved in severe acute respiratory syndrome, west nile virus, and food borne pathogens (e.g., E. coli).

III. DNA Methylation

In some embodiments, the present invention provides oligonucleotide therapeutics that are methylated at specific sites. 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]).

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-azacytodine 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.

The present invention is not limited to the use of methylated oligonucleotides. Indeed, the use of non-methylated oligonucleotides for the inhibition of gene expression is specifically contemplated by the present invention. Experiments conducted during the course of development of the present invention (See e.g., Example 8) demonstrated 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.

IV. Oligonucleotides

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

A. Oligonucleotide Design

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 50% GC content and at least 2 GC dinucleotides. It is preferred that oligonucleotides do not self hybridize. 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 18-24 nucleotides in length. In some embodiments, oligonucleotides comprise the universal protein binding sequences CGCCC and CGCG or 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).

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 Figures 2, 4, 6, 8, 10, and 12. 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 are shown in Figures 25-30. 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 in Examples 1 and 2 below, candidate oligonucleotides can be evaluated for their ability to prevent cell proliferation as a variety of concentrations. Particularly preferred oligonucleotides are those that inhibit gene expression of cell proliferation as a low concentration (*e.g.*, less that 20 μM, and preferably, less than 10 μM in the *in vitro* assays disclosed herein).

B. Preferred Oligonucleotide Zones

In some embodiments, 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 10 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 200 bp. Based on the above described criteria, exemplary hot zones were designed. These hot zones are shown in Table 1. Numbering is based on the sequences described in the Figures of the present invention.

Table 1

Exemplary Hot Zones

Gene	Hot Zones
Bcl-2	1-40 161-350 401-590 1002-1260
c-erbB-2	205-344 382-435
c-K-ras	1-289 432-658
c-Ha-ras	21-220 233-860 1411-1530 1631-1722
c-myc	3-124 165-629
TGF-α	1-90 175-219 261-367 431-930 964-1237

C. Preparation and Formulation of Oligonucleotides

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.

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 18 to about 30 nucleobases (*i.e.*, from about 18 to about 30 linked bases), although both longer and shorter sequences may find use with the present invention.

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.

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.

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 CH_2 component parts.

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).

In some embodiments, oligonucleotides of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH_2, --NH--O--CH_2--, --CH_2--N(CH_3)--O--CH_2-- [known as a methylene (methylimino) or MMI backbone], --CH_2--O--N(CH_3)--CH_2--, --CH_2--N(CH_3)--N(CH_3)--CH_2--, and --O--N(CH_3)--CH_2--CH_2-- [wherein the native phosphodiester backbone is represented as --O--P--O--CH_2--] 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.

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 C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, 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--$CH_2CH_2OCH_3$, 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'-dimethylaminooxyethoxy (*i.e.*, a $O(CH_2)_2ON(CH_3)_2$ 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--CH_2--O--CH_2--$N(CH_2)_2$.

Other preferred modifications include 2'-methoxy(2'-O--CH_3), 2'-aminopropoxy(2'-$OCH_2CH_2CH_2NH_2$) 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.

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-methylcytosine (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.

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-oxycholesterol moiety.

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.

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

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

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.

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

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

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 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.

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.*, Zuckennann *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).

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].

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. Nad. 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

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. The below discussion provides exemplary, non-limiting examples of formulations and dosages.

A. Pharmaceutical Compositions

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.

Pharmaceutical compositions and formulations for topical administration may include transdermal patches, 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.

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.

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.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, 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.

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.

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.

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.

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), also enhance the cellular uptake of oligonucleotides.

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 anti-inflammatory 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.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. 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. Prefered bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate, sodium glycodihydrofusidate. Prefered 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, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (*e.g.* sodium). Also prefered are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly prefered 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. 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(isohexylcynaoacrylate), 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, and polyethyleneglycol (PEG).

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, 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 and diethylstilbestrol (DES). Anti-inflammatory 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

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 phosphothiolates for delivery (See *e.g.*, U.S. Patent 6,169,177, herein incorporated by reference).

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).

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 (PharmaIn). 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).

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, cardiolipin based cationic liposomes (e.g., NEOPHECTIN, available from NeoPharm, Forest Lake, IL). In some preferred embodiments, the charge ration of NEOPHECTIN to oligonucleotide is 6:1. 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).

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

C. Dosages

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. The administering physician can easily 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 EC_{50}s found to be effective in *in vitro* and *in vivo* animal models or based on the examples described herein. In general, dosage is from 0.01 μg to 100 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 hours to several days or weeks. In some embodiments, treatment is given continuously 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).

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 0.01 μg to 100 g, preferably from 1mg to 50 mg, and even more preferably from 6 mg to 30 mg per kg of body weight, once or more daily, to once every 20 years.

VII. Combination Therapy

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.

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.

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 isirinotecan (CPT-11), *etc.*); 2) covalent DNA-binding agents (alkylating agents), including nitrogen mustards (*e.g.*, mechlorethamine, chlorambucil, cyclophosphamide, ifosphamide, and busulfan (MYLERAN), *etc.*), nitrosoureas (*e.g.*, carmustine, lomustine, and semustine, *etc.*), and other alkylating agents (*e.g.*, dacarbazine, hydroxymethylmelamine, thiotepa, 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 (idamycin), *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-α, *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) proteosome 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.

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 3 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 3

Aldesleukin (des-alanyl-1, serine-125 human interleukin-2)	Proleukin	Chiron Corp., Emeryville, CA
Alemtuzumab (IgG1κ anti CD52 antibody)	Campath	Millennium and ILEX Partners, LP,

		Cambridge, MA
Alitretinoin (9-cis-retinoic acid)	Panretin	Ligand Pharmaceuticals, Inc., San Diego CA
Allopurinol (1,5-dihydro-4 H -pyrazolo[3,4-d]pyrimidin-4-one monosodium salt)	Zyloprim	GlaxoSmithKline, Research Triangle Park, NC
Altretamine (N,N,N',N',N",N",- hexamethyl-1,3,5-triazine-2, 4, 6-triamine)	Hexalen	US Bioscience, West Conshohocken, PA
Amifostine (ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester))	Ethyol	US Bioscience
Anastrozole (1,3-Benzenediacetonitrile, a, a, a', a'-tetramethyl-5-(1H-1,2,4-triazol-1-ylmethyl))	Arimidex	AstraZeneca Pharmaceuticals, LP, Wilmington, DE
Arsenic trioxide	Trisenox	Cell Therapeutic, Inc., Seattle, WA
Asparaginase (L-asparagine amidohydrolase, type EC-2)	Elspar	Merck & Co., Inc., Whitehouse Station, NJ
BCG Live (lyophilized preparation of an attenuated strain of *Mycobacterium bovis* (*Bacillus Calmette-Gukin* [BCG], substrain Montreal)	TICE BCG	Organon Teknika, Corp., Durham, NC
bexarotene capsules (4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-napthalenyl) ethenyl] benzoic acid)	Targretin	Ligand Pharmaceuticals
bexarotene gel	Targretin	Ligand Pharmaceuticals
Bleomycin (cytotoxic glycopeptide antibiotics produced by *Streptomyces verticillus*; bleomycin A_2 and bleomycin B_2)	Blenoxane	Bristol-Myers Squibb Co., NY, NY
Capecitabine (5'-deoxy-5-fluoro-N-[(pentyloxy)carbonyl]-cytidine)	Xeloda	Roche
Carboplatin (platinum, diammine [1,1-cyclobutanedicarboxylato(2-)-0, 0']-,(SP-4-2))	Paraplatin	Bristol-Myers Squibb
Carmustine (1,3-bis(2-chloroethyl)-1-nitrosourea)	BCNU, BiCNU	Bristol-Myers Squibb
Carmustine with Polifeprosan 20 Implant	Gliadel Wafer	Guilford Pharmaceuticals, Inc., Baltimore, MD
Celecoxib (as 4-[5-(4-methylphenyl)-3- (trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide)	Celebrex	Searle Pharmaceuticals, England
Chlorambucil (4-[bis(2chlorethyl)amino]benzenebutanoic acid)	Leukeran	GlaxoSmithKline
Cisplatin ($PtCl_2H_6N_2$)	Platinol	Bristol-Myers Squibb
Cladribine (2-chloro-2'-deoxy-b-D-adenosine)	Leustatin, 2-CdA	R.W. Johnson Pharmaceutical

		Research Institute, Raritan, NJ
Cyclophosphamide (2-[bis(2-chloroethyl)amino] tetrahydro-2H-13,2-oxazaphosphorine 2-oxide monohydrate)	Cytoxan, Neosar	Bristol-Myers Squibb
Cytarabine (1-b-D-Arabinofuranosylcytosine, $C_9H_{13}N_3O_5$)	Cytosar-U	Pharmacia & Upjohn Company
cytarabine liposomal	DepoCyt	Skye Pharmaceuticals, Inc., San Diego, CA
Dacarbazine (5-(3,3-dimethyl-l-triazeno)-imidazole-4-carboxamide (DTIC))	DTIC-Dome	Bayer AG, Leverkusen, Germany
Dactinomycin, actinomycin D (actinomycin produced by *Streptomyces parvullus*, $C_{62}H_{86}N_{12}O_{16}$)	Cosmegen	Merck
Darbepoetin alfa (recombinant peptide)	Aranesp	Amgen, Inc., Thousand Oaks, CA
daunorubicin liposomal ((8S-cis)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-á-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride)	DanuoXome	Nexstar Pharmaceuticals, Inc., Boulder, CO
Daunorubicin HCl, daunomycin ((1 *S* ,3 *S*)-3-Acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl 3-amino-2,3,6-trideoxy-(alpha)-L-*lyxo* -hexopyranoside hydrochloride)	Cerubidine	Wyeth Ayerst, Madison, NJ
Denileukin diftitox (recombinant peptide)	Ontak	Seragen, Inc., Hopkinton, MA
Dexrazoxane ((S)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione)	Zinecard	Pharmacia & Upjohn Company
Docetaxel ((2R,3S)-N-carboxy-3-phenylisoserine, N-tert-butyl ester, 13-ester with 5b-20-epoxy-12a,4,7b,10b,13a-hexahydroxytax- 11-en-9-one 4-acetate 2-benzoate, trihydrate)	Taxotere	Aventis Pharmaceuticals, Inc., Bridgewater, NJ
Doxorubicin HCl (8S,10S)-10-[(3-amino-2,3,6-trideoxy-a-L-lyxo-hexopyranosyl)oxy] -8-glycolyl-7,8,9,10-tetrahydro-6,8,11- trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride)	Adriamycin, Rubex	Pharmacia & Upjohn Company
doxorubicin	Adriamycin PFS Intravenous injection	Pharmacia & Upjohn Company
doxorubicin liposomal	Doxil	Sequus Pharmaceuticals, Inc., Menlo park, CA
dromostanolone propionate (17b-Hydroxy-2a-methyl-5a-androstan-3-one propionate)	Dromostanolone	Eli Lilly & Company, Indianapolis, IN
dromostanolone propionate	Masterone injection	Syntex, Corp., Palo Alto, CA

Elliott's B Solution	Elliott's B Solution	Orphan Medical, Inc
Epirubicin ((8S-cis)-10-[(3-amino-2,3,6-trideoxy-a-L-arabino- hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8- (hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride)	Ellence	Pharmacia & Upjohn Company
Epoetin alfa (recombinant peptide)	Epogen	Amgen, Inc
Estramustine (estra-1,3,5(10)-triene-3,17-diol(17(beta))-, 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate, or estradiol 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate)	Emcyt	Pharmacia & Upjohn Company
Etoposide phosphate (4'-Demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-(beta)-D-glucopyranoside], 4'-(dihydrogen phosphate))	Etopophos	Bristol-Myers Squibb
etoposide, VP-16 (4'-demethylepipodophyllotoxin 9-[4,6-0-(R)-ethylidene-(beta)-D-glucopyranoside])	Vepesid	Bristol-Myers Squibb
Exemestane (6-methylenandrosta-1,4-diene-3, 17-dione)	Aromasin	Pharmacia & Upjohn Company
Filgrastim (r-metHuG-CSF)	Neupogen	Amgen, Inc
floxuridine (intraarterial) (2'-deoxy-5-fluorouridine)	FUDR	Roche
Fludarabine (fluorinated nucleotide analog of the antiviral agent vidarabine, 9-b -D-arabinofuranosyladenine (ara-A))	Fludara	Berlex Laboratories, Inc., Cedar Knolls, NJ
Fluorouracil, 5-FU (5-fluoro-2,4(1H,3H)-pyrimidinedione)	Adrucil	ICN Pharmaceuticals, Inc., Humacao, Puerto Rico
Fulvestrant (7-alpha-[9-(4,4,5,5,5-penta fluoropentylsulphinyl) nonyl]estra-1,3,5-(10)- triene-3,17-beta-diol)	Faslodex	IPR Pharmaceuticals, Guayama, Puerto Rico
Gemcitabine (2'-deoxy-2', 2'-difluorocytidine monohydrochloride (b-isomer))	Gemzar	Eli Lilly
Gemtuzumab Ozogamicin (anti-CD33 hP67.6)	Mylotarg	Wyeth Ayerst
Goserelin acetate (acetate salt of [D-Ser(But)^{6},Azgly^{10}]LHRH; pyro-Glu-His-Trp-Ser-Tyr-D-Ser(But)-Leu-Arg-Pro-Azgly-NH2 acetate [$C_{59}H_{84}N_{18}O_{14}$ •$(C_2H_4O_2)_x$	Zoladex Implant	AstraZeneca Pharmaceuticals
Hydroxyurea	Hydrea	Bristol-Myers Squibb
Ibritumomab Tiuxetan (immunoconjugate resulting from a thiourea covalent 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) -	Zevalin	Biogen IDEC, Inc., Cambridge MA

ethyl]glycine)		
Idarubicin (5, 12-Naphthacenedione, 9-acetyl-7-[(3-amino-2,3,6-trideoxy-(alpha)-L- lyxo -hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxyhydrochloride, (7S- cis))	Idamycin	Pharmacia & Upjohn Company
Ifosfamide (3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide)	IFEX	Bristol-Myers Squibb
Imatinib Mesilate (4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate)	Gleevec	Novartis AG, Basel, Switzerland
Interferon alfa-2a (recombinant peptide)	Roferon-A	Hoffmann-La Roche, Inc., Nutley, NJ
Interferon alfa-2b (recombinant peptide)	Intron A (Lyophilized Betaseron)	Schering AG, Berlin, Germany
Irinotecan HCl ((4S)-4,11-diethyl-4-hydroxy-9-[(4- piperi-dinopiperidino)carbonyloxy]-1H-pyrano[3', 4': 6,7] indolizino[1,2-b] quinoline-3,14(4H, 12H) dione hydrochloride trihydrate)	Camptosar	Pharmacia & Upjohn Company
Letrozole (4,4'-(1H-1,2,4 -Triazol-1-ylmethylene) dibenzonitrile)	Femara	Novartis
Leucovorin (L-Glutamic acid, N[4[[(2amino-5-formyl-1,4,5,6,7,8 hexahydro4oxo6-pteridinyl)methyl]amino]benzoyl], calcium salt (1:1))	Wellcovorin, Leucovorin	Immunex, Corp., Seattle, WA
Levamisole HCl ((-)-(S)-2,3,5, 6-tetrahydro-6-phenylimidazo [2,1-b] thiazole monohydrochloride $C_{11}H_{12}N_2S \cdot HCl$)	Ergamisol	Janssen Research Foundation, Titusville, NJ
Lomustine (1-(2-chloro-ethyl)-3-cyclohexyl-1-nitrosourea)	CeeNU	Bristol-Myers Squibb
Meclorethamine, nitrogen mustard (2-chloro-N-(2-chloroethyl)-N-methylethanamine hydrochloride)	Mustargen	Merck
Megestrol acetate 17α(acetyloxy)- 6- methylpregna- 4,6- diene-3,20- dione	Megace	Bristol-Myers Squibb
Melphalan, L-PAM (4-[bis(2-chloroethyl) amino]-L-phenylalanine)	Alkeran	GlaxoSmithKline
Mercaptopurine, 6-MP (1,7-dihydro-6 H -purine-6-thione monohydrate)	Purinethol	GlaxoSmithKline
Mesna (sodium 2-mercaptoethane sulfonate)	Mesnex	Asta Medica
Methotrexate (N-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-glutamic acid)	Methotrexate	Lederle Laboratories
Methoxsalen	Uvadex	Therakos, Inc., Way

(9-methoxy-7H-furo[3,2-g][1]-benzopyran-7-one)		Exton, Pa
Mitomycin C	Mutamycin	Bristol-Myers Squibb
mitomycin C	Mitozytrex	SuperGen, Inc., Dublin, CA
Mitotane (1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane)	Lysodren	Bristol-Myers Squibb
Mitoxantrone (1,4-dihydroxy-5,8-bis[[2- [(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione dihydrochloride)	Novantrone	Immunex Corporation
Nandrolone phenpropionate	Durabolin-50	Organon, Inc., West Orange, NJ
Nofetumomab	Verluma	Boehringer Ingelheim Pharma KG, Germany
Oprelvekin (IL-11)	Neumega	Genetics Institute, Inc., Alexandria, VA
Oxaliplatin (cis-[(1R,2R)-1,2-cyclohexanediamine-N,N'] [oxalato(2-)-O,O'] platinum)	Eloxatin	Sanofi Synthelabo, Inc., NY, NY
Paclitaxel (5ß, 20-Epoxy-1,2a, 4,7ß, 10ß, 13a-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R, 3 S)- N-benzoyl-3-phenylisoserine)	TAXOL	Bristol-Myers Squibb
Pamidronate (phosphonic acid (3-amino-1-hydroxypropylidene) bis-, disodium salt, pentahydrate, (APD))	Aredia	Novartis
Pegademase ((monomethoxypolyethylene glycol succinimidyl) 11 - 17 -adenosine deaminase)	Adagen (Pegademase Bovine)	Enzon Pharmaceuticals, Inc., Bridgewater, NJ
Pegaspargase (monomethoxypolyethylene glycol succinimidyl L-asparaginase)	Oncaspar	Enzon
Pegfilgrastim (covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol)	Neulasta	Amgen, Inc
Pentostatin	Nipent	Parke-Davis Pharmaceutical Co., Rockville, MD
Pipobroman	Vercyte	Abbott Laboratories, Abbott Park, IL
Plicamycin, Mithramycin (antibiotic produced by *Streptomyces plicatus*)	Mithracin	Pfizer, Inc., NY, NY
Porfimer sodium	Photofrin	QLT Phototherapeutics, Inc., Vancouver, Canada
Procarbazine (N-isopropyl-μ-(2-methylhydrazino)-p-toluamide monohydrochloride)	Matulane	Sigma Tau Pharmaceuticals, Inc., Gaithersburg, MD
Quinacrine	Atabrine	Abbott Labs

(6-chloro-9-(1 –methyl-4-diethyl-amine) butylamino-2-methoxyacridine)		
Rasburicase (recombinant peptide)	Elitek	Sanofi-Synthelabo, Inc.,
Rituximab (recombinant anti-CD20 antibody)	Rituxan	Genentech, Inc., South San Francisco, CA
Sargramostim (recombinant peptide)	Prokine	Immunex Corp
Streptozocin (streptozocin 2 –deoxy - 2 - [[(methylnitrosoamino)carbonyl]amino] - a(and b) - D - glucopyranose and 220 mg citric acid anhydrous)	Zanosar	Pharmacia & Upjohn Company
Talc ($Mg_3Si_4O_{10}(OH)_2$)	Sclerosol	Bryan, Corp., Woburn, MA
Tamoxifen ((Z)2-[4-(1,2-diphenyl-1-butenyl) phenoxy]-N, N-dimethylethanamine 2-hydroxy-1,2,3-propanetricarboxylate (1:1))	Nolvadex	AstraZeneca Pharmaceuticals
Temozolomide (3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-as-tetrazine-8-carboxamide)	Temodar	Schering
Teniposide, VM-26 (4'-demethylepipodophyllotoxin 9-[4,6-0-(R)-2-thenylidene-(beta)-D-glucopyranoside])	Vumon	Bristol-Myers Squibb
Testolactone (13-hydroxy-3-oxo-13,17-secoandrosta-1,4-dien-17-oic acid [dgr]-lactone)	Teslac	Bristol-Myers Squibb
Thioguanine, 6-TG (2-amino-1,7-dihydro-6 H - purine-6-thione)	Thioguanine	GlaxoSmithKline
Thiotepa (Aziridine, 1,1',1"-phosphinothioylidynetris-, or Tris (1-aziridinyl) phosphine sulfide)	Thioplex	Immunex Corporation
Topotecan HCl ((S)-10-[(dimethylamino) methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3', 4': 6,7] indolizino [1,2-b] quinoline-3,14-(4H,12H)-dione monohydrochloride)	Hycamtin	GlaxoSmithKline
Toremifene (2-(p-[(Z)-4-chloro-1,2-diphenyl-1-butenyl]-phenoxy)-N,N-dimethylethylamine citrate (1:1))	Fareston	Roberts Pharmaceutical Corp., Eatontown, NJ
Tositumomab, I 131 Tositumomab (recombinant murine immunotherapeutic monoclonal IgG_{2a} lambda anti-CD20 antibody (I 131 is a radioimmunotherapeutic antibody))	Bexxar	Corixa Corp., Seattle, WA
Trastuzumab (recombinant monoclonal IgG_1 kappa anti-HER2 antibody)	Herceptin	Genentech, Inc
Tretinoin, ATRA (all-trans retinoic acid)	Vesanoid	Roche
Uracil Mustard	Uracil Mustard Capsules	Roberts Labs
Valrubicin, N-trifluoroacetyladriamycin-14-	Valstar	Anthra --> Medeva

valerate ((2S-cis)-2- [1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy-7 methoxy-6,11-dioxo-[[4 2,3,6-trideoxy-3- [(trifluoroacetyl)-amino-α-L-*lyxo*-hexopyranosyl]oxyl]-2-naphthacenyl]-2-oxoethyl pentanoate)		
Vinblastine, Leurocristine ($C_{46}H_{56}N_4O_{10}\bullet H_2SO_4$)	Velban	Eli Lilly
Vincristine ($C_{46}H_{56}N_4O_{10}\bullet H_2SO_4$)	Oncovin	Eli Lilly
Vinorelbine (3',4'-didehydro-4'-deoxy-C'-norvincaleukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)])	Navelbine	GlaxoSmithKline
Zoledronate, Zoledronic acid ((1-Hydroxy-2-imidazol-1-yl-phosphonoethyl) phosphonic acid monohydrate)	Zometa	Novartis

VIII. Customized Patient Care

In some embodiments, the present invention provides customized patient care. The compositions of the present invention are targeted to specific genes unique to a patient's diseae (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.

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.

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 is utilized. In some embodiments, the analysis is performed by the clinical laboratory analyzing the patient sample and is transmitted to a second provider for formulation of the treatment cocktail. In some embodiments, the information is transmitted over the Internet, thus allowing for the shortest possible time in between diagnosis and the beginning of treatment.

A. Detection of RNA

In some embodiments, detection of oncogenes (*e.g.*, including but not limited to, those disclosed herein) is detected by measuring the expression of corresponding mRNA in a tissue sample (*e.g.*, cancer tissue). In other embodiments, expression of mRNA is measured in bodily fluids, including, but not limited to, blood, serum, mucus, and urine. In some preferred embodiments, the level of mRNA expression in measured quantitatively. RNA expression may be measured by any suitable method, including but not limited to, those disclosed below.

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. 5,846,717, 6,090,543; 6,001,567; 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 hybridization of overlapping oligonucleotide probes.

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 AMPLITAQ GOLD DNA polymerase. A probe consisting of an oligonucleotide with a 5'-reporter dye (*e.g.*, a fluorescent dye) and a 3'-quencher dye is included in the PCR reaction. During PCR, if the probe is bound to its target, the 5'-3' nucleolytic activity of the AMPLITAQ GOLD polymerase cleaves the probe between the reporter and the quencher dye. The separation of the reporter dye from the quencher dye results in an increase of fluorescence. The signal accumulates with each cycle of PCR and can be monitored with a fluorimeter.

In yet other embodiments, reverse-transcriptase PCR (RT-PCR) is used to detect the expression of RNA. In RT-PCR, RNA is enzymatically converted 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 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.

B. Detection of Protein

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. In other embodiments, 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.

Antibody binding is detected by techniques known in the art (*e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (*e.g.*, using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.

In one embodiment, antibody binding is detected by detecting a label on 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 binding in an immunoassay and are within the scope of the present invention.

In some embodiments, an automated detection assay is 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, software that generates an expression profile based on the presence or absence of a series of proteins corresponding to oncogenes is utilized.

In other embodiments, the immunoassay described in U.S. Patents 5,599,677 and 5,672,480; each of which is herein incorporated by reference.

EXPERIMENTAL

The following 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.

In the experimental disclosure which follows, the following abbreviations apply: N (normal); M (molar); mM (millimolar); μM (micromolar); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); pmol (picomoles); g (grams); mg (milligrams); μg (micrograms); ng (nanograms); l or L (liters); ml (milliliters); μl (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); and ^{O}C (degrees Centigrade).

Example 1

Materials and Methods

This Example describes experimental methods utilized in the below examples.

A. Cell Lines

Cell lines used in experiments of the present invention are described below.

MDA-MB-231

Tissue:	adenocarcinoma; mammary gland; breast; pleural effusion
Tumorigenic:	forms adenocarcinoma grade III
Receptors expressed:	Epidermal Growth Factor (EGF) and Transforming growth factor (**TGF-alpha**)
Oncogene:	wnt3 + and wnt7h +
References:	

Siciliano MJ, Barker PE, Cailleau R. Mutually exclusive genetic signatures of human breast tumor cell lines with a common chromosomal marker.
Cancer Res. 1979 Mar;39(3):919-22.

Calleau R, Olive M, Cruciger QV. Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization.
In vitro. 1978 Nov;14(11):911-5.

Cruciger QV, Pathak S, Calleau R. Human breast carcinomas: marker chromosomes involving 1q in seven cases.
Cytogenet Cell Genet. 1976;17(4):231-5.

Satya-Prakash KL, Pathak S, Hsu TC, Olive M, Cailleau R.
Cytogenetic analysis on eight human breast tumor cell lines: high frequencies of 1q, 11q and HeLa-like marker chromosomes. Cancer Genet Cytogenet 1981 Jan;3(1):61-73

MCF7

Tissue: adenocarcinoma, mammary gland, breast

Metastatic site: pleural effusion

Receptors: estrogen receptor +

Oncogenes: wnt7h +

This cell line is also known to moderately express c-**erbB-2** and overexpress **c-myc** oncogene

Cellular product: Insulin like growth factor binding protein (IGFBP)

References:

Soule HD *et al.* Ahuman cell line from a pleural effusion derived from a breast carcinoma. J. Natl. Cancer Inst. 51: 1409-1416, 1973

Landers JE *et al.* Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. Cancer Res. 57:

3562-3568, 1997

Bacus SS *et al.* Differentiation of cultured human cancer cells (AU-565 and MCF7) associated with loss of cell surface HER-2/neu oligonucleotide. Mol. Carcinog. 3: 350-362, 1990

MCF10CA1

MCF10 cells are derived from benign breast tissue from a woman with fibrocystic disease. MCF10 lines consists of several lines, one is MCF10A, an immortalized normal human breast cell line. MCF10A was transformed with T24 Ha-ras to make MCF10AneoT cells. MCF10AT with neoplastic progression potential was derived from xenograft passaged MCF10-AneoT. MCF10AT generates carcinoma in about 25% of xenografts. Fully malignant MCF10CA1 lines were derived from several xenograft passages of MCF10AT. MCF10CA1a forms tumors 100% of the time and it metastasizes. A kariotype of MCF10CA1a shows an extra copy of chromosome 1. It metastasizes into the lung 36 days after IV injection of the cells.

References:

Santner SJ *et al.* Malignant MCF10CA1 cell lines derived from premalignant human breast epithelial MCF10AT cells. Breast Cancer Research and treatment 65: 101-110, 2001.

MYC-MT-1

A female MMTV-C-MYC transgenic mouse developed a mammary tumor. The tumor was isolated and a small fresh tissue is put into culture with a medium conditioned by Dr. Jushoa Liao at Karmanos Cancer Institute. This tumor cell line was established after 10 passages.

NMuMG

Tissue:	Mouse normal mammary gland, epithelial
Strain:	NAMRU, female
Tumorigenic:	produce benign tumor in mice

References:

Owens RB. Glandular epithelial cells from mice: a method for selective cultivation. J. Natl. Cancer Inst. 52: 1375-1378, 1974

Owens RB *et al.* Epithelial cell cultures from normal glandular tissue of mice. J. Natl. Cancer Inst. 53: 261-269, 1974

Yingling JM *et al.* Mammalian dwarfins are phosphorylated in response to transforming growth factor beta and are implicated in control of cell growth. Proc. Natl. Acad. Sci. USA 93: 8940-8944, 1996

BxPC-3

Tissue: adenocarcinoma, pancreas

Cellular product: mucin, pancreatic cancer specific antigen; CEA, carcinoma embryonic antigen.

Source: 61 year old female

Tumorigenic: yes

Oncogenes: c-Ki-ras

References:

Tan MH *et al.* Characterization of a new primary human pancreatic tumor line. Cancer Invest. 4: 15-23, 1986

Loor R *et al.* Use of pancreas-specific antigen in immunodiagnosis of pancreatic cancer. Clin. Lab. Med. 2: 567-578, 1982

Lan MS *et al.* Polypeptide core of a human pancreatic tumor mucin antigen. Cancer Res. 50: 2997-3001, 1990

Chambers JA and Harris A. Expression of the cystic fibrosis gene and the major pancreatic mucin gene, MUC1, in human ductal epithelial cells. J. Cell Sci. 105: 417-422, 1993

T-47D

Tissue: ductal carcinoma, mammary gland, breast, duct

Metastatic site: pleural effusion

Source:	pleural effusion of a 54 years old female with infiltrating ductal carcinoma of the breast
Receptor expression:	estrogen, androgen, calcitonin, progesteron, glucocorticoid and prolactin positive.
Oncogenes:	wnt3+ and wnt7h+ This cell line is also know to overexpress **c-erbB-2**

References:

Keydar I *et al.*, Establishment and characterization of a cell line of human breast carcinoma origin. Eur. J. Cancer 15: 659-670, 1979

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Sher E *et al.* Whole-cell uptake and nuclear localization of 1,25-dihydroxy-cholecalciferol by breast cancer cells **(T-47D)** in culture. Biochem. J. 200: 315-320, 1981

Freake HC *et al.* 1,25-Dihydroxyvitamin D3 specifically binds to a human breast cancer cell line **(T-47D)** and stimulates growth. Biochem. Biophys. Res. Commun. 101: 1131-1138, 1981

Faust JB and Meeker TC. Amplification and expression of the bcl-1 gene in human solid tumor cell lines. Cancer Res. 52: 2460-2463, 1992 RF33514:

Huguet EL *et al.* Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue. Cancer Res. 54: 2615-2621, 1994

BT-474

Tissue: ductal carcinoma, mammary gland, breast

Source: 60 year old female

Oncogene: c-erbB-2

References:

Lasfargues EY *et al.* Isolation of two human tumor epithelial cell lines from solid breast carcinomas. J. Natl. Cancer Inst. 61: 967-978, 1978

Lasfargues EY *et al.* A human breast tumor cell line **(BT-474)** that supports mouse mammary tumor virus replication. *In vitro* 15: 723-729, 1979

Littlewood-Evans AJ *et al.* The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. Cancer Res. 57: 5386-5390, 1997

WSU-FSCCL

Human B- cell line established in 1993

Source: from peripheral blood of a male patient with low grade follicular small cleaved cell lymphoma in leukemic phase.

Oncogenes: exhibiting chromosomal translocation for both **c-myc** and **bcl-2**

Reference:

Mohammad RM, Mohamed AN, Smith MR, Jawadi NS, AL-Khatib A. A unique EBV-Negative Low Grade Lymphoma Line (WSU-FSCCL) Exhibiting both t(14;18) and t(8;11). Cancer Genet Cytogenet 70:62-67, 1993

B. Cell Culture

Human breast cancer cells, MCF7, MCF10CAla, MDA-MB 231, MDA-MB 435.eB, and human normal breast cells, MCF10A were all 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. 5% horse serum and insulin (10 μg/ml) was used for MCF10Cala, and 10% fetal calf serum was used for MDA-MB 231 and 435 lines. MCF 1.0A culture was supplemented with 5% horse serum, insulin. (10 μg/ml), 100 ng/ml cholera enterotoxin (Calbiochem, CA), 0.5 μg/ml hydrocortisone (Sigma Chemical) and 20 ng/ml epidermal growth factor (Sigma Chemical). All flasks and plates were incubated in a humidified atmosphere of 95% air and 5% $C0_2$ at 37°C.

MYC-MT-1 cells were also cultured in DMEM/F12 media containing 10 ng/ml EGF (epithelial growth factor), 1 nM estradiol, 10 μg/ml insulin and 10% FBS (fetal bovine serum). BxPC-3 pancreatic carcinoma cell line and BT-474, breast tumor cell line were cultured in RPMI 1640 with 10% FBS. Breast tumor cell line, T-47D was cultured in the same media as BT-474 with the addition of 2.5 μg/ml insulin. 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.

All the above cells were seeded at 2500 to 5,000 cells/well in 96 well plates. The cells were treated with oligonucleotide compounds in fresh media (100 μl 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.

Human follicular lymphoma cell line, WSU-FSCCL was used to evaluate the effect of antic-myc oligonucleotides as well as anti-Bcl-2 oligonucleotides. FSCCL cells grow as a single cell suspension in tissue culture. The culture was maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 1% L-glutamine, 100 units/ml penicillin and 1.00 μg/ml streptomycin. FSCCL cells were treated in 24 well plates ($2x10^5$ cells/well/ml) with oligonucleotide compounds and incubated in a humidified atmosphere of 95% air and 5% $C0_2$ at 37°C. The cells were counted every 24 hours using a hemocytometer.

C. Oligonucleotide Preparation

All oligonucleotides were synthesized, gel purified anal 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.

D. Lipofectin Encapsulation

20 μg lipofectin (Invitrogen) and 16 μg oligonucleotides were each incubated with 200 μl Opti-MEM (Invitrogen) media in separate sterile tubes at room temperature for 45

min. They were then combined and incubated for an additional 15 min. 1.6 ml Opti-MEM media was then added to a final volume of 2 ml and a final concentration 1 μM oligonucleotide. The concentration of lipofectin and oligonucleotides can be adjusted based on their molecular weight and desired concentration of compounds. There was no cytotoxic effect at this level.

E. Cell Growth Inhibition Assay

Cell growth inhibition was assessed using 3-[4,5-Dimethyl-thiazol-2-yl]-2,5diphenyltetrazolium bromide (MTT) purchased from Sigma Chemical (St. Louis, MO). Cells were resuspended in culture media at 50,000 cells /ml and 100 μl 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 μl 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 μl 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 μl 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.

F. Protein Extraction and Western Blot Analysis

The cells were seeded and cultured in T25 tissue culture flasks (Costar, Corning, NY, USA) at 200,000 cells/flask. The cells were allowed to attach for 24 hours. The media was replaced with fresh media containing 10 to 20 μM oligonucleotides and incubated for 24 hours. The media was changed every 48 hours without further addition of inhibitors and cell cultures were continued until the control flasks were confluent (6-7 days). Cells were harvested using 1x trypsin:EDTA (Invitrogen, Gibco, MD) and collected by centrifugation at 2000 rpm for 5 min. Cells were resuspended in 125 mM Tris-HCL buffer (pH 6.8), sonicated with 10-20% output and lysed in an equal volume of 8% SDS for a final concentration of 4% SDS. Cells extracts were boiled for 10 min, chilled on ice and centrifuged at 2,000 rpm for 5 min before collecting the supernatant. The protein was quantitated using BCA protein assay kit (Pierce, Rockford, IL). 50 to 100 μg of proteins were subjected to 10 to 15% gel (depending on molecular weight of each protein) electrophoresis and transferred to nitrocellulose membrane (Schleicher & Schuell, Kence, NH). Each membrane was blocked with 10% dry milk in TBSTe (Tris buffered saline, Tween 20) for 2 hr, prior to incubation with primary antibodies in TBST overnight. Antibodies to human c-myc, c-ha-ras and erbB-2 were mice IgG (Pharmingen San Diego, CA). Membranes were washed 3 times, 15 min each in TBST, then incubated with secondary antibodies conjugated with peroxidase for 1 hr. The membranes were washed 5 times, 10 min each in TBST and incubated with 2 ml each of Lumino/Enhancer and Stable peroxide solution (PIERCE) for 1 min. The membranes were exposed to X-ray film for 2 min (exposure time is adjusted from 10 seconds up to 24 hr if necessary).

Example 2

c-ki-RAS

This example describes the ability of oligonucleotide compounds targeted against the promoter of the c-ki-Ras gene to inhibit the growth of cancer cell lines. Experiments were performed as described in Example 1. The results are shown in Figures 13 and 19. The sequences of the oligonucleotides targeted against c-ki-Ras as well as the sequence of c-ki-Ras gene are shown in Figures 5 and 6.

Example 3

Bcl-2

This example describes the ability of oligonucleotide compounds targeted against the promoter of the bcl-2 gene to inhibit the growth of cancer cell lines. Experiments were performed as described in Example 1. The results are shown in Figures 14 and 20. The sequences of the oligonucleotides targeted against bcl-2 as well as the sequence of bcl-2 gene are shown in Figures 1 and 2.

Example 4

c-ha-RAS

This example describes the ability of oligonucleotide compounds targeted against the promoter of the c-ha-Ras gene to inhibit the growth of cancer cell lines. Experiments were performed as described in Example 1. The results are shown in Figures 16 and 22.

The sequences of the oligonucleotides targeted against c-ha-Ras as well as the sequence of c-ha-Ras gene are shown in Figures 7 and 8.

Example 5

c-erbB-2

This example describes the ability of oligonucleotide compounds targeted against the promoter of the c-erbB-2 gene to inhibit the growth of cancer cell lines. Experiments were performed as described in Example 1. The results are shown in Figures 15 and 21. The sequences of the oligonucleotides targeted against c-erbB-2 as well as the sequence of c-erbB-2 gene are shown in Figures 3 and 4.

Example 6

c-myc

This example describes the ability of oligonucleotide compounds targeted against the promoter of the c-myc gene to inhibit the growth of cancer cell lines. Experiments were performed as described in Example 1. The results are shown in Figures 17 and 23. The sequences of the oligonucleotides targeted against c-myc as well as the sequence of c-myc gene are shown in Figures 9 and 10.

Example 7

TGF-α

This example describes the ability of oligonucleotide compounds targeted against the promoter of the TGF-α gene to inhibit the growth of cancer cell lines. Experiments were performed as described in Example 1. The results are shown in Figures 18 and 24. The sequences of the oligonucleotides targeted against TGF-α as well as the sequence of TGF-α gene are shown in Figures 11 and 12.

Example 8

Inhibition of cell growth by Non-methylated oligonucleotides

This example describes the inhibition of growth of lymphoma cell lines by non-methylated oligonucleotides targeted towards Bcl-2. WSU-FSCCL cells were plated in 24 well plates at $2x10^5$ cells /well at t=-24hr. For each time point to be harvested, triplicate wells were treated at t=0 with the oligos at the concentrations indicated. Controls were plated in triplicate. Plates were incubated at 37°C. All cultures were monitored through out the experiment by cell count and viability every 24 hr for 4 days using trypan blue stain and hemacytometer.

The MABL2 oligonucleotide is targeted to the promoter region of Bcl-2 [5'- CAX GCA XGX GCA TCC CXG CCX GTG -3' (SEQ ID NO: 3)]. Pho-Mabl-2 is an unmethylated version of MABL-2 [5'- CAC GCA CGC GCA TCC CCG CCC GTG -3' (SEQ ID NO: 1438)]. WSU-FSCCL – derived from human B cell lymphoma (low-grade follicular small-cleaved cell lymphoma). The experimental protocol is shown in Table 2.

Table 2

Group	Target Gene	Compound	Cells	Conc.	Formulation	Viability Assay	Harvest for Methyl
1	Bcl-2	MABL2	FSCCL	10uM	none	n=3 @ 24, 48 & 72 hr	n=3 @ 72 hr
2	Bcl-2	MABL2	FSCCL	3uM	none	n=3 @ 24, 48 & 72 hr	n=3 @72 hr
3	Bcl-2	PhoMABL2	FSCCL	10uM	none	n=3 @ 24, 48 & 72 hr	n=3 @72 hr
4	none	none	FSCCL	n/a	none	n=3 @ 24, 48 & 72 hr	n=3 @72 hr

The results are shown in Figure 31. The results demonstrate that the unmethylated oligonucleotide directed against Bcl-2 is as effective as the methylated oligonucleotide in inhibiting cell growth.

Example 9

In Vivo Inhibition of Tumor Growth

This example describes the inhibition of tumor growth by oligonucleotides of the present invention in a human prostate carcinoma model.

Animals: The human PC-3 GFP prostate carcinoma subcutaneous model was utilized (See e.g., Yang et al., Cancer Research 59, 781-786, [1999]; Glinskii et al., Cancer Research 63, 4239-4243, [2003]; and Kalikin et al., Cancer Biology and Therapy 2:6, 17-21 [2003]). Male athymic NCr nude mice between 5 and 6 weeks of age were used. The animals were bred and maintained in a HEPA filtered environment with cages, food and bedding sterilized by autoclaving. The breeding pairs were obtained from Taconic Quality Laboratory Animals and Services for Research (Germantown, NY). The animal diets (5010 autoclavable rodent diet) were obtained from PMI nutrition International Inc. (Brentwood, MO). A total of 60 male animals were used for the study.

Study drugs: Nucleic acid based oligo compound PNT100 and scrambled oligo control PNT-C with cationic liposomal delivery (LDV) formulation.

GFP expression vector: pLEIN was purchased from Clontech (Palo Alto, CA). The vector expresses enhanced GFP and the neomycin resistance gene on the same bicistronic message that contains an internal ribosome entry site.

Cell culture, vector production, transfection, and subcloning: PT67, an NIH3T3 derived packaging cell line, expressing the 10 AI viral envelopes, was purchased from Clontech. PT67 cells were cultured in DMEM supplemented with 10% fetal bovine serum. For vector production, packaging cells (PT67), at 70% confluence, were incubated with a precipitated mixture of N- [1- (2 ,3 - dioleoyloxyl) propyl]-N, N, trimethylammoniummethyl sulfate reagent and saturating amounts of pLEIN plasmid for 18 h. Fresh medium was replenished at this time. The cells were examined by fluorescence microscopy 48 h posttransfection. For selection, the cells were cultured in the presence of 200-1000 μg/ml G418 for 7 days.

GFP gene transduction of PC-3-GFP cells: For GFP gene fransduction, 20% confluent PC-3 cells (ATCC, CRL 1435) were incubated with a 1:1 precipitated mixture of retroviral supernatants of PT67 cells and Ham's F-12 K containing 7% fetal bovine serum for 72 h. Fresh medium was replenished at this time. PC-3 cells were harvested by trypsin EDTA 72 h posttransduction and subcultured at a ratio of 1:15 into selective medium that contained 200 μg/ml 6418. The level of 6418 was increased to 1000 Ng/ml stepwise. The brightest PC-3 cell clones expressing GFP were selected, combined, and then amplified and transferred by conventional culture methods.

Subcutaneous Tumor Growth: Tumor stocks were made by subcutaneously injecting PC-3-GFP cells at the concentration of 5 x 10^6 cells /200 μl into the flank of nude mice. The strong GFP expression of tumors grown in the subcutis of mice was certified before harvest. The tumor tissues harvested from subcutaneous growth in nude mice were inspected, and any grossly necrotic or suspected necrotic or non GFP tumor tissues were removed. Tumor tissues were subsequently cut into small fragments of approximately 2 mm^3.

Subcutaneous tissue fragment implantation: Tumor stock of the prostate cancer PC-3 GFP was established by subcutaneously injecting PC-3 GFP cells to the flank of nude mice. The tumor was maintained in nude mice subcutaneously as tumor stock prior to use. Before implantation, strong GFP expression of the PC-3 GFP tumor tissue was confirmed by the fluorescent light. On the day of implantation, the tumor was harvested from the subcutaneous site and placed in RPMI-1640 medium. Necrotic tissues were removed and viable tissues were cut into 2 mm^3 pieces. The tissue fragments were then implanted subcutaneously to right flank of the nude nice.

Whole body optical imaging of green fluorescent protein expressing tumors and metastases: A Leica stereo fluorescence microscope model LZ12 equipped with a mercury lamp power supply was used. Selective excitation of GFP was produced through a D425/60 band pass filter and 470 DCXR dichroic mirror. Emitted fluorescence was collected through a long pass filter GG475 (Chroma Technology, Brattleboro, VT) on a ST-133 Micromax High Speed TEA/CCD-1317K1 thermoelectrically cooled camera (Princeton Instruments, Trenton, NJ), with a 1317x1035 pixel chip. Experiments were controlled and images were processed for contrast and brightness and analyzed with the help of Image Pro Plus 3.1 software (Media Cybernetics, Silver Spring, Maryland). High resolution images were captured directly on the computer or continuously through video output.

Study animals: A total of 60 mice used for the study were divided into 6 groups 12 days after surgery. Groups for each of the cohort conditions were randomly chosen.

Treatment Initiation: When primary tumors reach between 50-100 mm^3 estimated volume.

The study design is shown in Table 4.

Table 4

Group ID	Subgroup ID	Description	Dose (mg/kg)	Schedule	Route	N
1	A	PBS Control	200 μl	qd X 5	s.c	10
1	B	PNT-C (5'-NNNNNNNNNNNNNNNNNNNNNNNNNN-3'; SEQ ID NO:1439) + LDV	5	qd X 5	s.c.	10

1	C	PNT-100 (PhoMabl2; SEQ ID NO:1438) + LDV	2.5	qd X 5	s.c.	10
1	D	PNT-100 + LDV	5	qd X 5	s.c.	10
1	E	TAXOTERE	10 and 5	Day 2 and 5	i.v.	10
1	F	TAXOTERE + PNT-100/LDV	10 and 5 + 5	Day 2 and 5 + qd X 5	i.v. + s.c.	10

Data collection:

Tumor sizes: Each animal was checked once a week for tumor growth by caliper measurements until the end of the study. Measurements over a 40 days period were taken to calculate tumor volume response over time. An approximate tumor volume was calculated using the formula 1/2 (a x b), where b is the smaller of two perpendicular diameters. Approximate tumor volume was calculated by the formula (Wx L) x 1/2.

GFP Imaging: 12 days after implantation, whole body optical imaging of GFPexpressing tumors was taken once a week.

Body weights: Body weights for all animals was weighed once per week for the duration of the study. An electronic balance was used to measure the body weight.

Termination: The final tumor weights were acquired after animals were sacrificed at the fortysixth day of the study. Each tumor was weighed using an electronic balance.

Statistical methods used in efficacy evaluation: Tumor volumes and final tumor weights of all 6 groups were analyzed using the Student's t^2 test with an = 0.05 (twosided).

Results: The results are shown in Figures 32-35. Figure 32 shows mean tumor volume of tumors in the PC-3 GFP prostate carcinoma subcutaneous model following treatment with PNT-100 and/or TAXOTERE. Figure 33 shows mean body weight of tumors in the PC-3 GFP prostate carcinoma subcutaneous model following treatment with PNT-100 and/or TAXOTERE. Figure 34 shows mean tumor volume of tumors in the PC-3

GFP prostate carcinoma subcutaneous model following treatment with PNT-100 and/or TAXOTERE. Figure 35 shows mean final tumor volume of tumors in the PC-3 GFP prostate carcinoma subcutaneous model following treatment with PNT-100 and/or TAXOTERE. The results indicate that PNT-100 decreased tumor size. The effect was increased in the presence of TAXOTERE.

Example 10

In Vivo Inhibition of Tumor Growth

This example describes the inhibition of tumor growth by in vivo delivery of oligonucleotides of the present invention in a human prostate carcinoma model.

I) PC-3 prostate Xenograft tumor response to PNT100 administered i.v. with randomized xenografts at different vascularization states was investigated. This experiment was performed with the two step Neophectin formulation with five daily doses of 1 mg/kg PNT100. All mice survived the dosing regiment without noticeable toxicity responses.

II) WSU-DLCL2 Xenograft i.v. PNT100 study: A second study was performed to establish i.v. delivery and efficacy of PNT100-Neophectin in a non-Hodgkin's-lymphoma model (NHL). The study was designed to administer five daily doses of 5 mg/kg PNT100, and in certain cohorts, combination therapy with vincristine. After one dose of PNT100, noticeable weight loss in the animals injected with PNT100 and PNT-C (Scrambled control) was observed. The data shows substantial effect of combination therapy with PNT100 and PNT-C. Results showing tumor burden 20 days post WSU-DLCL2 transplantation indicate that PNT100, alone and in combination with vincristine, shrinks tumors in mice.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

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The claims defining the invention are as follows:

1. A composition comprising an oligonucleotide that hybridizes under physiological conditions to a promoter region of a bcl-2 gene, wherein said first oligonucleotide is SEQ ID NO: 1438 and complements thereof.

2. The composition of claim 1, further comprising an additional oligonucleotide.

3. The composition of claim 2, wherein said additional oligonucleotide is selected from the group consisting of SEQ ID NOs: 2, 3, 7, and complements thereof.

4. The composition of claim 2, wherein said additional oligonucleotide hybridizes to a promoter region of a gene is selected from the group consisting of c-ki-Ras, c-Ha-Ras, c-myc, Her-2, and TGF-α.

5. A method, comprising

a) providing

i) a composition of any one of claims 1–4; and

ii) a cell capable of expressing a bcl-2 gene, wherein said cell is capable of proliferation; and

b) introducing said oligonucleotide to said cell, wherein said administering results in a reduction of proliferation of said cell.

6. The method of claim 5, wherein said cell is a cancer cell selected from the group consisting of pancreatic cancer, colon cancer, breast cancer, bladder cancer, lung cancer, leukemia, prostate cancer, lymphoma, ovarian cancer, and melanoma.

7. The method of claim 5, wherein said cell is in an animal.

8. The method of claim 7, wherein said animal is a human.

9. The method of claim 5, further comprising the step of introducing a test compound to said cell.

10. The method of claim 9, wherein said test compound is a known chemotherapy agent.

11. The method of claim 5, further comprising providing a drug delivery system for introducing said oligonucleotide to said cell.

12. The method of claim 11, wherein said drug delivery system comprises a liposome.

13. The composition of claim 1 further comprising a known chemotherapy agent.

14. The composition of claim 13, wherein said chemotherapy agent is docetaxel ((2R,3S)-N-carboxy-3-phenylisoserine, N-tert-butyl ester, 13-ester with 5, 20-epoxy-1, 2, 30 4, 7, 10, 13-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate).

15. The method of claim 12, wherein said liposome is a cardiolipin based cationic liposome.

16. The method of claim 12, wherein said liposome comprises N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP).

17. The method of claim 10, wherein said known chemotherapeutic agent is docetaxel ((2R,3S)-N-carboxy-3-phenylisoserine, N-tert-butyl ester, 13-ester with 5, 20epoxy-1, 2, 4, 7, 10, 13-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate).

18. A pharmaceutical composition comprising an oligonucleotide having the nucleic acid sequence of SEQ ID NO: 1438 and a liposome.

19. The composition of claim 18, wherein said liposome is a cardiolipin based cationic liposome.

20. The composition of claim 18, wherein said liposome comprises N-[1-(2,3 Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP).

Dated 23 January, 2012
Pronai Therapeutics, Inc.

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

Figure 1
SEQ ID NO:1

ctcgagccct attaagtaag ccgctgtgct tctagaagac ctttttcttt tcttggtgct ttttgtcaaa
gactcttgga gataaaaata cacacgtgca acttgtttgt cctcttgtcc ttttttgcta ggggctattc
atgctgatta atttaaaact gtctgcttgc gcgtacacac gtctgcgagt gtgaatgtgt atgtgtgtat
ctatgtacct catttgagaa agtgcggcca actaggattg gctacgaggc aaaggtggag acctttagga
gcccacccac cccagcgtta ggacggtggg cctgaaagtt actatatgga agtcctcatc gtgtagcact
aaaccagtgt aaaaggtgtt agggacagag ggaaaacatt gacttaaact gtcgtaaagc ccttgataaa
ccccttttccc tggagctgct gagttctgca tggcctgggc acggactagg tgttcaggtg gacacgggcg
gggatgcgcg tgcgtgtgta gtgcgcggac acctaggaag ctacttgaaa gtaaacacca cgctcggggc
gtccctagac attgcttaaa acgtgcagag tcacctgtct tcacagcagg gcagcgctga ggtctcactg
ctgggggcgg tgggggggcgg cattggcctg ggtcttccgc gcgccgagcg ccggtaacac aacgtgtgtg
tgtgtagcgc gtgtacacac tctcatacac ggctagaaag ggtccaggcg acacacacac tcccacatac
acggccagaa aaggtccagg cgagacacac acacacacac acacacacac actccacaca cactcacacg
gccagaaagg gtccaggcgg ttggcggcgc ttttccagcc cttccgcccg gcccggcgcg tcttttcatg
gcgcaccctc ccgccagccg ccccccctccg cactccgtcg tgcgattccc cgggagcccc caccccgtcg
cggaccccag cgaccaccaa gtcgcaccgg cctccgcagg cctgagcaga aggccccgcg cacacccacg
cgcggggccc gcggggaggc ctgtgccgcc cgcgccaccc actggccggg ccccgcgggc gcagcggagc
gggcgggtgg ccggcccgga ggcgccctcc ccggcccggc cccgcgcgcc atgtgccccc ggcgggacgg
ccactcccgg gcctgccgcg gcgcctttaa cccgggccag ggagcggggc ggagggggcg gtcggggtgg
ctcagaggag ggctctttct ttcttctttt tttgaatgaa ccgtgtgacg ttacgcacag gaaaccggtc
gggctgtgca gagaatgaag taagaggaca ggcaccacag ccccgctccc gccccttcc tccc

Figure 2
Exemplary bcl-2 Antigenes

- *Bl1 168-190*

5'- ACTXG CAGAX GTGTG TAXGX GCA -3‘ (SEQ ID NO:2)

- *Bl2 483-506***

5'- CAXGC AXGXG CATCC CXGCC XGTG -3‘ (SEQ ID NO:3)

- *Bl3 666-685*

5'- ACXGG XGCTX GGXGX GXGGA -3‘ (SEQ ID NO:4)

- *Bl4 883-902*

5'- GAXGX GCXGG GCXGG GXGGA -3‘ (SEQ ID NO:5)

- *Bl5 1035-1056*

5'- CCXGX GXGTG GGTGT GXGXG GG -3‘(SEQ ID NO:6)

- *Bl6 1102-1127*

5'- CCXGC CXGCT CXGCT GXGCC XGXGG G -3‘(SEQ ID NO:7)

- *Bl7 1150-1170*

5'- GGXGX GXGGG GCXGG GCXGG G –3’ (SEQ ID NO:8)

- *Bl8 1148-1174*

5'- ACATG GXGXG XGGGG CXGGG CXGGG GA-3’ (SEQ ID NO:9)

Figure 2 (cont.)

- *NBL1 1-24*

5'- GCGG CTTACTTAAT AGGGCTCGAG -3' (SEQ ID NO:10)

- *NBL2 232-259*

5'- CCTCGTAGCCAATCCTAGTTGGCCGCAC -3' (SEQ ID NO:11)

- *NBL3 291-314*

5'- CAGGCCCACCGTCCTAACGCTGGG -3' (SEQ ID NO:12)

- *NBL4 546-570*

5'- GTCTAGGGAC GCCCCGAGCGTGGTG -3' (SEQ ID NO:13)

- *NBL5 614-649*

5'- CGCCCCCCA CCGCCCCCAG CAGTGAGACCTCAGCGC -3' (SEQ ID NO:14)

- *NBL6 707-732*

5'- CCGTGTATGAGAGTGTGTACACGCGC -3' (SEQ ID NO:15)

- *NBL7 839-870*

5'- GCGCCGCCAA CCGCCTGGAC CCTTTCTGGCCG -3' (SEQ ID NO:16)

Figure 2 (cont.)

- *NBL8 921-954*

5'- CGCA CGACGGAGTG CGGAGGGGGG CGGCTGGCGG -3' (SEQ ID NO:17)

- *NBL9 961-983*

5'- CCG CGACGGGGTG GGGGCTCCCG -3' (SEQ ID NO:18)

- *NBL10 991-1017*

5'- CCG CGACGGGGTG GGGGCTCCCG -3' (SEQ ID NO:19)

- · *NBL11 1057-1098*

5'- CGGCCAGTGGGTGGCGCGGGCGGCACAG GCCTCCCCGCGGGC -3' (SEQ ID NO:20)

- *NBL12 1180-1214*

5'- GCGCCGCGGCAGGCCCGGGAGTGG CCGTCCCGCCG -3' (SEQ ID NO:21)

- *NBL13 1223-1258*

5'- ACCCCGAC CGCCCCCTCC GCCCCGCTCC CTGGCCCG -3' (SEQ ID NO:22)

- *NBL14 1301-1334*

5'- GCCCGACCGGTTTCCTGTGCGTAA CGTCACACGG -3' (SEQ ID NO: 23)

Figure 2 (cont.)

- *NBL15 91-120*

5'- GGACAAGAGG ACAAACAAGT TGCACGTGTG -3' (SEQ ID NO: 24)

- *NBL16 328-357*

5'- CTGGTTT AGTGCTACAC GATGAGGACT TCC -3' (SEQ ID NO: 25)

- *NBL17 401-432*

5'- CA GGGAAAGGGG TTTATCAAGG GCTTTACGAC -3' (SEQ ID NO: 26)

- *NBL18 452-480*

5'- CACCTGAACA CCTAGTCCGT GCCCAGGCC -3' (SEQ ID NO: 27)

- *NBL19 1131-1149*

5'- GAGGGCGCC TCCGGGCCGG -3' (SEQ ID NO: 28)

Figure 3
SEQ ID NO:29

1 cccgggggtc ctggaagcca caaggtaaac acaacacatc cccctccttg actatcaatt
61 ttactagagg atgtggtggg aaaaccatta tttgatatta aaacaaatag gcttgggatg
121 gagtaggatg caagctccca ggaaagttta agataaaacc tgagacttaa aagggtgtta
181 agagtggcag cctagggaat ttatcccgga ctccggggga gggggcagag tcaccagcct
241 ctgcatttag ggattctccg aggaaaagtg tgagaacggc tgcaggcaac ccagcttccc
301 ggcgctagga gggacgcacc caggcctgcg cgaagagagg gagaaagtga agctgggagt
361 tgccactccc agacttgttg gaatgcagtt ggaggggggcg agctgggagc gcgcttgctc
421 ccaatcacag gagaaggagg aggtggagga ggagggctgc ttgaggaagt ataagaatga
481 agttgtgaag ctgagattcc cctccattgg gaccggagaa accagggagc ccccccggg

Figure 4

- *<u>HR1 393-416</u>*

5'-AAGXG XGCTC CCAGC TXGCC CCCT-3' (SEQ ID NO:30)

- *<u>HR2 298-322</u>*

5'-TGGGT *G*XGTC CCTCC TAGXG CXGGG-3' (SEQ ID NO:31)

- *<u>HR3 310-334</u>*

5'-TTXGC XGCAG GCCTG GGT*G*X GTCCC T-3' (SEQ ID NO:32)

- *<u>HR4 300-332</u>*

5'-XGXGC AGGCC TGGGT *G*XGTC CCTCC TAGXG CXG-3' (SEQ ID NO:33)

- *<u>HR5 257-281</u>*

5'-AGCXG TTCTC ACACT TTTCC TXGGA-3' (SEQ ID NO:34)

- *<u>HR6 204-227</u>*

5'-TGCCC CCTCC CCXGG AGTCX GGGA-3' (SEQ ID NO:35)

- *<u>HR11 393-425</u>*

5'-ATTGG GAGCA AGXGX GCTCC CAGCT XGCCC CCT-3' (SEQ ID NO:36)

- *<u>HR23 299-333</u>*

5'-TXGXG CAGGC CTGGG T*G*XGT CCCTC CTAGX GCXGG-3' (SEQ ID NO:37)

- *<u>HR23* 299-333</u>*

5'-TXGXG CAGGC CTGGG TXGT CCCTC CTAGX GCXGG-3' (SEQ ID NO:38)

Figure 4 (cont.)

- *HR-New1 1-21*

5'-G TGGCT TCCAG GACCC CCGGG-3' (SEQ ID NO:39)

- *HR6 204-227*

5'-TGCCC CCTCC CCXGG AGTCX GGGA-3' (SEQ ID NO:40)

- *HR11 394-425*

5'-ATTGG GAGCA AGXGX GCTCC CAGCT XGCCC CCT-3' (SEQ ID NO:41)

- *HR23 299-333*

5'-TXGXG CAGGC CTGGG T*G*XGT CCCTC CTAGX GCXGG-3' (SEQ ID NO:42)

- *HR23* 299-333*

5'-TXGXG CAGGC CTGGG TXGT CCCTC CTAGX GCXGG-3' (SEQ ID NO:43)

- *HR23-New 18-mer 299-317*

5'-*G*XGT CCCTC CTAGX GCXGG -3' (SEQ ID NO:44)

- *HR-New2 511-539*

5'-CCCG GGGGG GCTCC CTGGT TTCTC CGGTC-3' (SEQ ID NO:45)

Figure 5
SEQ ID NO:46

1 cctgagggtg gcggggtgct cttcgcagct tctctgtgga gaccggtcag cggggcggcg
61 tggccgctcg cggcgtctcc ctgtggcatc cgcacagccc gccgcggtcc ggtcccgctc
121 cgggtcagaa ttggcggctg cggggacagc cttgcggcta ggcagggggc gggccgccgc
181 gtgggtccgg cagtccctcc tcccgccaag gcgccgccca gacccgctct ccagccggcc
241 cggctcgcca ccctagaccg ccccagccac cccttcctcc gccggcccgg cccccgctcc
301 tcccccgccg gcccggcccg gccccctcct tctccccgcc ggcgctcgct gcctccccct
361 cttccctctt cccacaccgc cctcagccgc tccctctcgt acgcccgtct gaagaagaat
421 cgagcgcgga acgcatcgat agctctgccc tctgcggccg cccggccccg aactcatcgg
481 tgtgctcgga gctcgatttt cctaggcggc ggccgcggcg gcggaggcag cagcggcggc
541 ggcagtggcg gcggcgaagg tggcggcggc tcggccagta ctcccggccc ccgccatttc
601 ggactgggag cgagcgcggc gcaggcactg aaggcggcgg cggggccaga ggctcagcgg
661 ctcccaggtg cgggagagag gtacggagcg gaccacccct cctgggcccc tgcccggg

Figure 6

- *Kr1 102-124*

5' - CCXGG AGXGG GACXG GACXG XGG -3' (SEQ ID NO:47)

- *Kr2 168-191*

5' - GCXGG ACCCA XGXGG XGGCC XGCC -3' (SEQ ID NO:48)

- *Kr3 505-527*

5' - CCTCX GCXGC XGXGG CXGCX GCC -3' (SEQ ID NO:49)

- *Kr4 532-558*

5' - TTXGC XGCXG CCACT GCXGC XGCXG CT -3' (SEQ ID NO:50)

- *Kr5 547-574*

5'- CXGAG CXGCX GCCAC CTTXG CXGCX GC -3' (SEQ ID NO:51)

- *Kr6 598-623*

5'- TGXGX GXGCT XGCTC CCAGT CXGAA -3' (SEQ ID NO:52)

- *Kr7 40-67*

5' - GCGGC CACGC CGCCC CGCTG ACCGG TCT -3' (SEQ ID NO:53)

Figure 6 (cont.)

- *NKr1 11-39*

5' - CCACAGAGA AGCTGCGAAG AGCACCCCGC -3' (SEQ ID NO:54)

- *NKr2 69-102*

5' - GC GGGCTGTGCG GATGCCACAG GGAGACGCCGCG -3' (SEQ ID NO:55)

- *NKr3 133-158*

5' - GCCGCAAGGCTGTCCCCGCAGCCGCC -3' (SEQ ID NO:56)

- *NKr4 203-227*

5' - GCGGGTCTGGGCGGCGCCTTGGCGG -3' (SEQ ID NO:57)

- *NKr5 234-260*

5'- CGGTCTAGGG TGGCGAGCCGGGCCGGC -3' (SEQ ID NO:58)

- *NKr6 280-312*

5'- GC CGGCGGGGGA GGAGCGGGGG CCGGGCCGGCG -3' (SEQ ID NO:59)

- *NKr7 313-349*

5' - GCGAGCGCC GGCGGGGAGA AGGAGGGGGC CGGGCCGG - 3' (SEQ ID NO:60)

Figure 6 (cont.)

- *NKr8 421-443*

5' - GCT ATCGATGCGT TCCGCGCTCG -3‘ (SEQ ID NO:61)

- *NKr9 454-495*

5' - CGAGC TCCGAGCACA CCGATGAGTT CGGGGCCGGG CGGCCGC -3‘ (SEQ ID NO:62)

- *NKr10 377-409*

5' - GACGGGCGT ACGAGAGGGA GCGGCTGAGG GCGG -3‘(SEQ ID NO:63)

- *NKr11 630-660*

5' - CCGCTGAGCC TCTGGCCCCG CCGCCGCCTT C -3' (SEQ ID NO:64)

- *NKr12 670-690*

5' - CGCTCCGTAC CTCTCTCCCG C -3' (SEQ ID NO:65)

Figure 7
SEQ ID NO:66

ggatcccagc ctttccccag cccgtagccc cgggacctcc gcggtgggcg gcgccgcgct
gccggcgcag ggagggcctc tggtgcaccg gcaccgctga gtcgggttct ctcgccggcc
tgttcccggg agagcccggg gccctgctcg gagatgccgc cccgggcccc cagacaccgg
ctccctggcc ttcctcgagc aaccccgagc tcggctccgg tctccagcca agcccaaccc
cgagaggccg cggccctact ggctccgcct cccgcgttgc tcccggaagc cccgcccgac
cgcggctcct gacagacggg ccgctcagcc aaccggggtg gggcggggcc cgatggcgcg
cagccaatgg taggccgcgc ctggcagacg gacgggcgcg gggcggggcg tgcgcaggcc
cgcccgagtc tccgccgccc gtgccctgcg cccgcaaccc gagccgcacc cgccgcggac
ggagcccatg cgcggggcga accgcgcgcc cccgcccccg ccccgccccg gcctcggccc
cggccctggc cccgggggca gtcgcgcctg tgaacggtga gtgcgggcag ggatcggccg
ggccgcgcgc cctcctcgcc cccaggcggc agcaatacgc gcggcgcggg ccgggggcgc
ggggccggcg ggcgtaagcg gcggcggcgg cggcgggtgg gtggggccgg gcggggcccg
cgggcacagg tgagcgggcg tcgggggctg cggcgggcgg gggccccttc ctccctgggg
cctgcgggaa tccgggcccc acccgtggcc tcgcgctggg cacggtcccc acgccggcgt
acccgggagc ctcgggcccg gcgccctcac acccgggggc gtctgggagg aggcggccgc
ggccacggca cgcccgggca cccccgattc agcatcacag gtcgcggacc aggccggggg
cctcagcccc agtgcctttt ccctctccgg gtctcccgcg ccgcttctcg gccccttcct
gtcgctcagt ccctgcttcc caggagctcc tctgtcttct ccagctttct gtggctgaaa
gatgccccg gttccccgcc gggggtgcgg ggcgctgccc gggtctgccc tcccctcggc
ggcgcctagt acgcagtagg cgctcagcaa atacttgtcg gaggcaccag cgccgcgggg
cctgcaggct ggcactagcc tgcccgggca cgccgtggcg cgctccgccg tggccagacc
tgttctggag gacggtaacc tcagccctcg ggcgcctccc tttagccttt ctgccgaccc
agcagcttct aatttgggtg cgtggttgag agcgctcagc tgtcagccct gcctttgagg
gctgggtccc ttttcccatc actgggtcat taagagcaag tgggggcgag gcgacagccc
tcccgcacgc tgggttgcag ctgcacaggt aggcacgctg cagtccttgc tgcctggcgt
tggggcccag ggaccgctgt gggtttgccc ttcagatggc cctgccagca gctgccctgt
ggggcctggg gctgggcctg ggcctggctg agcagggccc tccttggcag gtggggcagg
agaccctgta ggaggacccc gggccgcagg cccctgagga gcgatgacgg aatataagct
ggtggtggtg ggcgccggcg gtgtgggcaa gagtgcgctg accatccagc tgatccagaa

Figure 8

- *RZ1X** 485-510*

5'-GGXGX GXGGT TXGCC CXGXG CATGG G-3' (SEQ ID NO:67)

- *rz1 485-510*

5'-GGCGC GCGGT TCGCC CCGCG CATGG G-3' (SEQ ID NO:68)

- *rz4x 1641-1672*

5' -TTCXG TCATX GCTCC TCA*G*G GGCCT GXGGC CC-3' (SEQ ID NO:69)

- *rzx** 388-415*

5' -GXGCA XGCCC XGCCC XGXGC CXGTC XGT - 3' (SEQ ID NO:70)

- *rz2x** 501-533*

5' -GGXGG GGXGG GGGXG GGGGX GXGXG GT - 3' (SEQ ID NO:71)

- *rz3x 1271-1290*

5' -GGGAG GXGCC XGAGG GCTGA GGTTA CXGTC-3' (SEQ ID NO:72)

- *erz1x** 481-510*

5'-CGGGG GCGGG GGXGX GXGGT TXGCC CXGXG CATGG GCTCC-3' (SEQ ID NO:73)

- *srz1x 491-510*

5'-GGXGX GXGGT TXGCC CXGXG-3' (SEQ ID NO:74)

- *rz5x 333-361*

5' -GXGXG CCATX GGGCC CXGCC CCACC CXGG-3' (SEQ ID NO:75)

Figure 8 (cont.)

- *rz6x** 665-696*

5'- CXGCX GCXGC XGCXG CXGCT TAXGC CXGCX GG- 3' (SEQ ID NO:76)

- *rz7x 1224-1251*

5'- AXGGX GGAGX GXGCC AXGGX GTGCC XGG -3' (SEQ ID NO:77)

- *rz8x 1421-1451*

5'- AGXGT GXGGG AGGGC TGTXG CCTCG CCCCC A-3' (SEQ ID NO:78)

- *RZ4X(New) 1639-1672*

5'- CXGTC ATXGC TCCTC AGGGG CCTGX GGCCX GG -3' (SEQ ID NO:79)

- *RZ9X** 733-760*

5'- CXGCC XGCXG CAGCC CCXGA XGCCX GCT -3' (SEQ ID NO:80)

- *RZ10X** 333-361*

5'- GXGXG CCATX GGGCC CXGCC CCACC CXGG -3' (SEQ ID NO:81)

- *LRZ1X 485-539*

5'- GGCXG AGGCX GGGGX GGGGX GGGGG XGGGG GXGXG XGGTT XGCCC XGXGC
ATGGG -3' (SEQ ID NO:82)

- *RZ1uX 468-500*

5'- TXGCC CXGXG CATGG GCTCX GTCXG XGGXG GGT -3' (SEQ ID NO:83)

- *RZ11X 442-467*

5'- GXGGC TXGGG TTGXG GGXGC AGGGC A -3' (SEQ ID NO:84)

Figure 8 (cont.)

- *Nras-1 31-68*

5'-GCGCCGGC AGCGCGGCGC CGCCCACCGC GGAGGTCCCG -3' (SEQ ID NO:85)

- *N-ras-2 85-117*

5'-CGGCGAG AGAACCCGAC TCAGCGGTGC CGGTGC -3' (SEQ ID NO:86)

- *Nras-3 127-160*

5' -GCGGCATCTC CGAGCAGGGC CCCGGGCTCT CCCG -3' (SEQ ID NO:87)

- *Nras-4 196-220*

5' -CCGGAGCCGA GCTCGGGGTT GCTCG - 3' (SEQ ID NO:88)

- *Nras-5 241-268*

5' -GCGGAGCC AGTAGGGCCG CGGCCTCTCG - 3' (SEQ ID NO:89)

- *Nras-6 273-294*

5' -CGGG GCTTCCGGGA GCAACGCG -3' (SEQ ID NO:90)

- *Nras-7 297-323*

5'-CGG CCCGTCTGTC AGGAGCCGCG GTCG -3' (SEQ ID NO:91)

- *Nras-8 418-441*

5'-C GGGCGGCGGA GACTCGGGCG GGC -3' (SEQ ID NO:92)

- *Nras-9 540-577*

5' -CCGTTCA CAGGCGCGAC TGCCCCCGGG GCCAGGGCCG G - 3'(SEQ ID NO:93)

Figure 8 (cont.)

- *Nras-10 595-618*

5'- CGAGGAGG GCGCGCGGCC CGGCCG - 3' (SEQ ID NO:94)

- *Nras-11 781-815*

5'- CGCGA GGCCACGGGT GGGGCCCGGA TTCCCGCAGG - 3'(SEQ ID NO:95)

- *Nras-12 820-845*

5'- CGGGT ACGCCGGCGT GGGGACCGTG C -3' (SEQ ID NO:96)

- *Nras-13 851-881*

5'- C GCCCCCGGGT GTGAGGGCGC CGGGCCCGAG -3' (SEQ ID NO:97)

- *Nras-14 892-916*

5'- CGGGCG TGCCGTGGCC GCGGCCGCC -3' (SEQ ID NO:98)

- *Nras-15 981-1010*

5'- CGAGAAGCGG CGCGGGAGAC CCGGAGAGGG -3' (SEQ ID NO:99)

- *Nras-16 1088-1115*

5'- GCGCC CCGCACCCCC GGCGGGGAAC CGG -3' (SEQ ID NO:100)

- *Nras-17 1120-1154*

5'- GCGT ACTAGGCGCC GCCGAGGGGA GGGCAGACCC G -3' (SEQ ID NO:101)

- *Nras-18 1160-1197*

5'- CGCGGCG CTGGTGCCTC CGACAAGTAT TTGCTGAGCG C - 3'(SEQ ID NO:102)

Figure 8 (cont.)

- *Nras-19 161-185*

5'- GGGAG CCGGTGTCTG GGGGCCCGGG - 3' (SEQ ID NO:103)

- *Nras-20 363-387*

5'- CTGCCAG GCGCGGCCTA CCATTGGC -3' (SEQ ID NO:104)

- *Nras-21 706-729*

5'- CTGTGCCCG CGGGCCCCGC CCGGC -3' (SEQ ID NO:105)

- *Nras-22 918-956*

5'- CGGCCT GGTCCGCGAC CTGTGATGCT GAATCGGGGG TGC - 3' (SEQ ID NO:106)

- *Nras-23 1691-1720*

5'- CAGCGCACTC TTGCCCACAC CGCCGGCGCC -3' (SEQ ID NO:107)

Figure 9
SEQ ID NO:108

1 ctgcagcctg gtacgcgcgt ggctggcggt gggcgcgcag tggttctcgg tgtggagggc
61 agctgttccc cctgcgatga tttatactca caggacaagg atgcgatttg tcaaacacta
121 cgaggagtag cagagaaagg gagagggttt gaaagggagc aaaagaaaat ggtaggcgcg
181 cgtagttaat tcatgcggct ctcttactct gtttacatcc tagaggtaga gtgctcggct
241 gcccggctga gtctcctccc caccttcccc accctcccca ccctccccat aagcgccctc
301 ccgggttccc aaagcagagg gcgtggggga aaagaaaaaa gatcctctct cgctaatctc
361 cgcccaccgg ccctttataa tgcgagggtc tggacggctg aggacccccg agctgtgctg
421 ctcgcggccg ccaccgcggg ccccggccgt ctttggctcc cctcctgcct cgagaagggc
481 agggcttctc agaggcttgg cgggaaaaag aacggaggga gggatcgctc tgagtataaa
541 agccgatttt cggggcttta tctaactcgc tgtagtaatt ttaccgagag gcagagggag
601 cgagcgggcg gccgcctagg gtggaagagc cgggcgagta gagttgcact tgggaaggga
661 gatccggagc gaatcggggg cttcgcctct ggcccagccc tcccgctgat cccccaggca
721 gtggtccgca atccttgccg cattcacgaa aatttggcca taacacgggg cggtcacttt
781 acactggaac gtccaacacc cgagcaagga cgcgagtctc ccgancgcga ggagactatt
841 gagatgtgtc gattggctac atagggtgtc ttttctccca ctcctgcgcc atcgacagtt
901 ttctcagaat acctttggta agtggggctg gggtgggcgt tatttcagaa ctgtatcggg
961 caaattgtat tcctcaccgc cacctcccgc ggcttcttaa gggcgccagg gccgctttcg
1021 attcctctgc cgctgcgggg ccgacgttcg ggactgagca ctgcgcgctg cgccaggttt
1081 ccgcaccaag accccttaa ctcaagactg cctcccgctt tgtgtgcccc gctccagcag

Figure 10

- *cm1 423-449*

5'-XGGCX GGGGC CXGX GGTGG XGGCX GXG-3' (SEQ ID NO:109)

- *cm2 175-199*

5'-GCXGC ATGA ATTAA CTAXG XGXGC C-3' (SEQ ID NO:110)

- *cm3** 349-371*

5'-GCXGG TGGGX GGAGA TTAG XGAG-3' (SEQ ID NO:111)

- *cm4** 501-529*

5'-AGXGA TCCCT CCCTC XGTTC TTTTT CCXG-3' (SEQ ID NO:112)

- *cm5 47-77*

5'- TXGCA GGGGG AACAG CTGCC CTCCA CACXG A -3' (SEQ ID NO:113)

- *cm6 13-39*

5'- TGXGX GCCCA CXGCC AGCC AXGXG XGT -3' (SEQ ID NO:114)

- *cm7*

5'- GGGXG CCTXG CTAAG GCTGG GGAAA GGGCX GXGC -3' (SEQ ID NO:115)

Figure 10 (cont.)

- *Ncm1 101-130*

5'- CTACTCCTCG TAGTGTTTGA CAAATCGCAT -3' (SEQ ID NO:116)

- *Ncm2 225-251*

5'-C TCAGCCGGGC AGCCGAGCAC TCTACC -3' (SEQ ID NO:117)

- *Ncm3 293-326*

5'-CCACGC CCTCTGCTTT GGGAACCCGG GAGGGCGC -3' (SEQ ID NO:118)

- *Ncm4 542-569*

5'-CGAGTTAGA TAAAGCCCCG AAAATCGGC -3' (SEQ ID NO:119)

- *Ncm5 600-632*

5'- CG GCTCTTCCAC CCTAGGCGGC CGCCCGCTCG C -3' (SEQ ID NO:120)

- *Ncm6 664-687*

5'- GGCGAAG CCCCCGATTC GCTCCGG -3' (SEQ ID NO:121)

- *Ncm7 800-836*

5'- GTCTCC TCGCGNTCGG GAGACTCGCG TCCTTGCTCG G -3'(SEQ ID NO:122)

Figure 10 (cont.)

- *Ncm8 957-993*

5'-GCC GCGGGAGGTG GCGGTGAGGA ATACAATTTG CCCG -3' (SEQ ID NO:123)

- *Ncm9 1011-1046*

5'-CGTCGG CCCCGCAGCG GCAGAGGAAT CGAAAGCGGC -3' (SEQ ID NO:124)

- *Ncm10 1061-1090*

5'-CTTGGTGCGG AAACCTGGCG CAGCGCGCAG -3' (SEQ ID NO:125)

- *Ncm11 396-420*

5'-CAGCACAGCT CGGGGGTCCT CAGCC-3' (SEQ ID NO:126)

- *Ncm12 721-748*

5'-CGTGAATG CGGCAAGGAT TGCGGACCAC-3' (SEQ ID NO:127)

- *Ncm13 758-777*

5'-GTGACCG CCCCGTGTTA TGG-3' (SEQ ID NO:128)

- *Ncm14 875-896*

5'-GTCGAT GGCGCAGGAG TGGGAG-3' (SEQ ID NO:129)

- *Ncm15 1110-1140*

5'- CTGCTGGAGC GGGGCACACA AAGCGGGAGG C-3' (SEQ ID NO:130)

Figure 11
SEQ ID NO:131

gagctccggg tacctggaga acatgaatct cccgggtgta ctgtccccat gagggtcacc
gaggaaccta tggatcttcc actcttcgcc cagaatgtct tccctctatc ccctccacct
ccccaaattc cagcaccagg ggccacctca gagccacaaa tcctgaaagg accacccagg
tgactcgggc ccacacaccc ctctttcggg gtacacttgg tggcaggcta ggagcttttg
gcctcagctg gtggtgccac acaccagatt cgtcacagag acccattttt tatcagtccc
aggccagaag tacgaagact gatcttaacg atgtggcctg cctgggaggt aaggcggcag
gcgttgcaga attgatggga actgtggcac aggtgggaaa cctggtttaa caaattcttc
attgattcag gggaccactt tccttgagcc aagtcttggc aagcggccgg cgaaactcac
aggtcccttt cctggctgcg tccccagcct ccagccttcc ccgccccaga gatgccccag
gagcggcccc tcggtgtagg taacgggtgc ccgggcggct ccgtccgccg cctagagcct
ggaagccgcc actgcggccc aggacaatcc ggctacgcgg ccggcgccga ccccgcacgc
tggagtccgc tgccgcacgg cgctggcagt cgggggtggt gtctgaagtc aggcgcttcc
tgccttttcg tcggcccggg tgcccggctc gcgccgccag gctctgggat cccaggtcgc
cccgcccagc agcccgcgcc ctgctcggtg cgctcagcgt ccccgcccct taccccaaac
ccccaccctc tgtgccctca ggggggcacc cccatcgggg cgggaggggg gggtcagctg
tgccccggtc gccgagtggc gaggaggtga cggtagccgc cttcctattt ccgcccggcg
ggcagcgctg cggggcgagt gccagcagag aggcgctcgg tcctccctcc gccctcccgc
gccgggggca ggccctgcct agtctgcgtc tttttccccc gcaccgcggc gccgctccgc
cactcgggca ccgcaggtag ggcaggaggc tggagagcct gctgcccgcc cgcccgtaaa
atggtcccct cggctggaca gctcgccctg ttcgctctgg gtattgtgtt ggctgcgtgc
caggccttgg agaacagcac gtccccgctg agtgcagacc cgcccgtggc tgcagcagtg
gtgtcccatt ttaatgactg cccagattcc cacactcagt tctgcttcca tgga

Figure 12

- *tg1 997-1025*

5'- CXGGX GXGGG AGGGX GGAGG GAGGA CXGT -3' (SEQ ID NO:132)

- *tg2 951-978*

5'- TXGCC CXGCA GXGCT GCCXG CXGGG XGG -3' (SEQ ID NO:133)

- *tg3 805-830*

5'- AGGGG XGGGG AXGCT GAGXG CACXG A -3' (SEQ ID NO:134)

- *tg4 777-802*

5'- AGGGX GXGGG CTGCT GGGXG GGXGA -3' (SEQ ID NO:135)

- *tg5 728-762*

5'- GCCTG GXGGX GXGAG CXGGG CACCX GGGCX GAXGA -3' (SEQ ID NO:136)

- *tg6 641-661*

5'- AGXGT GXGGG GTXGG XGCXG GCXGX GTA-3' (SEQ ID NO:137)

- *tg7 563-591*

5'- GXGGX GGAXG GAGCX GCCXG GGCAC CXGT -3' (SEQ ID NO:138)

- *tg8** 754-787*

5'- GGGXG GGGXG ACCTG GGATC CCAGG CCTGG XGG -3' (SEQ ID NO:139)

- *tg9 521-546*

5'- GXGCT CCTGG GGCAT CTCTG GGGXG G -3' (SEQ ID NO:140)

Figure 12 (cont.)

- *tg10** 1111-1137*

5'- AXGGG XGGGX GGGCA GCAGG CTCTC CA -3' (SEQ ID NO:141)

- *tg11** 328-357*

5'-CXGCC TTACC TCCCA GGCAG GCCAC ATXGT -3' (SEQ ID NO:142)

- *tg12** 185-209*

5'- CXGAA AGAGG GGTGT GTGGG CCXGA -3' (SEQ ID NO:143)

- *tg13** 1064-1094*

5'- GXGGT GCCXG AGTGG XGGAG XGGXG CXGXG G -3' (SEQ ID NO:144)

Figure 12 (cont.)

- *Ntg1 1-25*

5'-CATGT TCTCCAGGTA CCCGGAGCTC -3' (SEQ ID NO:145)

- *Ntg2 31-60*

5'-C GGTGACCCTC ATGGGGACAG TACACCCGGG -3' (SEQ ID NO:146)

- *Ntg3 81-110*

5'-GATAGAGGGA AGACATTCTG GGCGAAGAGT -3' (SEQ ID NO:147)

- *Ntg4 453-480*

5'-GTGAGTTTCG CCGGCCGCTT GCCAAGAC -3' (SEQ ID NO:148)

- *Ntg5 614-640*

5'- CCGCGTAGCC GGATTGTCCT GGGCCGC -3' (SEQ ID NO:149)

- *Ntg6 662-682*

5'- CG CCGTGCGGCA GCGGACTCC -3' (SEQ ID NO:150)

- *Ntg7 691-716*

5'- GCGCCT GACTTCAGAC ACCACCCCCG -3' (SEQ ID NO:151)

- *Ntg8 876-912*

5'- GC GACCGGGGCA CAGCTGACCC CCCCCTCCCG CCCCG - 3' (SEQ ID NO:152)

- *Ntg9 913-940*

5'- GCGGCTACCG TCACCTCCTC GCCACTCG -3'(SEQ ID NO:153)

Figure 12 (cont.)

- *Ntg10 1151-1175*

5'- GCGAA CAGGGCGAGC TGTCCAGCCG -3' (SEQ ID NO:154)

- *Ntg11 1226-1250*

5'- GCCACGGGCG GGTCTGCACT CAGCG -3'(SEQ ID NO:155)

Ntg12 257-284

5'-GGGT CTCTGTGACG AATCTGGTGT GTGG -3' (SEQ ID NO:156)

- *Ntg13 361-384*

5'-CAGT TCCCATCAAT TCTGCAACGC -3' (SEQ ID NO:157)

- *Ntg14 1038-1062*

5'-GC GGGGGAAAAA GACGCAGACT AGG -3' (SEQ ID NO:158)

- *Ntg15 1193-1223*

5'-GAC GTGCTGTTCT CCAAGGCCTG GCACGCAG -3'(SEQ ID NO:159)

Figure 13

BxPC3

Lead	Concentration	average % Inhib	1 st dev
kr1	5 uM	93%	1.6%
kr1	15 uM	99%	0.4%
kr1	30 uM	99%	0.1%
kr2	5 uM	12%	5.9%
Kr2	10 uM	14%	10.2%
Kr2	10 uM	20%	6.9%
kr2	15 uM	87%	6.0%
Kr2	20 uM	87%	1.7%
Kr2	20 uM	91%	1.7%
kr2	30 uM	99%	0.1%
Kr3	10 uM	14%	5.0%
Kr3	20 uM	49%	14.3%
kr3	50 uM	99%	0.1%
kr4	5 uM	53%	2.6%
Kr4	10 uM	18%	4.5%
kr4	15 uM	99%	0.1%
Kr4	20 uM	93%	1.4%
kr4	30 uM	99%	0.1%
Kr5	10 uM	16%	4.5%
Kr5	20 uM	17%	3.9%
kr5	50 uM	99%	0.4%
Kr6	10 uM	15%	3.9%
Kr6	10 uM	16%	6.5%
Kr6	20 uM	25%	6.9%
Kr6	20 uM	18%	6.8%
kr6	50 uM	68%	3.9%

Myc-MT-1

Lead	Concentration	average % Inhib	1 st dev
kr1	10 uM	77%	2.5%

Figure 14

Lead	Concentration	average % Inhib	repeats	1 st dev
BL1	10 uM	12%	8	6%
BL2	10 uM	76%	8	3%
BL2	20 uM	94%	4	3%
BL3	10 uM	20%	8	4%
BL4	10 uM	9%	8	7%
BL5	10 uM	26%	8	5%
BL6	10 uM	47%	8	2%
BL7	10 uM	15%	8	8%
BL8	20 uM	81%	4	4%

M14

Lead	Concentration	average % Inhib	repeats	1 st dev
BL1	10 uM	27%	4	2%
BL2	10 uM	65%	4	3%
BL2	10 uM	35%	4	6%
BL2	20 uM	98%	4	2%
BL3	10 uM	20%	4	10%
BL4	10 uM	21%	4	11%
BL5	10 uM	18%	4	5%
BL6	10 uM	60%	4	1%
BL7	10 uM	97%	4	1%
BL7	10 uM	98%	4	0%
BL7	20 uM	98%	4	0%

BT474

BL1	10 uM	45%	4	5%
BL2	10 uM	67%	4	8%
BL3	10 uM	85%	4	6%
BL4	10 uM	45%	4	14%
BL5	10 uM	84%	4	6%
BL6	10 uM	71%	4	5%
BL7		76%	4	2%

T47D

BL2	10 uM	42%	4	10%
BL2	20 uM	88%	4	3%
BL7	10 uM	95%	4	1%
BL7	20 uM	96%	4	1%
BL8	2 uM	25%	4	12%
BL8	6 uM	33%	4	7%
BL8	20 uM	59%	4	24%

FSCCL

BL2	10 uM post 8 hrs	60%	4	7%
BL2	10 uM post 16 hrs	86.50%	4	6%
BL2	10 uM post 24 hrs	95%	4	4%
BL2	10 uM post 32 hrs	98.90%	4	2%
BL2	10 uM post 40 hrs	100%	4	0%

BL2	10 uM post 48 hrs	100%	4	0%
BL6	10 uM	40%	4	13%
BL6	10 uM	71.60%	4	8%
BL6	10 uM	96.30%	4	5%
BL6	10 uM	100%	4	0%
BL6	10 uM	100%	4	0%
BL6	10 uM	100%	4	0%
BL7	1 uM post 8 hrs	-14%	4	9%
BL7	3 uM post 8 hrs	14%	4	16%
BL7	10 uM post 8 hrs	5%	4	26%
BL7	1 uM post 16 hrs	6%	4	11%
BL7	3 uM post 16 hrs	54%	4	7%
BL7	10 uM post 16 hrs	86%	4	6%
BL7	1 uM post 24 hrs	28%	4	21%
BL7	3 uM post 24 hrs	76%	4	5%
BL7	10 uM post 24 hrs	100%	4	0%
BL7	1 uM post 32 hrs	37%	4	12%
BL7	3 uM post 32 hrs	93%	4	4%
BL7	10 uM post 32 hrs	100%	4	0%
BL7	1 uM post 48 hrs	40%	4	16%
BL7	3 uM post 48 hrs	100%	4	0%
BL7	10 uM post 48 hrs	100%	4	0%
BL8	1 uM post 8 hrs	3.80%	4	10%
BL8	3 uM post 8 hrs	19%	4	16%
BL8	10 uM post 8 hrs	38%	4	14%
BL8	1 uM post 16 hrs	13.40%	4	10%
BL8	3 uM post 16 hrs	18%	4	3%
BL8	10 uM post 16 hrs	57%	4	6%
BL8	1 uM post 24 hrs	31.30%	4	8%
BL8	3 uM post 24 hrs	42%	4	7%
BL8	10 uM post 24 hrs	82%	4	7%
BL8	1 uM post 32 hrs	33%	4	9%
BL8	3 uM post 32 hrs	42.50%	4	4%
BL8	10 uM post 32 hrs	95.70%	4	3%
BL8	1 uM post 40 hrs	36%	4	3%
BL8	3 uM post 40 hrs	45.40%	4	8%
BL8	10 uM post 40 hrs	100%	4	0%
BL8	1 uM post 48 hrs	35%	4	3%
BL8	3 uM post 48 hrs	49%	4	9%
BL8	10 uM post 48 hrs	100%	4	0%

NMuMG

BL2	10 uM	4%	4	6%
BL2	20 uM	5.50%	4	6%
BL7	No concentration	52%	8	14%
BL8	20 uM	27%	4	6%

MCF7

BL8	5 uM	41%	4	4%
BL8	10 uM	61%	4	1%
BL8	20 uM	83%	4	5%

Myc-MT-1

BL2	1 uM	4%	4	8%
BL2	3 uM	6%	4	2%
BL2	10 uM	37%	4	8%

Figure 15

MCF10a

Lead	Concentration	average % Inhib	1 st dev	repeats
HR3	10 uM	12%	8%	8
HR4	10 uM	-5%	8%	8

MDA-MB-231

Lead	Concentration	average % Inhib	1 st dev	repeats
HR23	1 uM	18%	4%	4
HR23	2.5 uM	19%	4%	4
HR23	5 uM	32%	2%	4
HR23	10 uM	98%	1%	4
HR23	10 uM	74%	4%	4
HR23	20 uM	78%	1%	4

T47D

Lead	Concentration	average % Inhib	1 st dev	repeats
HR2	10 uM	92%	1%	5
HR2	10 uM	86%	3%	4
HR2	10 uM	58%	2%	4
HR2	20 uM	88%	4%	4
HR3	10 uM	89%	5%	4
HR3	10 uM	45%	5%	4
HR3	20 uM	97%	2%	4
HR5	10 uM	-15%	18%	5
HR6	10 uM	66%	28%	5
HR11	10 uM	46%	7%	5
HR23	1 uM	0%	3%	4
HR23	1 uM	26%	15%	4
HR23	2.5 uM	37%	6%	4
HR23	3 uM	22%	9%	4
HR23	5 uM	75%	2%	4
HR23	10 uM	96%	2%	2
HR23	10 uM	92%	1%	4
HR23	10 uM	99%	1%	4
HR23	10 uM	68%	2%	4
HR23	20 uM	94%	3%	4
HR23	20 uM	69%	2%	4
*HR23**	10 uM	89%	1%	4
*HR23**	20 uM	91%	0%	4

BT-474

Lead	Concentration	average % Inhib	1 st dev	repeats
HR2	10 uM	84%	2%	5
HR5	10 uM	37%	13%	5
HR6	10 uM	71%	1%	4
HR11	10 uM	75%	7%	4
HR23	10 uM	98%	1%	4

MCF7

Lead	Concentration	average % Inhib	1 st dev	repeats
HR6	no concentration	94%	2%	4
HR11	no concentration	96%	2%	4

NMuMG

Lead	Concentration	average % Inhib	1 st dev	repeats
HR2	10 uM	19%	10%	4
HR2	20 uM	35%	12%	4
HR23	no concentration	75%	4%	8

Figure 16

Lead	Concentration	average % Inhib	1 st dev	repeats
RZ1X	5 uM:2159-5	80%	4%	4
RZ1X	5 uM:2159-6	76%	4%	4
RZ1X	5 uM:2159-5	49%	7%	4
RZ1X	5 uM:2159-6	61%	11%	4
RZ1X	10 uM	96%	2%	7
RZ1X	10 uM:2159-1	97%	1%	4
RZ1X	10 uM:2159-1	86%	2%	4
RZ1X	20 uM	88%	N/A	
RZ1X	20 uM:2159-5	97%	3%	4
RZ1X	20 uM:2159-6	98%	1%	4
RZ1X	20 uM:2159-5	95%	1%	4
RZ1X	20 uM:2159-6	97%	1%	4
rz1	20 uM	67%	N/A	
rz4x	20 uM	-3%	N/A	
rh1x	20 uM	0%	N/A	
rzmx / rmx	20 uM	-2%	N/A	
rz6x	10 uM	91%	2%	4
RZ9X	10 uM	88%	4%	4
RZ10X	10 uM	58%	3%	4
RZ1X**	10 uM	20%	6%	4

Lead	Concentration	average % Inhib	1 st dev	repeats
RZ1X	10 uM	78%	1%	8
RZ1X	10 uM:1979-1	91%	5%	4
RZ1X	10 uM:1979-1	97%	2%	3
RZ1X	10 uM:2159-6	92%	3%	4
RZ1X	20 uM:2159-1	82%	5%	4
RZ1X	20 uM:2159-6	93%	1%	4
RZ1X	20 uM:2159-1	99%	0%	4
RZ1X	20 uM:2159-6	99%	0%	4
rz6x	10 uM	60%	3%	4
rz6x	20 uM	72%	22%	4
RZ9X	10 uM	86%	1%	4
RZ9X	10 uM	98%	1%	4
RZ9X	20 uM	93%	2%	4
RZ10X	10 uM	44%	32%	4
RZ10X	20 uM	83%	2%	4
RZ1X**	10 uM	23%	14%	4
RZ1X**	20 uM	60%	11%	4

Lead	Concentration	average % Inhib	1 st dev	repeats
RZ1X	10 uM	-1%	9%	8
RZ1X	10 uM	12%	6%	8

Lead	Concentration	average % Inhib	1 st dev	repeats
RZ1X	1 uM	0.40%	2%	4
RZ1X	3 uM	2.70%	2%	4
RZ1X	10 uM	95%	3%	8
RZ1X	10 uM:1979-1	97%	1%	3
RZ1X	10 uM:1979-1	90%	3%	4
RZ1X	10 uM:1979-1	65%	3%	4
RZ1X	10 uM:2159-6	99%	0%	4
RZ1X	20 uM:1979-1	90%	7%	3
RZ1X	20 uM:1979-1	97%	1%	4
RZ1X	20 uM:2159-6	99%	0%	4
ARZ1X	10 uM	9%	4%	4
G5RZ1X	10 uM	52%	6%	4
G21RZ1X	10 uM	14%	5%	4
RZ1*X	10 uM	1%	1.2%	4
RZ1*X	20 uM	4%	2.9%	4
RZ1X**	10 uM	35%	10%	4
RZ1X**	10 uM	65%	33%	3
RZ1X**	20 uM	89%	5%	3
RZ1-X	10 uM	70%	6.2%	4
RZ1-X	20 uM	99%	0.2%	4
RZ1uX	10 uM	1%	6.8%	4
RZ1uX	20 uM	-1%	3.4%	4
rzx	10 uM	77%	2%	8
rzmx / rmx	10 uM	29%	9%	8
erz1x	10 uM	87%	1%	8
srz1x	10 uM	65%	5%	8
drz1x	10 uM	-5%	12%	8
rz2x	10 uM	77%	2%	8
rz2x	10 uM	79%	1%	8
rz3x	10 uM	3%	6%	8
rz3x	10 uM	11%	6%	8
rz4x	10 uM	43%	4%	4
rz4x	10 uM	21%	8.6%	4
rz4x	20 uM	99%	0.1%	4
rz5x	10 uM	55%	4%	8
rz5x	10 uM	32%	3%	8
rz5x	10 uM	65%	8%	4
rz5x	20 uM	47%	25%	8
rz6x	1 uM	2%	11%	8
rz6x	2.5 uM	12%	11%	8
rz6x	5 uM	35%	10%	8
rz6x	5 uM	42%	11%	8
rz6x	10 uM	81%	3%	8
rz6x	10 uM	97%	2%	3
rz6x	10 uM	94%	4%	4
rz6x	20 uM	97%	3%	3
rz6x	36 uM	114%	8%	8
RZ6RX	10 uM	2%	3.3%	4
RZ6RX	20 uM	82%	1.6%	4

RZ6LX	10 uM	-5%	4.4%	4
RZ6LX	20 uM	99%	0.1%	4
RZ6UX	10 uM	-1%	5.9%	4
RZ6UX	20 uM	99%	0.2%	4
rz7x	10 uM	16%	23%	8
rz7x	10 uM	22%	2%	4
rz7x	10 uM	0%	5.2%	4
rz7x	20 uM	31%	1.5%	4
rz7x	20 uM	51%	12.7%	4
rz7x	40 uM	99%	0.1%	4
rz8x	10 uM	5%	8%	8
rz8x	10 uM	-3%	6.8%	4
rz8x	10 uM	15%	7.2%	4
rz8x	20 uM	98%	0.5%	4
rz8x	20 uM	97%	0.6%	4
RZ9X	10 uM	98%	0%	8
RZ9X	10 uM	99%	0%	4
RZ9X	10 uM	95%	0%	3
RZ9X	20 uM	93%	0%	3
RZ10X	10 uM	86%	14%	8
RZ10X	10 uM	96%	1%	3
RZ10X	10 uM	80%	7%	4
RZ10X	20 uM	94%	3%	3
RZ11X	10 uM	5%	5.0%	4
RZ11X	20 uM	99%	0.3%	4

Lead	Concentration	average % Inhib	1 st dev	repeats
RZ1X	10 uM	22%	11%	8

Lead	Concentration	average % Inhib	1 st dev	repeats
RZ1X	1 uM post 8 hrs	3.28%	16%	4
RZ1X	1 uM post 16 hrs	1.96%	5%	4
RZ1X	1 uM post 24 hrs	-0.81%	14%	4
RZ1X	1 uM post 32 hrs	5.20%	6%	4
RZ1X	1 uM post 40 hrs	5.51%	6%	4
RZ1X	1 uM post 48 hrs	6.49%	6%	4
RZ1X	3 uM post 8 hrs	9.84%	16%	4
RZ1X	3 uM post 16 hrs	6.86%	12%	4
RZ1X	3 uM post 24 hrs	5.65%	14%	4
RZ1X	3 uM post 32 hrs	5.20%	7%	4
RZ1X	3 uM post 40 hrs	6.99%	9%	4
RZ1X	3 uM post 48 hrs	11.36%	3%	4
RZ1X	5 uM day 1	53%	N/A	1
RZ1X	10 uM post 8 hrs	29.51%	3%	4
RZ1X	10 uM post 16 hr	35.29%	10%	4
RZ1X	10 uM post 24 hr	28.23%	14%	4
RZ1X	10 uM post 32 hr	38.15%	6%	4
RZ1X	10 uM post 40 hr	56.99%	5%	4
RZ1X	10 uM post 48 hr	62.01%	4%	4
RZ1**X	10 uM	-8%	N/A	
rz6x	10 uM	79%	N/A	

RZ9X	10 uM	90%	N/A	

Lead	Concentration	average % Inhib	1 st dev	repeats
erz1x	10 uM	83%	1%	8

Lead	Concentration	average % Inhib	1 st dev	repeats
RZ1X	10 uM: 1979-1	95%	4%	4
RZ1X	20 uM: 1979-1	98%	1%	4
RZ1X**	10 uM	15%	13%	4
RZ1X**	20 uM	37%	5%	4
RZ9X	10 uM	97%	1%	4
RZ9X	20 uM	98%	1%	4

Lead	Concentration	average % Inhib	1 st dev	repeats
RZ1X	10 uM:1979-1	77%	4%	4
RZ1X**	10 uM	2%	2%	4
rz6x	10 uM	56%	4%	4
rz6x	10 uM	91%	1%	4
RZ9X	10 uM	62%	4%	4
RZ10X	10 uM	21%	2%	4

Lead	Concentration	average % Inhib	1 st dev	repeats

Lead	Concentration	average % Inhib	1 st dev	repeats
RZ1X at 10 uM	10 uM	3.70%	7%	8
RZ1X	10 uM:2159-5	14%	7%	4
RZ1X	20 uM:2159-5	36%	24%	4
RZ1X	10 uM:2159-5, 2‹	13.00%	47%	4
RZ1X	10 uM:2159-5, 9(	36.00%	24%	4

Lead	Concentration	average % Inhib	1 st dev	repeats
RZ1X	1 uM	7%	2%	4
RZ1X	3 uM	23%	4%	4
RZ1X	10 uM	66%	4%	4

Figure 17

MCF7

Lead	Concentration	average % Inhib	1 st dev	repeats
cm1	10 uM	21%	15%	7
cm1	10 uM	38%	12%	8
cm2	10 uM	75%	17%	7
cm2	10 uM	54%	4%	8
cm3	10 uM	86%	3%	7
cm3	10 uM	71%	2%	8
cm3	10 uM	70%	2%	4
cm4	1 uM	6%	5%	4
cm4	3 uM	-9%	4%	4
cm4	6 uM	7%	17%	4
cm4	10 uM	87%	6%	7
cm4	10 uM	71%	2%	8
cm4	10 uM	84%	2%	4
cm4	10 uM	24%	9%	4
cm4	20 uM	45%	5%	4
cm5	10 uM	27%	9%	4
cm5	20 uM	57%	6%	4
cm6	10 uM	88%	2%	4
cm6	20 uM	93%	1%	4
cm7	10 uM	88%	4%	4
cm7	20 uM	93%	0%	4

MCF10a

Lead	Concentration	average % Inhib	1 st dev	repeats
cm1	10 uM	13%	16%	8
cm2	10 uM	69%	2%	8
cm3	10 uM	58%	5%	8
cm3	10 uM	73%	1%	8
cm4	10 uM	56%	4%	8
cm4	10 uM	74%	1%	8

MCF10ca1a

Lead	Concentration	average % Inhib	1 st dev	repeats
cm3	10 uM	55%	6%	4
cm3	20 uM	75%	9%	4
cm4	10 uM	83%	4%	4
cm4	10 uM	93%	4%	4
cm4	20 uM	89%	3%	4

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Lead	Concentration	average % Inhib	1 st dev	repeats
cm1	10 uM	-8%	10%	8
cm3	10 uM	17%	7%	4
cm3	20 uM	95%	1%	4
mac3G	5 uM	5%	4.9%	4
mac3G	10 uM	26%	4.5%	4
mac3G	20 uM	99%	0.1%	4
cm4	1 uM#3	6%	2%	4

cm4	3 uM#3	9%	4%	4
cm4	10 uM	33%	6%	4
cm4	10 uM#3	62%	6%	4
cm4	10 uM	12%	6.2%	4
cm4	20 uM#3	78%	3%	4
cm4	20 uM	64%	3.9%	4
cm5	10 uM	9%	9.0%	4
cm5	20 uM	80%	2.3%	4
cm6	10 uM	19%	2.6%	4
cm6	20 uM	100%	0.0%	4
cm7	10 uM	84%	9.2%	4
cm7	20 uM	100%	0.1%	4

MDA-MB 435 erb

Lead	Concentration	average % Inhib	1 st dev	repeats
cm3	10 uM	11%	4%	8

FSCCL

Lead	Concentration	average % Inhib	1 st dev	repeats
cm3	no concentration	66%	N/A	1
cm4	no concentration	71%	N/A	1
MAC-M4	1 uM post 8 hrs	-8%	10%	4
MAC-M4	1 uM post 16 hrs	22%	16%	4
MAC-M4	1 uM post 24 hrs	17%	16%	4
MAC-M4	1 uM post 32 hrs	19%	9%	4
MAC-M4	1 uM post 48 hrs	24%	13%	4
MAC-M4	3 uM post 8 hrs	-15%	15%	4
MAC-M4	3 uM post 16 hrs	29%	8%	4
MAC-M4	3 uM post 24 hrs	50%	8%	4
MAC-M4	3 uM post 32 hrs	49%	2%	4
MAC-M4	3 uM post 48 hrs	51%	8%	4
MAC-M4	10 uM post 8 hrs	8%	32%	4
MAC-M4	10 uM post 16 hr	94%	5%	4
MAC-M4	10 uM post 24 hr	100%	0%	4
MAC-M4	10 uM post 32 hr	100%	0%	4
MAC-M4	10 uM post 48 hr	100%	0%	4

T47D

Lead	Concentration	average % Inhib	1 st dev	repeats
cm3	no concentration	42%	5%	4
cm4	no concentration	61%	5%	4

Myc-mt-1

Lead	Concentration	average % Inhib	1 st dev	repeats
mac3G	5 uM	18%	7.6%	
mac3G	10 uM	42%	15.6%	
mac3G	20 uM	80%	1.2%	
cm4	1 uM#3	14.7%	6%	
cm4	3 uM#3	22.3%	5%	
cm4	10 uM#3	54.6%	2%	
cm4	20 uM#3	88.4%	1%	
cm7	20 uM	86%	1.2%	

Figure 18

Lead	Concentration	average % Inhib	1 st dev	repeats
tga1	10 uM	-3%	5%	8
tga2	10 uM	1%	2%	8
tga3	10 uM	63%	8%	8
tga3	10 uM	94%	1%	2
tga4	10 uM	-9%	9%	8
tga4	10 uM	-3%	1%	4
tga5	10 uM	-2%	3%	8
tga5	10 uM	69%	8.7%	4
tga5	20 uM	100%	0.1%	4
tga6	10 uM	-5%	2%	8
tga7	10 uM	7%	2%	8
tga8	1 uM	-7%	17%	8
tga8	1 uM	13%	10%	8
tga8	2.5 uM	0%	14%	8
tga8	5 uM	12%	10%	8
tga8	5 uM	79%	14%	8
tga8	10 uM	89%	5%	8
tga8	10 uM	88%	1%	3
tga8	10 uM	78%	6.1%	4
tga8	20 uM	94%	3%	3
tga8	20 uM	100%	0.1%	4
tga9	10 uM	67%	3%	8
tga10	1 uM	20%	3%	4
tga10	2.5 uM	27%	3%	4
tga10	5 uM	36%	2%	4
tga10	10 uM	84%	2%	8
tga10	10 uM	95%	2%	3
tga10	10 uM	61%	2%	4
tga10	20 uM	96%	2%	3
tga10	20 uM	79%	2%	4
tga11	1 uM	-1%	10%	8
tga11	1 uM	-3%	17%	8
tga11	1 uM	-19%	7%	8
tga11	2 uM	-12%	13%	8
tga11	2.5 uM	-3%	15%	8
tga11	5 uM	-4%	10%	8
tga11	5 uM	50%	23%	8
tga11	5 uM	-29%	20%	8
tga11	10 uM	86%	3%	8
tga11	10 uM	93%	6%	3
tga11	10 uM	98%	1%	4
tga11	20 uM	97%	2%	3
tga11	20 uM	99%	0.4%	4
tga12	10 uM	59%	2%	8
tga12	10 uM	99%	1%	2
tga13	10 uM	71%	17%	8
tga13	10 uM	99%	1%	3

Lead	Concentration	average % Inhib	1 st dev	repeats

tga5	20 uM	92%	1%	4
tga5	40 uM	99%	0%	4
tga8	10 uM	17%	2%	4
tga8	20 uM	93%	1%	4
tga8	40 uM	99%	0%	4
tga10	1 uM	6%	9%	4
tga10	2.5 uM	16%	6%	4
tga10	5 uM	53%	2%	4
tga10	10 uM	95%	2%	4
tga10	10 uM	76%	2%	4
tga10	20 uM	76%	2%	4
tga10	40 uM	84%	1%	4
tga11	2 uM	26%	7%	4
tga11	6 uM	24%	1%	4
tga11	10 uM	79%	5%	4
tga11	20 uM	61%	16%	4
tga11	20 uM	53%	7%	4
tga11	40 uM	93%	1%	4

Lead	**Concentration**	**average % Inhib**	**1 st dev**	**repeats**
tga10	10 uM	73%	11%	8

Figure 19

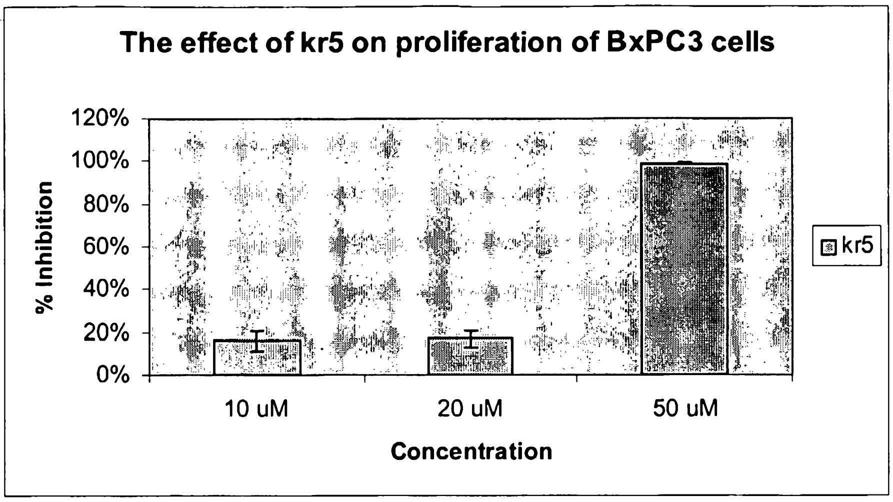
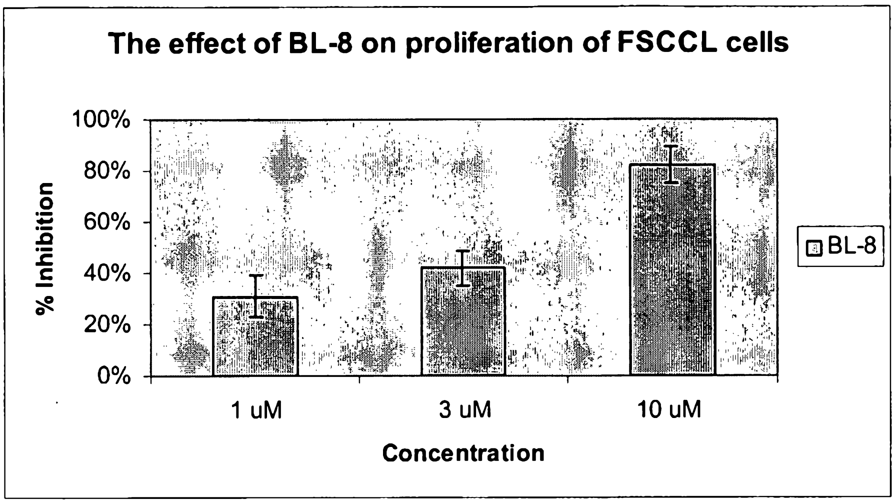
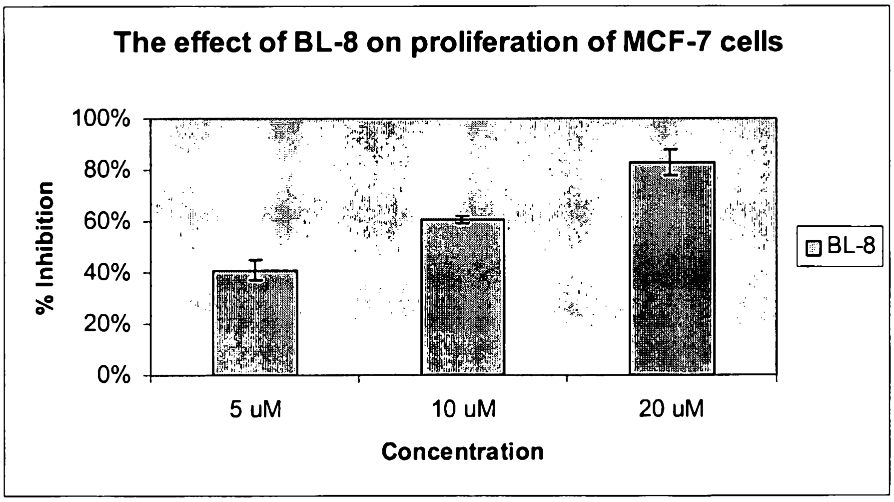

The effect of kr5 on proliferation of BxPC3 cells
120%
100%
80%
60%
40%
20%
0%
% Inhibition
10 uM
20 uM
50 uM
Concentration
kr5

Figure 20


The effect of BL-8 on proliferation of FSCCL cells
100%
80%
60%
40%
20%
0%
% Inhibition
1 uM
3 uM
10 uM
Concentration
BL-8

A


The effect of BL-8 on proliferation of MCF-7 cells
100%
80%
60%
40%
20%
0%
% Inhibition
5 uM
10 uM
20 uM
Concentration
BL-8

B

Figure 21

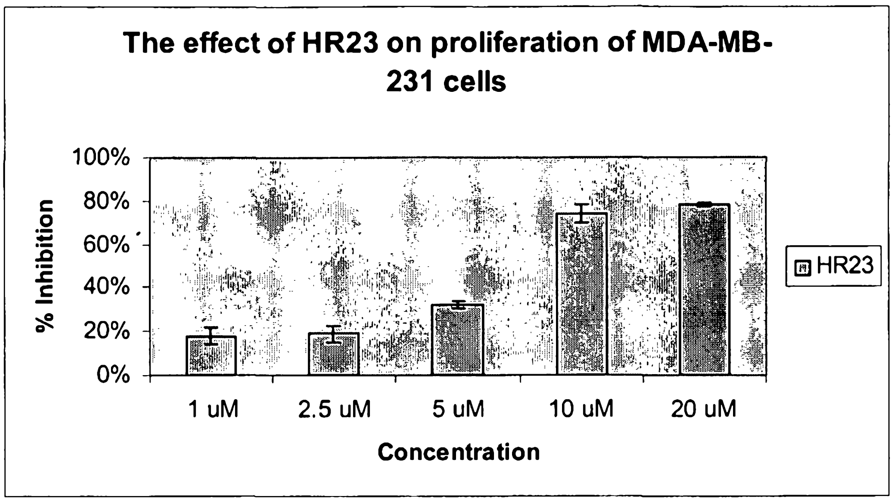

The effect of HR23 on proliferation of MDA-MB-231 cells
100%
80%
60%
40%
20%
0%
% Inhibition
1 uM
2.5 uM
5 uM
10 uM
20 uM
Concentration
HR23

Figure 22

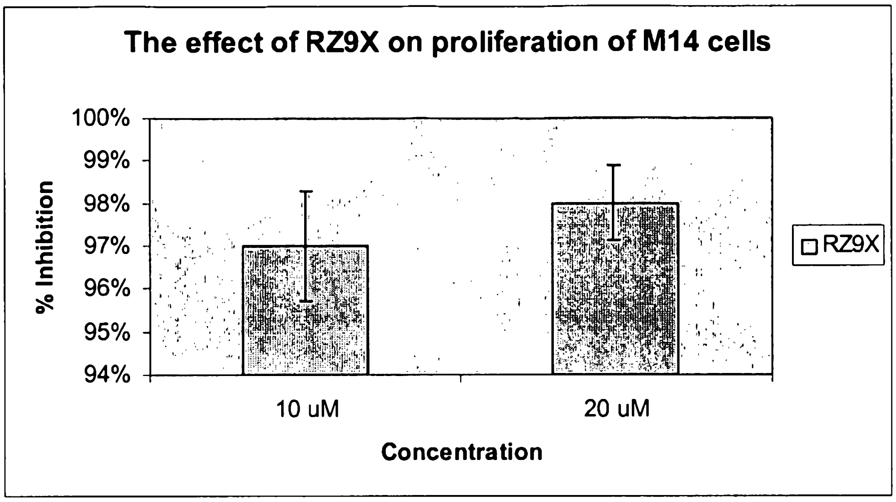

The effect of RZ9X on proliferation of M14 cells
100%
99%
98%
97%
96%
95%
94%
% Inhibition
10 uM
20 uM
Concentration
RZ9X

Figure 23

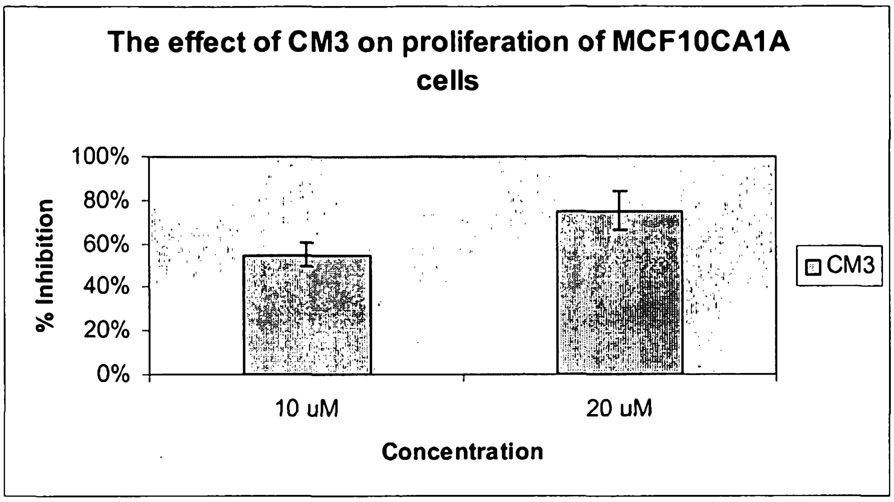
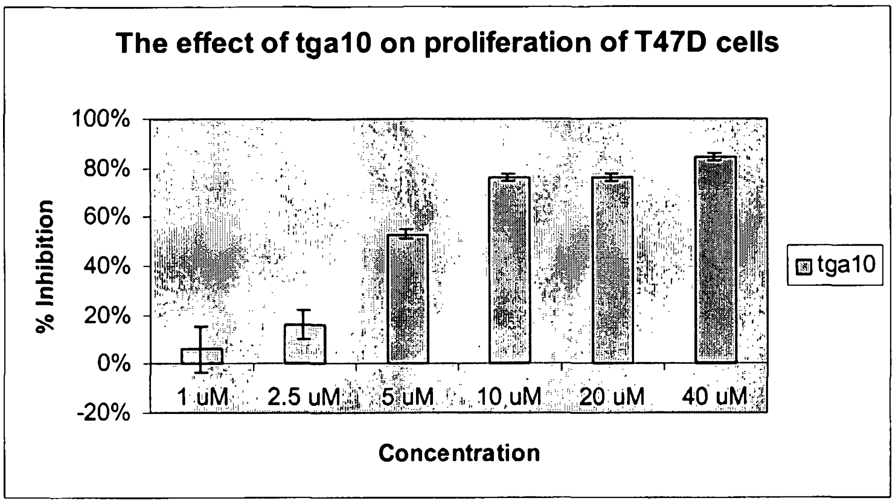
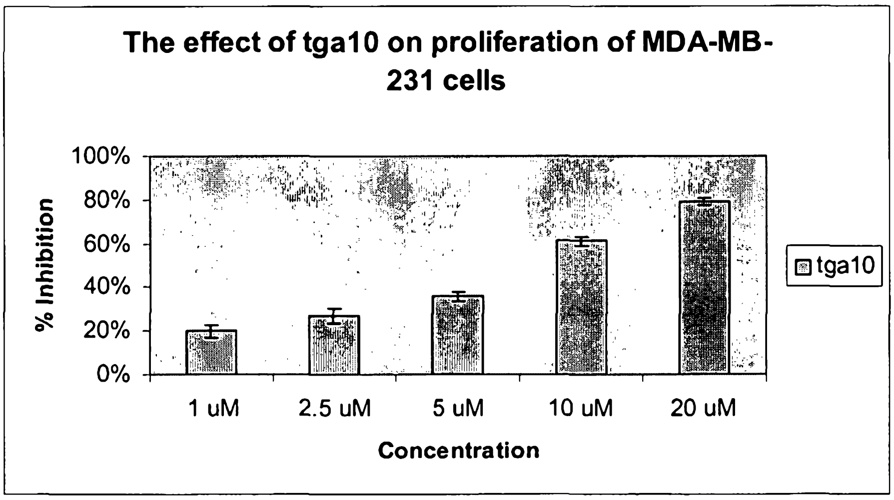

The effect of CM3 on proliferation of MCF10CA1A cells
100%
80%
60%
40%
20%
0%
% Inhibition
10 uM
20 uM
Concentration
CM3

Figure 24


The effect of tga10 on proliferation of T47D cells
100%
80%
60%
40%
20%
0%
-20%
% Inhibition
1 uM
2.5 uM
5 uM
10 uM
20 uM
40 uM
Concentration
tga10

A


The effect of tga10 on proliferation of MDA-MB-231 cells
100%
80%
60%
40%
20%
0%
% Inhibition
1 uM
2.5 uM
5 uM
10 uM
20 uM
Concentration
tga10

B

Figure 25

KR1 102-124
5'-CCXGGAGXGGGACXGGACXGXGG-3' (SEQ ID NO:707)
ACCXGGAGXGGGACXGGACXGXGG (SEQ ID NO:708)
GACCXGGAGXGGGACXGGACXGXGG (SEQ ID NO:709)
TGACCXGGAGXGGGACXGGACXGXGG (SEQ ID NO:710)
CTGACCXGGAGXGGGACXGGACXGXGG (SEQ ID NO:711)
TCTGACCXGGAGXGGGACXGGACXGXGG (SEQ ID NO:712)
CCXGGAGXGGGACXGGACXGXGGC (SEQ ID NO:713)
CCXGGAGXGGGACXGGACXGXGGCG (SEQ ID NO:714)
CCXGGAGXGGGACXGGACXGXGGCGG (SEQ ID NO:715)
CCXGGAGXGGGACXGGACXGXGGCGGG (SEQ ID NO:716)
CCXGGAGXGGGACXGGACXGXGGCGGGC (SEQ ID NO:717)
ACCXGGAGXGGGACXGGACXGXGGC (SEQ ID NO:718)
GACCXGGAGXGGGACXGGACXGXGGCG (SEQ ID NO:719)
TGACCXGGAGXGGGACXGGACXGXGGCGG (SEQ ID NO:720)
CTGACCXGGAGXGGGACXGGACXGXGGCGGG (SEQ ID NO:721)
TCTGACCXGGAGXGGGACXGGACXGXGGCGGGC (SEQ ID NO:722)
TGACCXGGAGXGGGACXGGACXG (SEQ ID NO:723)
TTCTGACCXGGAGXGGGACXGGA (SEQ ID NO:724)
CAATTCTGACCXGGAGXGGGACX (SEQ ID NO:725)
CGCCAATTCTGACCXGGAGXGGG (SEQ ID NO:726)
AGCCGCCAATTCTGACCXGGAGX (SEQ ID NO:727)
GGAGXGGGACXGGACXGXGGCGG (SEQ ID NO:728)
GXGGGACXGGACXGXGGCGGGCT (SEQ ID NO:729)
GGACXGGACXGXGGCGGGCTGTG (SEQ ID NO:730)
CXGGACXGXGGCGGGCTGTGCGG (SEQ ID NO:731)
GACXGXGGCGGGCTGTGCGGATG (SEQ ID NO:732)
XGGAGXGGGACXGGACXGXGG (SEQ ID NO:733)
GAGXGGGACXGGACXGXGG (SEQ ID NO:734)
GXGGGACXGGACXGXGG (SEQ ID NO:735)
GGGACXGGACXGXGG (SEQ ID NO:736)
CCXGGAGXGGGACXGGACXGX (SEQ ID NO:737)
CCXGGAGXGGGACXGGACX (SEQ ID NO:738)
CCXGGAGXGGGACXGGA (SEQ ID NO:739)
CCXGGAGXGGGACXG (SEQ ID NO:740)
CCXGGAGXCGGACXGGACXGXGG (SEQ ID NO:741)
CCXGGAGXGGGACXGGACXAXGG (SEQ ID NO:742)
CCXTGAGXGGGACXGGACXGXGG (SEQ ID NO:743)

KR2 168-191
5'-GCXGGACCCAXGXGGXGGCCXGCC-3' (SEQ ID NO:744)
TGCXGGACCCAXGXGGXGGCCXGCC (SEQ ID NO:745)
CTGCXGGACCCAXGXGGXGGCCXGCC (SEQ ID NO:746)
ACTGCXGGACCCAXGXGGXGGCCXGCC (SEQ ID NO:747)
GACTGCXGGACCCAXGXGGXGGCCXGCC (SEQ ID NO:748)
GGACTGCXGGACCCAXGXGGXGGCCXGCC (SEQ ID NO:749)
GCXGGACCCAXGXGGXGGCCXGCCC (SEQ ID NO:750)
GCXGGACCCAXGXGGXGGCCXGCCCC (SEQ ID NO:751)
GCXGGACCCAXGXGGXGGCCXGCCCCC (SEQ ID NO:752)
GCXGGACCCAXGXGGXGGCCXGCCCCCT (SEQ ID NO:753)
GCXGGACCCAXGXGGXGGCCXGCCCCCTG (SEQ ID NO:754)
TGCXGGACCCAXGXGGXGGCCXGCCC (SEQ ID NO:755)
CTGCXGGACCCAXGXGGXGGCCXGCCCC (SEQ ID NO:756)
ACTGCXGGACCCAXGXGGXGGCCXGCCCCC (SEQ ID NO:757)
GACTGCXGGACCCAXGXGGXGGCCXGCCCCCT (SEQ ID NO:758)
GGACTGCXGGACCCAXGXGGXGGCCXGCCCCCTG (SEQ ID NO:759)
ACTGCXGGACCCAXGXGGXGGCCX (SEQ ID NO:760)

GGGACTGCXGGACCCAXGXGGXGG (SEQ ID NO:761)
GGAGGGACTGCXGGACCCAXGXGG (SEQ ID NO:762)
GGAGGAGGGACTGCXGGACCCAXG (SEQ ID NO:763)
GCGGGAGGAGGGACTGCXGGACCC (SEQ ID NO:764)
GGACCCAXGXGGXGGCCXGCCCCC (SEQ ID NO:765)
CCCAXGXGGXGGCCXGCCCCCTGC (SEQ ID NO:766)
AXGXGGXGGCCXGCCCCCTGCCTA (SEQ ID NO:767)
XGGXGGCCXGCCCCCTGCCTAGCC (SEQ ID NO:768)
XGGCCXGCCCCCTGCCTAGCCGCA (SEQ ID NO:769)
XGGACCCAXGXGGXGGCCXGCC (SEQ ID NO:770)
GACCCAXGXGGXGGCCXGCC (SEQ ID NO:771)
CCCAXGXGGXGGCCXGCC (SEQ ID NO:772)
CAXGXGGXGGCCXGCC (SEQ ID NO:773)
AXGXGGXGGCCXGCC (SEQ ID NO:774)
GCXGGACCCAXGXGGXGGCCXG (SEQ ID NO:775)
GCXGGACCCAXGXGGXGGCC (SEQ ID NO:776)
GCXGGACCCAXGXGGXGG (SEQ ID NO:777)
GCXGGACCCAXGXGGX (SEQ ID NO:778)
GCXGGACCCAXGXGG (SEQ ID NO:779)
GCXGGGCCCAXGXGGXGGCCXGCC (SEQ ID NO:780)
GCXGGACCCAXGXTGXGGCCXGCC (SEQ ID NO:781)
GCXGGACCCAXGXGGXGGTCXGCC (SEQ ID NO:782)

KR4 532-558
5'-TTXGCXGCXGCCACTGCXGCXGCXGCT-3' (SEQ ID NO:783)
CTTXGCXGCXGCCACTGCXGCXGCXGCT (SEQ ID NO:784)
CCTTXGCXGCXGCCACTGCXGCXGCXGCT (SEQ ID NO:785)
ACCTTXGCXGCXGCCACTGCXGCXGCXGCT (SEQ ID NO:786)
CACCTTXGCXGCXGCCACTGCXGCXGCXGCT (SEQ ID NO:787)
CCACCTTXGCXGCXGCCACTGCXGCXGCXGCT (SEQ ID NO:788)
TTXGCXGCXGCCACTGCXGCXGCXGCTG (SEQ ID NO:789)
TTXGCXGCXGCCACTGCXGCXGCXGCTGC (SEQ ID NO:790)
TTXGCXGCXGCCACTGCXGCXGCXGCTGCT (SEQ ID NO:791)
TTXGCXGCXGCCACTGCXGCXGCXGCTGCTG (SEQ ID NO:792)
TTXGCXGCXGCCACTGCXGCXGCXGCTGCTGC (SEQ ID NO:793)
CTTXGCXGCXGCCACTGCXGCXGCXGCTG (SEQ ID NO:794)
CCTTXGCXGCXGCCACTGCXGCXGCXGCTGC (SEQ ID NO:795)
ACCTTXGCXGCXGCCACTGCXGCXGCXGCTGCT (SEQ ID NO:796)
CACCTTXGCXGCXGCCACTGCXGCXGCXGCTGCTG (SEQ ID NO:797)
CCACCTTXGCXGCXGCCACTGCXGCXGCXGCTGCTGC (SEQ ID NO:798)
ACCTTXGCXGCXGCCACTGCXGCXGCX (SEQ ID NO:799)
GCCACCTTXGCXGCXGCCACTGCXGCX (SEQ ID NO:800)
GCCGCCACCTTXGCXGCXGCCACTGCX (SEQ ID NO:801)
GCCGCCGCCACCTTXGCXGCXGCCACT (SEQ ID NO:802)
CGAGCCGCCGCCACCTTXGCXGCXGCC (SEQ ID NO:803)
GCXGCXGCCACTGCXGCXGCXGCTGCT (SEQ ID NO:804)
GCXGCCACTGCXGCXGCXGCTGCTGCC (SEQ ID NO:805)
GCCACTGCXGCXGCXGCTGCTGCCTCC (SEQ ID NO:806)
ACTGCXGCXGCXGCTGCTGCCTCCGCC (SEQ ID NO:807)
GCXGCXGCXGCTGCTGCCTCCGCCGCC (SEQ ID NO:808)
XGCXGCXGCCACTGCXGCXGCXGCT (SEQ ID NO:809)
CXGCXGCCACTGCXGCXGCXGCT (SEQ ID NO:810)
GCXGCCACTGCXGCXGCXGCT (SEQ ID NO:811)
XGCCACTGCXGCXGCXGCT (SEQ ID NO:812)
CCACTGCXGCXGCXGCT (SEQ ID NO:813)
ACTGCXGCXGCXGCT (SEQ ID NO:814)
TTXGCXGCXGCCACTGCXGCXGCXG (SEQ ID NO:815)
TTXGCXGCXGCCACTGCXGCXGC (SEQ ID NO:816)
TTXGCXGCXGCCACTGCXGCX (SEQ ID NO:817)
TTXGCXGCXGCCACTGCXG (SEQ ID NO:818)
TTXGCXGCXGCCACTGC (SEQ ID NO:819)

TTXGCXGCXGCCACT (SEQ ID NO:820)
TTXACXGCXGCCACTGCXGCXGCXGCT (SEQ ID NO:821)
TTXGCXGCXGCCACTCCXGCXGCXGCT (SEQ ID NO:822)
TTXGCXGCXGTCACTGCXGCXGCXGCT (SEQ ID NO:823)

KR7 40-67
5'-GCGGCCACGCCGCCCCGCTGACCGGTCT-3' (SEQ ID NO:824)
AGCGGCCACGCCGCCCCGCTGACCGGTCT (SEQ ID NO:825)
GAGCGGCCACGCCGCCCCGCTGACCGGTCT (SEQ ID NO:826)
CGAGCGGCCACGCCGCCCCGCTGACCGGTCT (SEQ ID NO:827)
GCGAGCGGCCACGCCGCCCCGCTGACCGGTCT (SEQ ID NO:828)
CGCGAGCGGCCACGCCGCCCCGCTGACCGGTCT (SEQ ID NO:820)
GCGGCCACGCCGCCCCGCTGACCGGTCTC (SEQ ID NO:830)
GCGGCCACGCCGCCCCGCTGACCGGTCTCC (SEQ ID NO:831)
GCGGCCACGCCGCCCCGCTGACCGGTCTCCA (SEQ ID NO:832)
GCGGCCACGCCGCCCCGCTGACCGGTCTCCAC (SEQ ID NO:833)
GCGGCCACGCCGCCCCGCTGACCGGTCTCCACA (SEQ ID NO:834)
AGCGGCCACGCCGCCCCGCTGACCGGTCTC (SEQ ID NO:835)
GAGCGGCCACGCCGCCCCGCTGACCGGTCTCC (SEQ ID NO:836)
CGAGCGGCCACGCCGCCCCGCTGACCGGTCTCCA (SEQ ID NO:837)
GCGAGCGGCCACGCCGCCCCGCTGACCGGTCTCCAC (SEQ ID NO:838)
CGCGAGCGGCCACGCCGCCCCGCTGACCGGTCTCCACA (SEQ ID NO:839)
CGAGCGGCCACGCCGCCCCGCTGACCGG (SEQ ID NO:840)
CCGCGAGCGGCCACGCCGCCCCGCTGAC (SEQ ID NO:841)
ACGCCGCGAGCGGCCACGCCGCCCCGCT (SEQ ID NO:842)
GAGACGCCGCGAGCGGCCACGCCGCCCC (SEQ ID NO:843)
AGGGAGACGCCGCGAGCGGCCACGCCGC (SEQ ID NO:844)
GCCACGCCGCCCCGCTGACCGGTCTCCA (SEQ ID NO:845)
ACGCCGCCCCGCTGACCGGTCTCCACAG (SEQ ID NO:846)
CCGCCCCGCTGACCGGTCTCCACAGAGA (SEQ ID NO:847)
CCCCGCTGACCGGTCTCCACAGAGAAGC (SEQ ID NO:848)
CGCTGACCGGTCTCCACAGAGAAGCTGC (SEQ ID NO:849)
GGCCACGCCGCCCCGCTGACCGGTCT (SEQ ID NO:850)
CCACGCCGCCCCGCTGACCGGTCT (SEQ ID NO:851)
ACGCCGCCCCGCTGACCGGTCT (SEQ ID NO:852)
GCCGCCCCGCTGACCGGTCT (SEQ ID NO:853)
CGCCCCGCTGACCGGTCT (SEQ ID NO:854)
CCCCGCTGACCGGTCT (SEQ ID NO:855)
CCCGCTGACCGGTCT (SEQ ID NO:856)
GCGGCCACGCCGCCCCGCTGACCGGT (SEQ ID NO:857)
GCGGCCACGCCGCCCCGCTGACCG (SEQ ID NO:858)
GCGGCCACGCCGCCCCGCTGAC (SEQ ID NO:858)
GCGGCCACGCCGCCCCGCTG (SEQ ID NO:860)
GCGGCCACGCCGCCCCGC (SEQ ID NO:861)
GCGGCCACGCCGCCCC (SEQ ID NO:862)
GCGGCCACGCCGCCC (SEQ ID NO:863)
GCGGCCACGACGCCCCGCTGACCGGTCT (SEQ ID NO:864)
GCGGCCACGCCGCTCCGCTGACCGGTCT (SEQ ID NO:865)
GCGGCCACGCCGCCCCGCGGACCGGTCT (SEQ ID NO:866)

Figure 26

BL2 483-506**
5'- CAXGC AXGXG CATCC CXGCC XGTG -3' (SEQ ID NO:163)
ACAXGC AXGXG CATCC CXGCC XGTG (SEQ ID NO:164)
CACAXGC AXGXG CATCC CXGCC XGTG (SEQ ID NO:165)
ACACAXGC AXGXG CATCC CXGCC XGTG (SEQ ID NO:166)
TACACAXGC AXGXG CATCC CXGCC XGTG (SEQ ID NO:167)
CTACACAXGC AXGXG CATCC CXGCC XGTG (SEQ ID NO:168)
CAXGC AXGXG CATCC CXGCC XGTGT (SEQ ID NO:169)
CAXGC AXGXG CATCC CXGCC XGTGTC (SEQ ID NO:170)
CAXGC AXGXG CATCC CXGCC XGTGTCC (SEQ ID NO:171)
CAXGC AXGXG CATCC CXGCC XGTGTCCA (SEQ ID NO:172)
CAXGC AXGXG CATCC CXGCC XGTGTCCAC (SEQ ID NO:173)
ACAXGC AXGXG CATCC CXGCC XGTGT (SEQ ID NO:174)
CACAXGC AXGXG CATCC CXGCC XGTGTC (SEQ ID NO:175)
ACACAXGC AXGXG CATCC CXGCC XGTGTCC (SEQ ID NO:176)
TACACAXGC AXGXG CATCC CXGCC XGTGTCCA (SEQ ID NO:177)
CTACACAXGC AXGXG CATCC CXGCC XGTGTCCAC (SEQ ID NO:178)
ACACAXGCAXGXGCATCCCXGCCX (SEQ ID NO:179)
ACTACACAXGCAXGXGCATCCCXG (SEQ ID NO:180)
CGCACTACACAXGCAXGXGCATCC (SEQ ID NO:181)
CCGCGCACTACACAXGCAXGXGCA (SEQ ID NO:182)
TGTCCGCGCACTACACAXGCAXGX (SEQ ID NO:183)
GCAXGXGCATCCCXGCCXGTGTCC (SEQ ID NO:184)
XGXGCATCCCXGCCXGTGTCCACC (SEQ ID NO:185)
GCATCCCXGCCXGTGTCCACCTGA (SEQ ID NO:186)
TCCCXGCCXGTGTCCACCTGAACA (SEQ ID NO:187)
CXGCCXGTGTCCACCTGAACACCT (SEQ ID NO:188)
XGCAXGXGCATCCCXGCCXGTG (SEQ ID NO:189)
CAXGXGCATCCCXGCCXGTG (SEQ ID NO:190)
XGXGCATCCCXGCCXGTG (SEQ ID NO:191)
XGCATCCCXGCCXGTG (SEQ ID NO:192)
GCATCCCXGCCXGTG (SEQ ID NO:193)
CAXGCAXGXGCATCCCXGCCXG (SEQ ID NO:194)
CAXGCAXGXGCATCCCXGCC (SEQ ID NO:195)
CAXGCAXGXGCATCCCXG (SEQ ID NO:196)
CAXGCAXGXGCATCCC (SEQ ID NO:197)
CAXGCAXGXGCATCC (SEQ ID NO:198)
CAXGCAXGXGTATCCCXGCCXGTG (SEQ ID NO:199)
CAXGCGXGXGCATCCCXGCCXGTG (SEQ ID NO:200)
CAXGCAXGXGCATCCCXGACXGTG (SEQ ID NO:201)

BL6 1102-1127
5'-CCXGC CXGCT CXGCT GXGCC XGXGG G-3' (SEQ ID NO:202)
ACCXGC CXGCT CXGCT GXGCC XGXGG G (SEQ ID NO:203)
CACCXGC CXGCT CXGCT GXGCC XGXGG G (SEQ ID NO:204)
CCACCXGC CXGCT CXGCT GXGCC XGXGG G (SEQ ID NO:205)
GCCACCXGC CXGCT CXGCT GXGCC XGXGG G (SEQ ID NO:206)
GGCCACCXGC CXGCT CXGCT GXGCC XGXGG G (SEQ ID NO:207)
CCXGC CXGCT CXGCT GXGCC XGXGG GG (SEQ ID NO:208)
CCXGC CXGCT CXGCT GXGCC XGXGG GGC (SEQ ID NO:209)
CCXGC CXGCT CXGCT GXGCC XGXGG GGCC (SEQ ID NO:210)
CCXGC CXGCT CXGCT GXGCC XGXGG GGCCC (SEQ ID NO:211)
CCXGC CXGCT CXGCT GXGCC XGXGG GGCCCG (SEQ ID NO:212)
ACCXGC CXGCT CXGCT GXGCC XGXGG GG (SEQ ID NO:213)
CACCXGC CXGCT CXGCT GXGCC XGXGG GGC (SEQ ID NO:214)
CCACCXGC CXGCT CXGCT GXGCC XGXGG GGCC (SEQ ID NO:215)

GCCACCXGC CXGCT CXGCT GXGCC XGXGG GGCCC (SEQ ID NO:216)
GGCCACCXGC CXGCT CXGCT GXGCC XGXGG GGCCCG (SEQ ID NO:217)
CCACCXGCCXGCTCXGCTGXGCCXGX (SEQ ID NO:218)
CGGCCACCXGCCXGCTCXGCTGXGCC (SEQ ID NO:219)
GGCCGGCCACCXGCCXGCTCXGCTGX (SEQ ID NO:220)
CCGGGCCGGCCACCXGCCXGCTCXGC (SEQ ID NO:221)
CCTCCGGGCCGGCCACCXGCCXGCTC (SEQ ID NO:222)
GCCXGCTCXGCTGXGCCXGXGGGGCC (SEQ ID NO:223)
XGCTCXGCTGXGCCXGXGGGGCCCGG (SEQ ID NO:224)
TCXGCTGXGCCXGXGGGGCCCGGCCA (SEQ ID NO:225)
GCTGXGCCXGXGGGGCCCGGCCAGTG (SEQ ID NO:226)
GXGCCXGXGGGGCCCGGCCAGTGGGT (SEQ ID NO:227)
XGCCXGCTCXGCTGXGCCXGXGGG (SEQ ID NO:228)
CCXGCTCXGCTGXGCCXGXGGG (SEQ ID NO:229)
XGCTCXGCTGXGCCXGXGGG (SEQ ID NO:230)
CTCXGCTGXGCCXGXGGG (SEQ ID NO:231)
CXGCTGXGCCXGXGGG (SEQ ID NO:232)
XGCTGXGCCXGXGGG (SEQ ID NO:233)
CCXGCCXGCTCXGCTGXGCCXGXG (SEQ ID NO:234)
CCXGCCXGCTCXGCTGXGCCXG (SEQ ID NO:235)
CCXGCCXGCTCXGCTGXGCC (SEQ ID NO:236)
CCXGCCXGCTCXGCTGXG (SEQ ID NO:237)
CCXGCCXGCTCXGCTG (SEQ ID NO:238)
CCXGCCXGCTCXGCT (SEQ ID NO:239)
CCXGCCXGCCCXGCTGXGCCXGXGGG (SEQ ID NO:240)
CCXGTCXGCTCXGCTGXGCCXGXGGG (SEQ ID NO:241)
CCXGCCXGCTCXGCTGXGGCXGXGGG (SEQ ID NO:242)

BL7 1150-1170
5'- GGXGX GXGGG GCXGG GCXGG G -3'(SEQ ID NO:243)
TGGXGX GXGGG GCXGG GCXGG G (SEQ ID NO:244)
ATGGXGX GXGGG GCXGG GCXGG G (SEQ ID NO:245)
CATGGXGX GXGGG GCXGG GCXGG G (SEQ ID NO:246)
ACATGGXGX GXGGG GCXGG GCXGG G (SEQ ID NO:247)
CACATGGXGX GXGGG GCXGG GCXGG G (SEQ ID NO:248)
GGXGX GXGGG GCXGG GCXGG GG (SEQ ID NO:249)
GGXGX GXGGG GCXGG GCXGG GGA (SEQ ID NO:250)
GGXGX GXGGG GCXGG GCXGG GGAG (SEQ ID NO:251)
GGXGX GXGGG GCXGG GCXGG GGAGG (SEQ ID NO:252)
GGXGX GXGGG GCXGG GCXGG GGAGGG (SEQ ID NO:253)
TGGXGX GXGGG GCXGG GCXGG GG (SEQ ID NO:254)
ATGGXGX GXGGG GCXGG GCXGG GGA (SEQ ID NO:255)
CATGGXGX GXGGG GCXGG GCXGG GGAG (SEQ ID NO:256)
ACATGGXGX GXGGG GCXGG GCXGG GGAGG (SEQ ID NO:257)
CACATGGXGX GXGGG GCXGG GCXGG GGAGGG (SEQ ID NO:258)
CATGGXGXGXGGGGCXGGGCX (SEQ ID NO:259)
GCACATGGXGXGXGGGGCXGG (SEQ ID NO:260)
GGGGCACATGGXGXGXGGGGC (SEQ ID NO:261)
CCGGGGGCACATGGXGXGXGG (SEQ ID NO:262)
GGCCCGGGGGCACATGGXGXG (SEQ ID NO:263)
GXGXGGGGCXGGGCXGGGGAG (SEQ ID NO:264)
XGGGGCXGGGCXGGGGAGGGC (SEQ ID NO:265)
GGCXGGGCXGGGGAGGGCGCC (SEQ ID NO:266)
XGGGCXGGGGAGGGCGCCTCC (SEQ ID NO:267)
GCXGGGGAGGGCGCCTCCGGG (SEQ ID NO:268)
XGXGXGGGGCXGGGCXGGG (SEQ ID NO:269)
XGXGGGGCXGGGCXGGG (SEQ ID NO:270)
XGGGGCXGGGCXGGG (SEQ ID NO:271)
GGXGXGXGGGGCXGGGCXG (SEQ ID NO:272)
GGXGXGXGGGGCXGGGC (SEQ ID NO:273)
GGXGXGXGGGGCXGG (SEQ ID NO:274)

GGXGXAXGGGGCXGGGCXGGG (SEQ ID NO:275)
GGXGXGXGGGGCXGGTCXGGG (SEQ ID NO:276)
GGXGXGXCGGGCXGGGCXGGG (SEQ ID NO:277)

BL8 1148-1174
5'-ACATGGXGXGXGGGGCXGGGCXGGGGA-3' (SEQ ID NO:278)
CACATGGXGXGXGGGGCXGGGCXGGGGA (SEQ ID NO:279)
GCACATGGXGXGXGGGGCXGGGCXGGGGA (SEQ ID NO:280)
GGCACATGGXGXGXGGGGCXGGGCXGGGGA (SEQ ID NO:281)
GGGCACATGGXGXGXGGGGCXGGGCXGGGGA (SEQ ID NO:282)
GGGGCACATGGXGXGXGGGGCXGGGCXGGGGA (SEQ ID NO:283)
ACATGGXGXGXGGGGCXGGGCXGGGGAG (SEQ ID NO:284)
ACATGGXGXGXGGGGCXGGGCXGGGGAGG (SEQ ID NO:285)
ACATGGXGXGXGGGGCXGGGCXGGGGAGGG (SEQ ID NO:286)
ACATGGXGXGXGGGGCXGGGCXGGGGAGGGC (SEQ ID NO:287)
ACATGGXGXGXGGGGCXGGGCXGGGGAGGGCG (SEQ ID NO:288)
CACATGGXGXGXGGGGCXGGGCXGGGGAG (SEQ ID NO:289)
GCACATGGXGXGXGGGGCXGGGCXGGGGAGG (SEQ ID NO:290)
GGCACATGGXGXGXGGGGCXGGGCXGGGGAGGG (SEQ ID NO:291)
GGGCACATGGXGXGXGGGGCXGGGCXGGGGAGGGC (SEQ ID NO:292)
GGGGCACATGGXGXGXGGGGCXGGGCXGGGGAGGGCG (SEQ ID NO:293)
GGCACATGGXGXGXGGGGCXGGGCXGG (SEQ ID NO:294)
GGGGGCACATGGXGXGXGGGGCXGGGC (SEQ ID NO:295)
GCCGGGGGCACATGGXGXGXGGGGCXG (SEQ ID NO:296)
CCCGCCGGGGGCACATGGXGXGXGGGG (SEQ ID NO:297)
CGTCCCGCCGGGGGCACATGGXGXGXG (SEQ ID NO:298)
TGGXGXGXGGGGCXGGGCXGGGGAGGG (SEQ ID NO:299)
XGXGXGGGGCXGGGCXGGGGAGGGCGC (SEQ ID NO:300)
GXGGGGCXGGGCXGGGGAGGGCGCCTC (SEQ ID NO:301)
GGGCXGGGCXGGGGAGGGCGCCTCCGG (SEQ ID NO:302)
CXGGGCXGGGGAGGGCGCCTCCGGGCC (SEQ ID NO:303)
ATGGXGXGXGGGGCXGGGCXGGGGA (SEQ ID NO:304)
GGXGXGXGGGGCXGGGCXGGGGA (SEQ ID NO:305)
XGXGXGGGGCXGGGCXGGGGA (SEQ ID NO:306)
XGXGGGGCXGGGCXGGGGA (SEQ ID NO:307)
XGGGGCXGGGCXGGGGA (SEQ ID NO:308)
GGGCXGGGCXGGGGA (SEQ ID NO:309)
ACATGGXGXGXGGGGCXGGGCXGGG (SEQ ID NO:310)
ACATGGXGXGXGGGGCXGGGCXG (SEQ ID NO:311)
ACATGGXGXGXGGGGCXGGGC (SEQ ID NO:312)
ACATGGXGXGXGGGGCXGG (SEQ ID NO:313)
ACATGGXGXGXGGGGCX (SEQ ID NO:314)
ACATGGXGXGXGGGG (SEQ ID NO:315)
ACATGGXTXGXGGGGCXGGGCXGGGGA (SEQ ID NO:316)
ACATGGXGXGXGGGGCXGGCCXGGGGA (SEQ ID NO:317)
ACATGGXGXAXGGGGCXGGGCXGGGGA (SEQ ID NO:318)

Figure 27

HR2 298-322
5'-TGGGTGXGTCCCTCCTAGXGCXGGG-3' (SEQ ID NO:441)
CTGGGTGXGTCCCTCCTAGXGCXGGG (SEQ ID NO:442)
CCTGGGTGXGTCCCTCCTAGXGCXGGG (SEQ ID NO:443)
GCCTGGGTGXGTCCCTCCTAGXGCXGGG (SEQ ID NO:444)
GGCCTGGGTGXGTCCCTCCTAGXGCXGGG (SEQ ID NO:445)
AGGCCTGGGTGXGTCCCTCCTAGXGCXGGG (SEQ ID NO:446)
TGGGTGXGTCCCTCCTAGXGCXGGGA (SEQ ID NO:447)
TGGGTGXGTCCCTCCTAGXGCXGGGAA (SEQ ID NO:448)
TGGGTGXGTCCCTCCTAGXGCXGGGAAG (SEQ ID NO:449)
TGGGTGXGTCCCTCCTAGXGCXGGGAAGC (SEQ ID NO:450)
TGGGTGXGTCCCTCCTAGXGCXGGGAAGCT (SEQ ID NO:451)
CTGGGTGXGTCCCTCCTAGXGCXGGGA (SEQ ID NO:452)
CCTGGGTGXGTCCCTCCTAGXGCXGGGAA (SEQ ID NO:453)
GCCTGGGTGXGTCCCTCCTAGXGCXGGGAAG (SEQ ID NO:454)
GGCCTGGGTGXGTCCCTCCTAGXGCXGGGAAGC (SEQ ID NO:455)
AGGCCTGGGTGXGTCCCTCCTAGXGCXGGGAAGCT (SEQ ID NO:456)
GCCTGGGTGXGTCCCTCCTAGXGCX (SEQ ID NO:457)
CAGGCCTGGGTGXGTCCCTCCTAGX (SEQ ID NO:458)
GCGCAGGCCTGGGTGXGTCCCTCCT (SEQ ID NO:459)
TTCGCGCAGGCCTGGGTGXGTCCCT (SEQ ID NO:460)
CTCTTCGCGCAGGCCTGGGTGXGTC (SEQ ID NO:461)
GTGXGTCCCTCCTAGXGCXGGGAAG (SEQ ID NO:462)
XGTCCCTCCTAGXGCXGGGAAGCTG (SEQ ID NO:463)
CCCTCCTAGXGCXGGGAAGCTGGGT (SEQ ID NO:464)
TCCTAGXGCXGGGAAGCTGGGTTGC (SEQ ID NO:465)
TAGXGCXGGGAAGCTGGGTTGCCTG (SEQ ID NO:466)
GGTGXGTCCCTCCTAGXGCXGGG (SEQ ID NO:467)
TGXGTCCCTCCTAGXGCXGGG (SEQ ID NO:468)
XGTCCCTCCTAGXGCXGGG (SEQ ID NO:469)
TCCCTCCTAGXGCXGGG (SEQ ID NO:470)
CCTCCTAGXGCXGGG (SEQ ID NO:472)
TGGGTGXGTCCCTCCTAGXGCXG (SEQ ID NO:473)
TGGGTGXGTCCCTCCTAGXGC (SEQ ID NO:474)
TGGGTGXGTCCCTCCTAGX (SEQ ID NO:475)
TGGGTGXGTCCCTCCTA (SEQ ID NO:476)
TGGGTGXGTCCCTCC (SEQ ID NO:477)
TGGGTGXATCCCTCCTAGXGCXGGG (SEQ ID NO:478)
TGTGTGXGTCCCTCCTAGXGCXGGG (SEQ ID NO:479)
TGGGTGXGTCCCTCCTACXGCXGGG (SEQ ID NO:480)

HR3 310-334
5'-TTXGCXGCAGGCCTGGGTGXGTCCCT-3' (SEQ ID NO:481)
CTTXGCXGCAGGCCTGGGTGXGTCCCT (SEQ ID NO:482)
TCTTXGCXGCAGGCCTGGGTGXGTCCCT (SEQ ID NO:483)
CTCTTXGCXGCAGGCCTGGGTGXGTCCCT (SEQ ID NO:484)
TCTCTTXGCXGCAGGCCTGGGTGXGTCCCT (SEQ ID NO:485)
CTCTCTTXGCXGCAGGCCTGGGTGXGTCCCT (SEQ ID NO:486)
TTXGCXGCAGGCCTGGGTGXGTCCCTC (SEQ ID NO:487)
TTXGCXGCAGGCCTGGGTGXGTCCCTCC (SEQ ID NO:488)
TTXGCXGCAGGCCTGGGTGXGTCCCTCCT (SEQ ID NO:489)
TTXGCXGCAGGCCTGGGTGXGTCCCTCCTA (SEQ ID NO:490)
TTXGCXGCAGGCCTGGGTGXGTCCCTCCTAG (SEQ ID NO:491)
CTTXGCXGCAGGCCTGGGTGXGTCCCTC (SEQ ID NO:492)
TCTTXGCXGCAGGCCTGGGTGXGTCCCTCC (SEQ ID NO:493)
CTCTTXGCXGCAGGCCTGGGTGXGTCCCTCCT (SEQ ID NO:494)
TCTCTTXGCXGCAGGCCTGGGTGXGTCCCTCCTA (SEQ ID NO:495)

CTCTCTTXGCXGCAGGCCTGGGTGXGTCCCTCCTAG (SEQ ID NO:496)
CTCTTXGCXGCAGGCCTGGGTGXGTC (SEQ ID NO:497)
CCTCTCTTXGCXGCAGGCCTGGGTGX (SEQ ID NO:498)
CTCCCTCTCTTXGCXGCAGGCCTGGG (SEQ ID NO:499)
TTTCTCCCTCTCTTXGCXGCAGGCCT (SEQ ID NO:500)
CACTTTCTCCCTCTCTTXGCXGCAGG (SEQ ID NO:501)
GCXGCAGGCCTGGGTGXGTCCCTCCT (SEQ ID NO:502)
GCAGGCCTGGGTGXGTCCCTCCTAGC (SEQ ID NO:503)
GGCCTGGGTGXGTCCCTCCTAGCGCC (SEQ ID NO:504)
CTGGGTGXGTCCCTCCTAGCGCCGGG (SEQ ID NO:505)
GGTGXGTCCCTCCTAGCGCCGGGAAG (SEQ ID NO:506)
XGCXGCAGGCCTGGGTGXGTCCCT (SEQ ID NO:507)
CXGCAGGCCTGGGTGXGTCCCT (SEQ ID NO:508)
GCAGGCCTGGGTGXGTCCCT (SEQ ID NO:509)
AGGCCTGGGTGXGTCCCT (SEQ ID NO:510)
GCCTGGGTGXGTCCCT (SEQ ID NO:511)
CCTGGGTGXGTCCCT (SEQ ID NO:512)
TTXGCXGCAGGCCTGGGTGXGTCC (SEQ ID NO:513)
TTXGCXGCAGGCCTGGGTGXGT (SEQ ID NO:514)
TTXGCXGCAGGCCTGGGTGX (SEQ ID NO:515)
TTXGCXGCAGGCCTGGGT (SEQ ID NO:516)
TTXGCXGCAGGCCTGG (SEQ ID NO:517)
TTXGCXGCAGGCCTG (SEQ ID NO:518)
TTXACXGCAGGCCTGGGTGXGTCCCT (SEQ ID NO:519)
TTXGCXGCAGGCCTGTGTGXGTCCCT (SEQ ID NO:520)
TTXGCXGCAGGCCTGGGTGXGTACCT (SEQ ID NO:521)

HR6 204-227
5'-TGCCCCCTCCCCXGGAGTCXGGGA-3' (SEQ ID NO:522)
CTGCCCCCTCCCCXGGAGTCXGGGA (SEQ ID NO:523)
TCTGCCCCCTCCCCXGGAGTCXGGGA (SEQ ID NO:524)
CTCTGCCCCCTCCCCXGGAGTCXGGGA (SEQ ID NO:525)
ACTCTGCCCCCTCCCCXGGAGTCXGGGA (SEQ ID NO:526)
GACTCTGCCCCCTCCCCXGGAGTCXGGGA (SEQ ID NO:527)
TGCCCCCTCCCCXGGAGTCXGGGAT (SEQ ID NO:528)
TGCCCCCTCCCCXGGAGTCXGGGATA (SEQ ID NO:529)
TGCCCCCTCCCCXGGAGTCXGGGATAA (SEQ ID NO:530)
TGCCCCCTCCCCXGGAGTCXGGGATAAA (SEQ ID NO:531)
TGCCCCCTCCCCXGGAGTCXGGGATAAAT (SEQ ID NO:532)
CTGCCCCCTCCCCXGGAGTCXGGGAT (SEQ ID NO:533)
TCTGCCCCCTCCCCXGGAGTCXGGGATA (SEQ ID NO:534)
CTCTGCCCCCTCCCCXGGAGTCXGGGATAA (SEQ ID NO:535)
ACTCTGCCCCCTCCCCXGGAGTCXGGGATAAA (SEQ ID NO:536)
GACTCTGCCCCCTCCCCXGGAGTCXGGGATAAAT (SEQ ID NO:537)
CTCTGCCCCCTCCCCXGGAGTCXG (SEQ ID NO:538)
TGACTCTGCCCCCTCCCCXGGAGT (SEQ ID NO:539)
TGGTGACTCTGCCCCCTCCCCXGG (SEQ ID NO:540)
GGCTGGTGACTCTGCCCCCTCCCC (SEQ ID NO:542)
AGAGGCTGGTGACTCTGCCCCCTC (SEQ ID NO:543)
CCCCTCCCCXGGAGTCXGGGATAA (SEQ ID NO:544)
CTCCCCXGGAGTCXGGGATAAATT (SEQ ID NO:545)
CCCXGGAGTCXGGGATAAATTCCC (SEQ ID NO:546)
XGGAGTCXGGGATAAATTCCCTAG (SEQ ID NO:547)
AGTCXGGGATAAATTCCCTAGGCT (SEQ ID NO:548)
CCCCCTCCCCXGGAGTCXGGGA (SEQ ID NO:549)
CCCTCCCCXGGAGTCXGGGA (SEQ ID NO:550)
CTCCCCXGGAGTCXGGGA (SEQ ID NO:551)
CCCCXGGAGTCXGGGA (SEQ ID NO:552)
CCCXGGAGTCXGGGA (SEQ ID NO:553)
TGCCCCCTCCCCXGGAGTCXGG (SEQ ID NO:554)
TGCCCCCTCCCCXGGAGTCX (SEQ ID NO:555)

TGCCCCCTCCCCXGGAGT (SEQ ID NO:556)
TGCCCCCTCCCCXGGA (SEQ ID NO:557)
TGCCCCCTCCCCXGG (SEQ ID NO:558)
TGCCCCCTCTCCXGGAGTCXGGGA (SEQ ID NO:559)
TGCCCCCTCCCCXGGAGTAXGGGA (SEQ ID NO:560)
TGCCCGCTCCCCXGGAGTCXGGGA (SEQ ID NO:561)

HR11 393-425
5'-ATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCT-3' (SEQ ID NO:562)
GATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:563)
TGATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:564)
GTGATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:565)
TGTGATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:566)
CTGTGATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:567)
ATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCTC (SEQ ID NO:568)
ATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCTCC (SEQ ID NO:569)
ATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCTCCA (SEQ ID NO:570)
ATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCTCCAA (SEQ ID NO:571)
ATTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCTCCAAC (SEQ ID NO:572)
GATTGGGAGCAAGCGCGCTCCCAGCTCGCCCCCTC (SEQ ID NO:573)
TGATTGGGAGCAAGCGCGCTCCCAGCTCGCCCCCTCC (SEQ ID NO:574)
GTGATTGGGAGCAAGCGCGCTCCCAGCTCGCCCCCTCCA (SEQ ID NO:575)
TGTGATTGGGAGCAAGCGCGCTCCCAGCTCGCCCCCTCCAA (SEQ ID NO:576)
CTGTGATTGGGAGCAAGCGCGCTCCCAGCTCGCCCCCTCCAAC (SEQ ID NO:577)
GTGATTGGGAGCAAGXGXGCTCCCAGCTXGCCC (SEQ ID NO:578)
CCTGTGATTGGGAGCAAGXGXGCTCCCAGCTXG (SEQ ID NO:579)
TCTCCTGTGATTGGGAGCAAGXGXGCTCCCAGC (SEQ ID NO:580)
CCTTCTCCTGTGATTGGGAGCAAGXGXGCTCCC (SEQ ID NO:581)
CCTCCTTCTCCTGTGATTGGGAGCAAGXGXGCT (SEQ ID NO:582)
GGGAGCAAGXGXGCTCCCAGCTXGCCCCCTCCA (SEQ ID NO:583)
AGCAAGXGXGCTCCCAGCTXGCCCCCTCCAACT (SEQ ID NO:584)
AAGXGXGCTCCCAGCTXGCCCCCTCCAACTGCA (SEQ ID NO:585)
XGXGCTCCCAGCTXGCCCCCTCCAACTGCATTC (SEQ ID NO:586)
GCTCCCAGCTXGCCCCCTCCAACTGCATTCCAA (SEQ ID NO:587)
TGGGAGCAAGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:588)
GGAGCAAGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:589)
AGCAAGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:590)
CAAGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:591)
AGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:592)
XGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:593)
XGCTCCCAGCTXGCCCCCT (SEQ ID NO:594)
CTCCCAGCTXGCCCCCT (SEQ ID NO:595)
CCCAGCTXGCCCCCT (SEQ ID NO:596)
ATTGGGAGCAAGXGXGCTCCCAGCTXGCCCC (SEQ ID NO:597)
ATTGGGAGCAAGXGXGCTCCCAGCTXGCC (SEQ ID NO:598)
ATTGGGAGCAAGXGXGCTCCCAGCTXG (SEQ ID NO:599)
ATTGGGAGCAAGXGXGCTCCCAGCT (SEQ ID NO:600)
ATTGGGAGCAAGXGXGCTCCCAG (SEQ ID NO:601)
ATTGGGAGCAAGXGXGCTCCC (SEQ ID NO:602)
ATTGGGAGCAAGXGXGCTC (SEQ ID NO:603)
ATTGGGAGCAAGXGXGC (SEQ ID NO:604)
ATTGGGAGCAAGXGX (SEQ ID NO:605)
GTTGGGAGCAAGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:606)
ATTGGGAGCAAGXGXGCTCCCTGCTXGCCCCCT (SEQ ID NO:607)
ATTGGGAGCACGXGXGCTCCCAGCTXGCCCCCT (SEQ ID NO:608)

HR23 299-333
5'-TXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGG-3' (SEQ ID NO:609)
TTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:610)
CTTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:611)
TCTTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:612)

CTCTTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:613)
TCTCTTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:614)
TXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGG (SEQ ID NO:615)
TXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGGA (SEQ ID NO:616)
TXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGGAA (SEQ ID NO:617)
TXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGGAAG (SEQ ID NO:618)
TXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGGAAGC (SEQ ID NO:619)
TTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGG (SEQ ID NO:620)
CTTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGGA (SEQ ID NO:621)
TCTTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGGAA (SEQ ID NO:622)
CTCTTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGGAAG (SEQ ID NO:623)
TCTCTTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGGAAGC (SEQ ID NO:624)
TCTTXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGC (SEQ ID NO:625)
CTCTCTTXGXGCAGGCCTGGGTGXGTCCCTCCTAG (SEQ ID NO:626)
TCCCTCTCTTXGXGCAGGCCTGGGTGXGTCCCTCC (SEQ ID NO:627)
TTCTCCCTCTCTTXGXGCAGGCCTGGGTGXGTCCC (SEQ ID NO:628)
ACTTTCTCCCTCTCTTXGXGCAGGCCTGGGTGXGT (SEQ ID NO:629)
XGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGGGAA (SEQ ID NO:630)
AGGCCTGGGTGXGTCCCTCCTAGXGCXGGGAAGCT (SEQ ID NO:631)
CCTGGGTGXGTCCCTCCTAGXGCXGGGAAGCTGGG (SEQ ID NO:632)
GGGTGXGTCCCTCCTAGXGCXGGGAAGCTGGGTTG (SEQ ID NO:633)
TGXGTCCCTCCTAGXGCXGGGAAGCTGGGTTGCCT (SEQ ID NO:634)
GXGCAGGCCTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:635)
GCAGGCCTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:636)
AGGCCTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:637)
GCCTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:638)
CTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:639)
GGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:640)
GTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:641)
GXGTCCCTCCTAGXGCXGG (SEQ ID NO:642)
GTCCCTCCTAGXGCXGG (SEQ ID NO:643)
CCCTCCTAGXGCXGG (SEQ ID NO:644)
TXGXGCAGGCCTGGGTGXGTCCCTCCTAGXGCX (SEQ ID NO:645)
TXGXGCAGGCCTGGGTGXGTCCCTCCTAGXG (SEQ ID NO:646)
TXGXGCAGGCCTGGGTGXGTCCCTCCTAG (SEQ ID NO:647)
TXGXGCAGGCCTGGGTGXGTCCCTCCT (SEQ ID NO:648)
TXGXGCAGGCCTGGGTGXGTCCCTC (SEQ ID NO:649)
TXGXGCAGGCCTGGGTGXGTCCC (SEQ ID NO:650)
TXGXGCAGGCCTGGGTGXGTC (SEQ ID NO:651)
TXGXGCAGGCCTGGGTGXG (SEQ ID NO:652)
TXGXGCAGGCCTGGGTG (SEQ ID NO:653)
TXGXGCAGGCCTGGG (SEQ ID NO:654)
TXGXACAGGCCTGGGTGXGTCCCTCCTAGXGCXGG (SEQ ID NO:655)
TXGXGCAGGCCTGGGTGXGCCCCTCCTAGXGCXGG (SEQ ID NO:656)
TXGXGCAGGCCTGGGTGXGTCCCTCCGAGXGCXGG (SEQ ID NO:657)

HR23* 299-333
5'-TXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGG-3' (SEQ ID NO:658)
TTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:659)
CTTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:660)
TCTTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:661)
CTCTTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:662)
TCTCTTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:663)
TXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGG (SEQ ID NO:664)
TXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGGA (SEQ ID NO:665)
TXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGGAA (SEQ ID NO:666)
TXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGGAAG (SEQ ID NO:667)
TXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGGAAGC (SEQ ID NO:668)
TTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGG (SEQ ID NO:669)
CTTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGGA (SEQ ID NO:670)
TCTTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGGAA (SEQ ID NO:671)

CTCTTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGGAAG (SEQ ID NO:672)
TCTCTTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGGAAGC (SEQ ID NO:673)
TCTTXGXGCAGGCCTGGGTXGTCCCTCCTAGXGC (SEQ ID NO:674)
CTCTCTTXGXGCAGGCCTGGGTXGTCCCTCCTAG (SEQ ID NO:675)
TCCCTCTCTTXGXGCAGGCCTGGGTXGTCCCTCC (SEQ ID NO:676)
TTCTCCCTCTCTTXGXGCAGGCCTGGGTXGTCCC (SEQ ID NO:677)
ACTTTCTCCCTCTCTTXGXGCAGGCCTGGGTXGT (SEQ ID NO:678)
XGCAGGCCTGGGTXGTCCCTCCTAGXGCXGGGAA (SEQ ID NO:679)
AGGCCTGGGTXGTCCCTCCTAGXGCXGGGAAGCT (SEQ ID NO:680)
CCTGGGTXGTCCCTCCTAGXGCXGGGAAGCTGGG (SEQ ID NO:681)
GGGTXGTCCCTCCTAGXGCXGGGAAGCTGGGTTG (SEQ ID NO:682)
TXGTCCCTCCTAGXGCXGGGAAGCTGGGTTGCCT (SEQ ID NO:683)
GXGCAGGCCTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:684)
GCAGGCCTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:685)
AGGCCTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:686)
GCCTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:687)
CTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:688)
GGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:689)
GTXGTCCCTCCTAGXGCXGG (SEQ ID NO:690)
XGTCCCTCCTAGXGCXGG (SEQ ID NO:691)
TCCCTCCTAGXGCXGG (SEQ ID NO:692)
CCCTCCTAGXGCXGG (SEQ ID NO:693)
TXGXGCAGGCCTGGGTXGTCCCTCCTAGXGCX (SEQ ID NO:694)
TXGXGCAGGCCTGGGTXGTCCCTCCTAGXG (SEQ ID NO:695)
TXGXGCAGGCCTGGGTXGTCCCTCCTAG (SEQ ID NO:696)
TXGXGCAGGCCTGGGTXGTCCCTCCT (SEQ ID NO:697)
TXGXGCAGGCCTGGGTXGTCCCTC (SEQ ID NO:698)
TXGXGCAGGCCTGGGTXGTCCC (SEQ ID NO:699)
TXGXGCAGGCCTGGGTXGTC (SEQ ID NO:700)
TXGXGCAGGCCTGGGTXG (SEQ ID NO:701)
TXGXGCAGGCCTGGGT (SEQ ID NO:702)
TXGXGCAGGCCTGGG (SEQ ID NO:703)
TXGXACAGGCCTGGGTXGTCCCTCCTAGXGCXGG (SEQ ID NO:704)
TXGXGCAGGCCTGGGTXGTTCCTCCTAGXGCXGG (SEQ ID NO:705)
TXGXGCAGGCCTGGGTXGTCCCTCCGAGXGCXGG (SEQ ID NO:706)

Figure 28

RZ1X 485-510**
5'-GGXGXGXGGTTXGCCCXGXGCATGGG-3' (SEQ ID NO:867)
GGGXGXGXGGTTXGCCCXGXGCATGGG (SEQ ID NO:868)
GGGGXGXGXGGTTXGCCCXGXGCATGGG (SEQ ID NO:869)
GGGGGXGXGXGGTTXGCCCXGXGCATGGG (SEQ ID NO:870)
CGGGGGXGXGXGGTTXGCCCXGXGCATGGG (SEQ ID NO:871)
GCGGGGGXGXGXGGTTXGCCCXGXGCATGGG (SEQ ID NO:872)
GGXGXGXGGTTXGCCCXGXGCATGGGC (SEQ ID NO:873)
GGXGXGXGGTTXGCCCXGXGCATGGGCT (SEQ ID NO:874)
GGXGXGXGGTTXGCCCXGXGCATGGGCTC (SEQ ID NO:875)
GGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:876)
GGXGXGXGGTTXGCCCXGXGCATGGGCTCCG (SEQ ID NO:877)
GGGXGXGXGGTTXGCCCXGXGCATGGGC (SEQ ID NO:878)
GGGGXGXGXGGTTXGCCCXGXGCATGGGCT (SEQ ID NO:879)
GGGGGXGXGXGGTTXGCCCXGXGCATGGGCTC (SEQ ID NO:880)
CGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:881)
GCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCG (SEQ ID NO:882)
GGGGGXGXGXGGTTXGCCCXGXGCAT (SEQ ID NO:883)
GGCGGGGGXGXGXGGTTXGCCCXGXG (SEQ ID NO:884)
GGGGGCGGGGGXGXGXGGTTXGCCCX (SEQ ID NO:885)
GGCGGGGGCGGGGGXGXGXGGTTXGC (SEQ ID NO:886)
CGGGGCGGGGGCGGGGGXGXGXGGTT (SEQ ID NO:887)
GXGXGGTTXGCCCXGXGCATGGGCTC (SEQ ID NO:888)
XGGTTXGCCCXGXGCATGGGCTCCGT (SEQ ID NO:889)
TTXGCCCXGXGCATGGGCTCCGTCCG (SEQ ID NO:890)
GCCCXGXGCATGGGCTCCGTCCGCGG (SEQ ID NO:891)
CXGXGCATGGGCTCCGTCCGCGGGCC (SEQ ID NO:892)
XGXGXGGTTXGCCCXGXGCATGGG (SEQ ID NO:893)
XGXGGTTXGCCCXGXGCATGGG (SEQ ID NO:894)
XGGTTXGCCCXGXGCATGGG (SEQ ID NO:895)
GTTXGCCCXGXGCATGGG (SEQ ID NO:896)
TXGCCCXGXGCATGGG (SEQ ID NO:897)
XGCCCXGXGCATGGG (SEQ ID NO:898)
GGXGXGXGGTTXGCCCXGXGCATG (SEQ ID NO:899)
GGXGXGXGGTTXGCCCXGXGCA (SEQ ID NO:900)
GGXGXGXGGTTXGCCCXGXG (SEQ ID NO:901)
GGXGXGXGGTTXGCCCXG (SEQ ID NO:902)
GGXGXGXGGTTXGCCC (SEQ ID NO:903)
GGXGXGXGGTTXGCC (SEQ ID NO:904)
GGXGXCXGGTTXGCCCXGXGCATGGG (SEQ ID NO:905)
GGXGXGXGGTTXGCTCXGXGCATGGG (SEQ ID NO:906)
GGXGXGXAGTTXGCCCXGXGCATGGG (SEQ ID NO:907)

RZ2X 501-533**
5'-GGXGGGGXGGGGGXGGGGGXGXGXGGT-3' (*SEQ ID NO:908*)
AGGXGGGGXGGGGGXGGGGGXGXGXGGT (SEQ ID NO:909)
GAGGXGGGGXGGGGGXGGGGGXGXGXGGT (SEQ ID NO:910)
CGAGGXGGGGXGGGGGXGGGGGXGXGXGGT (SEQ ID NO:911)
CCGAGGXGGGGXGGGGGXGGGGGXGXGXGGT (SEQ ID NO:912)
GCCGAGGXGGGGXGGGGGXGGGGGXGXGXGGT (SEQ ID NO:913)
GGXGGGGXGGGGGXGGGGGXGXGXGGTT (SEQ ID NO:914)
GGXGGGGXGGGGGXGGGGGXGXGXGGTTC (SEQ ID NO:915)
GGXGGGGXGGGGGXGGGGGXGXGXGGTTCG (SEQ ID NO:916)
GGXGGGGXGGGGGXGGGGGXGXGXGGTTCGC (SEQ ID NO:917)
GGXGGGGXGGGGGXGGGGGXGXGXGGTTCGCC (SEQ ID NO:918)
AGGXGGGGXGGGGGXGGGGGXGXGXGGTT (SEQ ID NO:919)

GAGGXGGGGXGGGGGXGGGGGXGXGXGGTTC (SEQ ID NO:920)
CGAGGXGGGGXGGGGGXGGGGGXGXGXGGTTCG (SEQ ID NO:921)
CCGAGGXGGGGXGGGGGXGGGGGXGXGXGGTTCGC (SEQ ID NO:922)
GCCGAGGXGGGGXGGGGGXGGGGGXGXGXGGTTCGCC (SEQ ID NO:923)
CGAGGXGGGGXGGGGGXGGGGGXGXGX (SEQ ID NO:924)
GGCCGAGGXGGGGXGGGGGXGGGGGXG (SEQ ID NO:925)
CGGGGCCGAGGXGGGGXGGGGGXGGGG (SEQ ID NO:926)
GGCCGGGGCCGAGGXGGGGXGGGGGXG (SEQ ID NO:927)
CAGGGCCGGGGCCGAGGXGGGGXGGGG (SEQ ID NO:928)
GGGGXGGGGGXGGGGGXGXGXGGTTCG (SEQ ID NO:929)
GXGGGGGXGGGGGXGXGXGGTTCGCCC (SEQ ID NO:930)
GGGGXGGGGGXGXGXGGTTCGCCCCGC (SEQ ID NO:931)
GXGGGGGXGXGXGGTTCGCCCCGCGCA (SEQ ID NO:932)
GGGGXGXGXGGTTCGCCCCGCGCATGG (SEQ ID NO:933)
XGGGGXGGGGGXGGGGGXGXGXGGT (SEQ ID NO:934)
GGGXGGGGGXGGGGGXGXGXGGT (SEQ ID NO:935)
GXGGGGGXGGGGGXGXGXGGT (SEQ ID NO:936)
GGGGGXGGGGGXGXGXGGT (SEQ ID NO:937)
GGGXGGGGGXGXGXGGT (SEQ ID NO:938)
GXGGGGGXGXGXGGT (SEQ ID NO:939)
GGXGGGGXGGGGGXGGGGGXGXGXG (SEQ ID NO:940)
GGXGGGGXGGGGGXGGGGGXGXG (SEQ ID NO:941)
GGXGGGGXGGGGGXGGGGGXG (SEQ ID NO:942)
GGXGGGGXGGGGGXGGGGG (SEQ ID NO:943)
GGXGGGGXGGGGGXGGG (SEQ ID NO:944)
GGXGGGGXGGGGGXG (SEQ ID NO:945)
GGXGAGGXGGGGGXGGGGGXGXGXGGT (SEQ ID NO:946)
GGXGGGGXGGGGGXGGGTGXGXGXGGT (SEQ ID NO:947)
GGXGGGGXGCGGGXGGGGGXGXGXGGT (SEQ ID NO:948)

RZ6X** 665-696
5'-CXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGG-3' (SEQ ID NO:949)
CCXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:950)
ACCXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:951)
CACCXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:952)
CCACCXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:953)
CCCACCXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:954)
CXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGGC (SEQ ID NO:955)
CXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGGCC (SEQ ID NO:956)
CXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGGCCC (SEQ ID NO:957)
CXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGGCCCC (SEQ ID NO:958)
CXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGGCCCCG (SEQ ID NO:959)
CCXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGGC (SEQ ID NO:960)
ACCXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGGCC (SEQ ID NO:961)
CACCXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGGCCC (SEQ ID NO:962)
CCACCXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGGCCCC (SEQ ID NO:963)
CCCACCXGCXGCXGCXGCXGCXGCTTAXGCCXGCXGGCCCCG (SEQ ID NO:964)
CACCXGCXGCXGCXGCXGCXGCTTAXGCCXGC (SEQ ID NO:965)
ACCCACCXGCXGCXGCXGCXGCXGCTTAXGCC (SEQ ID NO:966)
CCCACCCACCXGCXGCXGCXGCXGCXGCTTAX (SEQ ID NO:967)
GGCCCCACCCACCXGCXGCXGCXGCXGCXGCT (SEQ ID NO:968)
CCCGGCCCCACCCACCXGCXGCXGCXGCXGCX (SEQ ID NO:969)
CXGCXGCXGCXGCXGCTTAXGCCXGCXGGCCC (SEQ ID NO:970)
CXGCXGCXGCXGCTTAXGCCXGCXGGCCCCGC (SEQ ID NO:971)
CXGCXGCXGCTTAXGCCXGCXGGCCCCGCGCC (SEQ ID NO:972)
CXGCXGCTTAXGCCXGCXGGCCCCGCGCCCCC (SEQ ID NO:973)
CXGCTTAXGCCXGCXGGCCCCGCGCCCCCGGC (SEQ ID NO:974)
GCXGCXGCXGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:975)
XGCXGCXGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:976)
CXGCXGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:977)
GCXGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:978)

XGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:979)
CXGCXGCTTAXGCCXGCXGG (SEQ ID NO:980)
GCXGCTTAXGCCXGCXGG (SEQ ID NO:981)
XGCTTAXGCCXGCXGG (SEQ ID NO:982)
GCTTAXGCCXGCXGG (SEQ ID NO:983)
CXGCXGCXGCXGCXGCXGCTTAXGCCXGCX (SEQ ID NO:984)
CXGCXGCXGCXGCXGCXGCTTAXGCCXG (SEQ ID NO:985)
CXGCXGCXGCXGCXGCXGCTTAXGCC (SEQ ID NO:986)
CXGCXGCXGCXGCXGCXGCTTAXG (SEQ ID NO:987)
CXGCXGCXGCXGCXGCXGCTTA (SEQ ID NO:988)
CXGCXGCXGCXGCXGCXGCT (SEQ ID NO:989)
CXGCXGCXGCXGCXGCXG (SEQ ID NO:990)
CXGCXGCXGCXGCXGC (SEQ ID NO:991)
CXGCXGCXGCXGCXG (SEQ ID NO:992)
CXGCXGCXACXGCXGCXGCTTAXGCCXGCXGG (SEQ ID NO:993)
CXGCXGCXGCXGCXGCXGCTTCXGCCXGCXGG (SEQ ID NO:994)
CXGCXGCXGCXGCXACXGCTTAXGCCXGCXGG (SEQ ID NO:995)

RZ9X 733-760**
5'-CXGCCXGCXGCAGCCCCXGAXGCCXGCT-3' (SEQ ID NO:996)
CCXGCCXGCXGCAGCCCCXGAXGCCXGCT (SEQ ID NO:997)
CCCXGCCXGCXGCAGCCCCXGAXGCCXGCT (SEQ ID NO:998)
CCCCXGCCXGCXGCAGCCCCXGAXGCCXGCT (SEQ ID NO:999)
GCCCCXGCCXGCXGCAGCCCCXGAXGCCXGCT (SEQ ID NO:1000)
GGCCCCXGCCXGCXGCAGCCCCXGAXGCCXGCT (SEQ ID NO:1001)
CXGCCXGCXGCAGCCCCXGAXGCCXGCTC (SEQ ID NO:1002)
CXGCCXGCXGCAGCCCCXGAXGCCXGCTCA (SEQ ID NO:1003)
CXGCCXGCXGCAGCCCCXGAXGCCXGCTCAC (SEQ ID NO:1004)
CXGCCXGCXGCAGCCCCXGAXGCCXGCTCACC (SEQ ID NO:1005)
CXGCCXGCXGCAGCCCCXGAXGCCXGCTCACCT (SEQ ID NO:1006)
CCXGCCXGCXGCAGCCCCXGAXGCCXGCTC (SEQ ID NO:1007)
CCCXGCCXGCXGCAGCCCCXGAXGCCXGCTCA (SEQ ID NO:1008)
CCCCXGCCXGCXGCAGCCCCXGAXGCCXGCTCAC (SEQ ID NO:1009)
GCCCCXGCCXGCXGCAGCCCCXGAXGCCXGCTCACC (SEQ ID NO:1010)
GGCCCCXGCCXGCXGCAGCCCCXGAXGCCXGCTCACCT (SEQ ID NO:1011)
CCCCXGCCXGCXGCAGCCCCXGAXGCCX (SEQ ID NO:1012)
GGGCCCCXGCCXGCXGCAGCCCCXGAXG (SEQ ID NO:1013)
AAGGGGCCCCXGCCXGCXGCAGCCCCXG (SEQ ID NO:1014)
AGGAAGGGGCCCCXGCCXGCXGCAGCCC (SEQ ID NO:1015)
GGGAGGAAGGGGCCCCXGCCXGCXGCAG (SEQ ID NO:1016)
CCXGCXGCAGCCCCXGAXGCCXGCTCAC (SEQ ID NO:1017)
GCXGCAGCCCCXGAXGCCXGCTCACCTG (SEQ ID NO:1018)
GCAGCCCCXGAXGCCXGCTCACCTGTGC (SEQ ID NO:1019)
GCCCCXGAXGCCXGCTCACCTGTGCCCG (SEQ ID NO:1020)
CCXGAXGCCXGCTCACCTGTGCCCGCGG (SEQ ID NO:1021)
GCCXGCXGCAGCCCCXGAXGCCXGCT (SEQ ID NO:1022)
CXGCXGCAGCCCCXGAXGCCXGCT (SEQ ID NO:1023)
GCXGCAGCCCCXGAXGCCXGCT (SEQ ID NO:1024)
XGCAGCCCCXGAXGCCXGCT (SEQ ID NO:1025)
CAGCCCCXGAXGCCXGCT (SEQ ID NO:1026)
GCCCCXGAXGCCXGCT (SEQ ID NO:1027)
CCCCXGAXGCCXGCT (SEQ ID NO:1028)
CXGCCXGCXGCAGCCCCXGAXGCCXG (SEQ ID NO:1029)
CXGCCXGCXGCAGCCCCXGAXGCC (SEQ ID NO:1030)
CXGCCXGCXGCAGCCCCXGAXG (SEQ ID NO:1031)
CXGCCXGCXGCAGCCCCXGA (SEQ ID NO:1032)
CXGCCXGCXGCAGCCCCX (SEQ ID NO:1033)
CXGCCXGCXGCAGCCC (SEQ ID NO:1034)
CXGCCXGCXGCAGCC (SEQ ID NO:1035)
CXGCAXGCXGCAGCCCCXGAXGCCXGCT (SEQ ID NO:1036)
CXGCCXGCXGCAGCCCTXGAXGCCXGCT (SEQ ID NO:1037)

CXGCCXGCXGCAGCGCCXGAXGCCXGCT (SEQ ID NO:1038)

RZ10X 333-361**
5'-GXGXGCCATXGGGCCCXGCCCCACCCXGG-3' (SEQ ID NO:1039)
TGXGXGCCATXGGGCCCXGCCCCACCCXGG (SEQ ID NO:1040)
CTGXGXGCCATXGGGCCCXGCCCCACCCXGG (SEQ ID NO:1041)
GCTGXGXGCCATXGGGCCCXGCCCCACCCXGG (SEQ ID NO:1042)
GGCTGXGXGCCATXGGGCCCXGCCCCACCCXGG (SEQ ID NO:1043)
TGGCTGXGXGCCATXGGGCCCXGCCCCACCCXGG (SEQ ID NO:1044)
GXGXGCCATXGGGCCCXGCCCCACCCXGGT (SEQ ID NO:1045)
GXGXGCCATXGGGCCCXGCCCCACCCXGGTT (SEQ ID NO:1046)
GXGXGCCATXGGGCCCXGCCCCACCCXGGTTG (SEQ ID NO:1047)
GXGXGCCATXGGGCCCXGCCCCACCCXGGTTGG (SEQ ID NO:1048)
GXGXGCCATXGGGCCCXGCCCCACCCXGGTTGGC (SEQ ID NO:1049)
TGXGXGCCATXGGGCCCXGCCCCACCCXGGT (SEQ ID NO:1050)
CTGXGXGCCATXGGGCCCXGCCCCACCCXGGTT (SEQ ID NO:1051)
GCTGXGXGCCATXGGGCCCXGCCCCACCCXGGTTG (SEQ ID NO:1052)
GGCTGXGXGCCATXGGGCCCXGCCCCACCCXGGTTGG (SEQ ID NO:1053)
TGGCTGXGXGCCATXGGGCCCXGCCCCACCCXGGTTGGC (SEQ ID NO:1054)
GCTGXGXGCCATXGGGCCCXGCCCCACCC (SEQ ID NO:1055)
TTGGCTGXGXGCCATXGGGCCCXGCCCCA (SEQ ID NO:1056)
CCATTGGCTGXGXGCCATXGGGCCCXGCC (SEQ ID NO:1057)
CTACCATTGGCTGXGXGCCATXGGGCCCX (SEQ ID NO:1058)
GGCCTACCATTGGCTGXGXGCCATXGGGC (SEQ ID NO:1059)
XGCCATXGGGCCCXGCCCCACCCXGGTTG (SEQ ID NO:1060)
CATXGGGCCCXGCCCCACCCXGGTTGGCT (SEQ ID NO:1061)
XGGGCCCXGCCCCACCCXGGTTGGCTGAG (SEQ ID NO:1062)
GCCCXGCCCCACCCXGGTTGGCTGAGCGG (SEQ ID NO:1063)
CXGCCCCACCCXGGTTGGCTGAGCGGCCC (SEQ ID NO:1064)
GXGCCATXGGGCCCXGCCCCACCCXGG (SEQ ID NO:1065)
GCCATXGGGCCCXGCCCCACCCXGG (SEQ ID NO:1066)
CATXGGGCCCXGCCCCACCCXGG (SEQ ID NO:1067)
TXGGGCCCXGCCCCACCCXGG (SEQ ID NO:1068)
GGGCCCXGCCCCACCCXGG (SEQ ID NO:1069)
GCCCXGCCCCACCCXGG (SEQ ID NO:1070)
CCXGCCCCACCCXGG (SEQ ID NO:1071)
GXGXGCCATXGGGCCCXGCCCCACCCX (SEQ ID NO:1072)
GXGXGCCATXGGGCCCXGCCCCACC (SEQ ID NO:1073)
GXGXGCCATXGGGCCCXGCCCCA (SEQ ID NO:1074)
GXGXGCCATXGGGCCCXGCCC (SEQ ID NO:1075)
GXGXGCCATXGGGCCCXGC (SEQ ID NO:1076)
GXGXGCCATXGGGCCCX (SEQ ID NO:1077)
GXGXGCCATXGGGCC (SEQ ID NO:1078)
GXGXGCCATXGGGCACXGCCCCACCCXGG (SEQ ID NO:1079)
GXGXGCCATXGGGCCCXGCCCTACCCXGG (SEQ ID NO:1080)
GXGXGCGATXGGGCCCXGCCCCACCCXGG (SEQ ID NO:1081)

ERZ1X 481-510**
5'-CGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC-3' (SEQ ID NO:1082)
GCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1083)
GGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1084)
GGGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1085)
CGGGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1086)
GCGGGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1087)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCG (SEQ ID NO:1088)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGT (SEQ ID NO:1089)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGTC (SEQ ID NO:1090)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGTCC (SEQ ID NO:1091)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGTCCG (SEQ ID NO:1092)
GCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCG (SEQ ID NO:1093)
GGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGT (SEQ ID NO:1094)

GGGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGTC (SEQ ID NO:1095)
CGGGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGTCC (SEQ ID NO:1096)
GCGGGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGTCCG (SEQ ID NO:1097)
GGGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGC (SEQ ID NO:1098)
GGCGGGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATG (SEQ ID NO:1099)
GGGGGCGGGCGGGGGCGGGGGXGXGXGGTTXGCCCXGXGC (SEQ ID NO:1100)
GGCGGGGGCGGGCGGGGGCGGGGGXGXGXGGTTXGCCCXG (SEQ ID NO:1101)
CGGGGCGGGGGCGGGCGGGGGCGGGGGXGXGXGGTTXGCC (SEQ ID NO:1102)
GGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGTC (SEQ ID NO:1103)
CGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGTCCGC (SEQ ID NO:1104)
GGGXGXGXGGTTXGCCCXGXGCATGGGCTCCGTCCGCGGC (SEQ ID NO:1105)
XGXGXGGTTXGCCCXGXGCATGGGCTCCGTCCGCGGCGGG (SEQ ID NO:1106)
GXGGTTXGCCCXGXGCATGGGCTCCGTCCGCGGCGGGTGC (SEQ ID NO:1107)
GGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1108)
GGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1109)
CGGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1110)
GGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1111)
GGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1112)
XGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1113)
XGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1114)
XGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1115)
GTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1116)
TXGCCCXGXGCATGGGCTCC (SEQ ID NO:1117)
GCCCXGXGCATGGGCTCC (SEQ ID NO:1118)
CCXGXGCATGGGCTCC (SEQ ID NO:1119)
CCXGXGCATGGGCTC (SEQ ID NO:1120)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGGCT (SEQ ID NO:1121)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATGGG (SEQ ID NO:1122)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCATG (SEQ ID NO:1123)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXGCA (SEQ ID NO:1124)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXG (SEQ ID NO:1125)
CGGGGGCGGGGGXGXGXGGTTXGCCCXG (SEQ ID NO:1126)
CGGGGGCGGGGGXGXGXGGTTXGCCC (SEQ ID NO:1127)
CGGGGGCGGGGGXGXGXGGTTXGC (SEQ ID NO:1128)
CGGGGGCGGGGGXGXGXGGTTX (SEQ ID NO:1129)
CGGGGGCGGGGGXGXGXGGT (SEQ ID NO:1130)
CGGGGGCGGGGGXGXGXG (SEQ ID NO:1131)
CGGGGGCGGGGGXGXG (SEQ ID NO:1132)
CGGGGGCGGGGGXGX (SEQ ID NO:1133)
CGGGGGCAGGGGXGXGXGGTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1134)
CGGGGGCGGGGGXGXGXGTTTXGCCCXGXGCATGGGCTCC (SEQ ID NO:1135)
CGGGGGCGGGGGXGXGXGGTTXGCCCXGXACATGGGCTCC (SEQ ID NO:1136)

SRZ1X 491-510
5′-GGXGXGXGGTTXGCCCXGXG-3′ (SEQ ID NO:1137)
GGGXGXGXGGTTXGCCCXGXG (SEQ ID NO:1138)
GGGGXGXGXGGTTXGCCCXGXG (SEQ ID NO:1139)
GGGGGXGXGXGGTTXGCCCXGXG (SEQ ID NO:1140)
CGGGGGXGXGXGGTTXGCCCXGXG (SEQ ID NO:1141)
GCGGGGGXGXGXGGTTXGCCCXGXG (SEQ ID NO:1142)
GGXGXGXGGTTXGCCCXGXGC (SEQ ID NO:1143)
GGXGXGXGGTTXGCCCXGXGCA (SEQ ID NO:1144)
GGXGXGXGGTTXGCCCXGXGCAT (SEQ ID NO:1145)
GGXGXGXGGTTXGCCCXGXGCATG (SEQ ID NO:1146)
GGXGXGXGGTTXGCCCXGXGCATGG (SEQ ID NO:1147)
GGGXGXGXGGTTXGCCCXGXGC (SEQ ID NO:1148)
GGGGXGXGXGGTTXGCCCXGXGCA (SEQ ID NO:1149)
GGGGGXGXGXGGTTXGCCCXGXGCAT (SEQ ID NO:1150)
CGGGGGXGXGXGGTTXGCCCXGXGCATG (SEQ ID NO:1151)
GCGGGGGXGXGXGGTTXGCCCXGXGCATGG (SEQ ID NO:1152)
GGGGGXGXGXGGTTXGCCCX (SEQ ID NO:1153)

GGCGGGGGXGXGXGGTTXGC (SEQ ID NO:1154)
GGGGGCGGGGGXGXGXGGTT (SEQ ID NO:1155)
GGCGGGGGCGGGGGXGXGXG (SEQ ID NO:1156)
CGGGGCGGGGGCGGGGGXGX (SEQ ID NO:1157)
GXGXGGTTXGCCCXGXGCAT (SEQ ID NO:1158)
XGGTTXGCCCXGXGCATGGG (SEQ ID NO:1159)
TTXGCCCXGXGCATGGGCTC (SEQ ID NO:1160)
GCCCXGXGCATGGGCTCCGT (SEQ ID NO:1161)
CXGXGCATGGGCTCCGTCCG (SEQ ID NO:1162)
XGXGXGGTTXGCCCXGXG (SEQ ID NO:1163)
XGXGGTTXGCCCXGXG (SEQ ID NO:1164)
GXGGTTXGCCCXGXG (SEQ ID NO:1165)
GGXGXGXGGTTXGCCCXG (SEQ ID NO:1166)
GGXGXGXGGTTXGCCC (SEQ ID NO:1167)
GGXGXGXGGTTXGCC (SEQ ID NO:1168)
GGXGXAXGGTTXGCCCXGXG (SEQ ID NO:1169)
GGXGXGXGGTTXGTCCXGXG (SEQ ID NO:1170)
GGXGXGXGGTTXGCCGXGXG (SEQ ID NO:1171)

RZ1 485-510
5'-GGCGCGCGGTTCGCCCCGCGCATGGG-3' (SEQ ID NO:1172)
GGGCGCGCGGTTCGCCCCGCGCATGGG (SEQ ID NO:1173)
GGGGCGCGCGGTTCGCCCCGCGCATGGG (SEQ ID NO:1174)
GGGGGCGCGCGGTTCGCCCCGCGCATGGG (SEQ ID NO:1175)
CGGGGGCGCGCGGTTCGCCCCGCGCATGGG (SEQ ID NO:1176)
GCGGGGGCGCGCGGTTCGCCCCGCGCATGGG (SEQ ID NO:1177)
GGCGCGCGGTTCGCCCCGCGCATGGGC (SEQ ID NO:1178)
GGCGCGCGGTTCGCCCCGCGCATGGGCT (SEQ ID NO:1179)
GGCGCGCGGTTCGCCCCGCGCATGGGCTC (SEQ ID NO:1180)
GGCGCGCGGTTCGCCCCGCGCATGGGCTCC (SEQ ID NO:1181)
GGCGCGCGGTTCGCCCCGCGCATGGGCTCCG (SEQ ID NO:1182)
GGGCGCGCGGTTCGCCCCGCGCATGGGC (SEQ ID NO:1183)
GGGGCGCGCGGTTCGCCCCGCGCATGGGCT (SEQ ID NO:1184)
GGGGGCGCGCGGTTCGCCCCGCGCATGGGCTC (SEQ ID NO:1185)
CGGGGGCGCGCGGTTCGCCCCGCGCATGGGCTCC (SEQ ID NO:1186)
GCGGGGGCGCGCGGTTCGCCCCGCGCATGGGCTCCG (SEQ ID NO:1187)
GGGGGCGCGCGGTTCGCCCCGCGCAT (SEQ ID NO:1188)
GGCGGGGGCGCGCGGTTCGCCCCGCG (SEQ ID NO:1189)
GGGGGCGGGGGCGCGCGGTTCGCCCC (SEQ ID NO:1190)
GGCGGGGGCGGGGGCGCGCGGTTCGC (SEQ ID NO:1191)
CGGGGCGGGGGCGGGGGCGCGCGGTT (SEQ ID NO:1192)
GCGCGGTTCGCCCCGCGCATGGGCTC (SEQ ID NO:1193)
CGGTTCGCCCCGCGCATGGGCTCCGT (SEQ ID NO:1194)
TTCGCCCCGCGCATGGGCTCCGTCCG (SEQ ID NO:1195)
GCCCCGCGCATGGGCTCCGTCCGCGG (SEQ ID NO:1196)
CCGCGCATGGGCTCCGTCCGCGGGCC (SEQ ID NO:1197)
CGCGCGGTTCGCCCCGCGCATGGG (SEQ ID NO:1198)
CGCGGTTCGCCCCGCGCATGGG (SEQ ID NO:1199)
CGGTTCGCCCCGCGCATGGG (SEQ ID NO:1200)
GTTCGCCCCGCGCATGGG (SEQ ID NO:1201)
TCGCCCCGCGCATGGG (SEQ ID NO:1202)
CGCCCCGCGCATGGG (SEQ ID NO:1203)
GGCGCGCGGTTCGCCCCGCGCATG (SEQ ID NO:1204)
GGCGCGCGGTTCGCCCCGCGCA (SEQ ID NO:1205)
GGCGCGCGGTTCGCCCCGCG (SEQ ID NO:1206)
GGCGCGCGGTTCGCCCCG (SEQ ID NO:1207)
GGCGCGCGGTTCGCCC (SEQ ID NO:1208)
GGCGCGCGGTTCGCC (SEQ ID NO:1209)
GGCGCGCTGTTCGCCCCGCGCATGGG (SEQ ID NO:1210)
GGCGCGCGGTTCGCCCCGCGCCTGGG (SEQ ID NO:1211)
GGCGCGCGGTTCGACCCGCGCATGGG (SEQ ID NO:1212)

RZ8X 1421-1451
5'-AGXGTGXGGGAGGGCTGTXGCCTCGCCCCCA-3' (SEQ ID NO:1213)
CAGXGTGXGGGAGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1214)
CCAGXGTGXGGGAGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1215)
CCCAGXGTGXGGGAGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1216)
ACCCAGXGTGXGGGAGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1217)
AACCCAGXGTGXGGGAGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1218)
AGXGTGXGGGAGGGCTGTXGCCTCGCCCCCAC (SEQ ID NO:1219)
AGXGTGXGGGAGGGCTGTXGCCTCGCCCCCACT (SEQ ID NO:1220)
AGXGTGXGGGAGGGCTGTXGCCTCGCCCCCACTT (SEQ ID NO:1221)
AGXGTGXGGGAGGGCTGTXGCCTCGCCCCCACTTG (SEQ ID NO:1222)
AGXGTGXGGGAGGGCTGTXGCCTCGCCCCCACTTGC (SEQ ID NO:1223)
CAGXGTGXGGGAGGGCTGTXGCCTCGCCCCCAC (SEQ ID NO:1224)
CCAGXGTGXGGGAGGGCTGTXGCCTCGCCCCCACT (SEQ ID NO:1225)
CCCAGXGTGXGGGAGGGCTGTXGCCTCGCCCCCACTT (SEQ ID NO:1226)
ACCCAGXGTGXGGGAGGGCTGTXGCCTCGCCCCCACTTG (SEQ ID NO:1227)
AACCCAGXGTGXGGGAGGGCTGTXGCCTCGCCCCCACTTGC (SEQ ID NO:1228)
CCCAGXGTGXGGGAGGGCTGTXGCCTCGCCC (SEQ ID NO:1229)
CAACCCAGXGTGXGGGAGGGCTGTXGCCTCG (SEQ ID NO:1230)
CTGCAACCCAGXGTGXGGGAGGGCTGTXGCC (SEQ ID NO:1231)
CAGCTGCAACCCAGXGTGXGGGAGGGCTGTX (SEQ ID NO:1232)
GTGCAGCTGCAACCCAGXGTGXGGGAGGGCT (SEQ ID NO:1233)
GTGXGGGAGGGCTGTXGCCTCGCCCCCACTT (SEQ ID NO:1234)
XGGGAGGGCTGTXGCCTCGCCCCCACTTGCT (SEQ ID NO:1235)
GAGGGCTGTXGCCTCGCCCCCACTTGCTCTT (SEQ ID NO:1236)
GGCTGTXGCCTCGCCCCCACTTGCTCTTAAT (SEQ ID NO:1237)
TGTXGCCTCGCCCCCACTTGCTCTTAATGAC (SEQ ID NO:1238)
XGTGXGGGAGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1239)
TGXGGGAGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1240)
XGGGAGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1241)
GGAGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1242)
AGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1243)
GGCTGTXGCCTCGCCCCCA (SEQ ID NO:1244)
CTGTXGCCTCGCCCCCA (SEQ ID NO:1245)
GTXGCCTCGCCCCCA (SEQ ID NO:1246)
AGXGTGXGGGAGGGCTGTXGCCTCGCCCC (SEQ ID NO:1247)
AGXGTGXGGGAGGGCTGTXGCCTCGCC (SEQ ID NO:1248)
AGXGTGXGGGAGGGCTGTXGCCTCG (SEQ ID NO:1249)
AGXGTGXGGGAGGGCTGTXGCCT (SEQ ID NO:1250)
AGXGTGXGGGAGGGCTGTXGC (SEQ ID NO:1251)
AGXGTGXGGGAGGGCTGTX (SEQ ID NO:1252)
AGXGTGXGGGAGGGCTG (SEQ ID NO:1253)
AGXGTGXGGGAGGGC (SEQ ID NO:1254)
AGXGAGXGGGAGGGCTGTXGCCTCGCCCCCA (SEQ ID NO:1255)
AGXGTGXGGGAGGGATGTXGCCTCGCCCCCA (SEQ ID NO:1256)
AGXGTGXGGGAGGGCTGTXGCGTCGCCCCCA (SEQ ID NO:1257)

RZ11X 442-467
5'-GXGGCTXGGGTTGXGGGXGCAGGGCA-3' (SEQ ID NO:1258)
TGXGGCTXGGGTTGXGGGXGCAGGGCA (SEQ ID NO:1259)
GTGXGGCTXGGGTTGXGGGXGCAGGGCA (SEQ ID NO:1260)
GGTGXGGCTXGGGTTGXGGGXGCAGGGCA (SEQ ID NO:1261)
GGGTGXGGCTXGGGTTGXGGGXGCAGGGCA (SEQ ID NO:1262)
CGGGTGXGGCTXGGGTTGXGGGXGCAGGGCA (SEQ ID NO:1263)
GXGGCTXGGGTTGXGGGXGCAGGGCAC (SEQ ID NO:1264)
GXGGCTXGGGTTGXGGGXGCAGGGCACG (SEQ ID NO:1265)
GXGGCTXGGGTTGXGGGXGCAGGGCACGG (SEQ ID NO:1266)
GXGGCTXGGGTTGXGGGXGCAGGGCACGGG (SEQ ID NO:1267)
GXGGCTXGGGTTGXGGGXGCAGGGCACGGGC (SEQ ID NO:1268)
TGXGGCTXGGGTTGXGGGXGCAGGGCAC (SEQ ID NO:1269)

GTGXGGCTXGGGTTGXGGGXGCAGGGCACG (SEQ ID NO:1270)
GGTGXGGCTXGGGTTGXGGGXGCAGGGCACGG (SEQ ID NO:1271)
GGGTGXGGCTXGGGTTGXGGGXGCAGGGCACGGG (SEQ ID NO:1272)
CGGGTGXGGCTXGGGTTGXGGGXGCAGGGCACGGGC (SEQ ID NO:1273)
GGTGXGGCTXGGGTTGXGGGXGCAGG (SEQ ID NO:1274)
GCGGGTGXGGCTXGGGTTGXGGGXGC (SEQ ID NO:1275)
GCGGCGGGTGXGGCTXGGGTTGXGGG (SEQ ID NO:1276)
TCCGCGGCGGGTGXGGCTXGGGTTGX (SEQ ID NO:1277)
CCGTCCGCGGCGGGTGXGGCTXGGGT (SEQ ID NO:1278)
GCTXGGGTTGXGGGXGCAGGGCACGG (SEQ ID NO:1279)
XGGGTTGXGGGXGCAGGGCACGGGCG (SEQ ID NO:1280)
GTTGXGGGXGCAGGGCACGGGCGGCG (SEQ ID NO:1281)
GXGGGXGCAGGGCACGGGCGGCGGAG (SEQ ID NO:1282)
GGXGCAGGGCACGGGCGGCGGAGACT (SEQ ID NO:1283)
GGCTXGGGTTGXGGGXGCAGGGCA (SEQ ID NO:1284)
CTXGGGTTGXGGGXGCAGGGCA (SEQ ID NO:1285)
XGGGTTGXGGGXGCAGGGCA (SEQ ID NO:1286)
GGTTGXGGGXGCAGGGCA (SEQ ID NO:1287)
TTGXGGGXGCAGGGCA (SEQ ID NO:1288)
TGXGGGXGCAGGGCA (SEQ ID NO:1289)
GXGGCTXGGGTTGXGGGXGCAGGG (SEQ ID NO:1290)
GXGGCTXGGGTTGXGGGXGCAG (SEQ ID NO:1291)
GXGGCTXGGGTTGXGGGXGC (SEQ ID NO:1292)
GXGGCTXGGGTTGXGGGX (SEQ ID NO:1293)
GXGGCTXGGGTTGXGG (SEQ ID NO:1294)
GXGGCTXGGGTTGXG (SEQ ID NO:1295)
GXGACTXGGGTTGXGGGXGCAGGGCA (SEQ ID NO:1296)
GXGGCTXGGGTTGXGGTXGCAGGGCA (SEQ ID NO:1297)
GXGGCTXGGGTTCXGGGXGCAGGGCA (SEQ ID NO:1298)

Figure 29

CM2 175-199
5'-GCXGCATTGAATTAACTAXGXGXGCC-3' (SEQ ID NO:319)
AGCXGCATTGAATTAACTAXGXGXGCC (SEQ ID NO:320)
GAGCXGCATTGAATTAACTAXGXGXGCC (SEQ ID NO:321)
AGAGCXGCATTGAATTAACTAXGXGXGCC (SEQ ID NO:322)
GAGAGCXGCATTGAATTAACTAXGXGXGCC (SEQ ID NO:323)
AGAGAGCXGCATTGAATTAACTAXGXGXGCC (SEQ ID NO:324)
GCXGCATTGAATTAACTAXGXGXGCCT (SEQ ID NO:325)
GCXGCATTGAATTAACTAXGXGXGCCTA (SEQ ID NO:326)
GCXGCATTGAATTAACTAXGXGXGCCTAC (SEQ ID NO:327)
GCXGCATTGAATTAACTAXGXGXGCCTACC (SEQ ID NO:328)
GCXGCATTGAATTAACTAXGXGXGCCTACCA (SEQ ID NO:329)
AGCXGCATTGAATTAACTAXGXGXGCCT (SEQ ID NO:330)
GAGCXGCATTGAATTAACTAXGXGXGCCTA (SEQ ID NO:331)
AGAGCXGCATTGAATTAACTAXGXGXGCCTAC (SEQ ID NO:332)
GAGAGCXGCATTGAATTAACTAXGXGXGCCTACC (SEQ ID NO:333)
AGAGAGCXGCATTGAATTAACTAXGXGXGCCTACCA (SEQ ID NO:334)
AGAGCXGCATTGAATTAACTAXGXGX (SEQ ID NO:335)
AAGAGAGCXGCATTGAATTAACTAXG (SEQ ID NO:336)
AGTAAGAGAGCXGCATTGAATTAACT (SEQ ID NO:337)
CAGAGTAAGAGAGCXGCATTGAATTA (SEQ ID NO:338)
AAACAGAGTAAGAGAGCXGCATTGAA (SEQ ID NO:339)
GCATTGAATTAACTAXGXGXGCCTAC (SEQ ID NO:340)
TTGAATTAACTAXGXGXGCCTACCAT (SEQ ID NO:341)
AATTAACTAXGXGXGCCTACCATTTT (SEQ ID NO:342)
TAACTAXGXGXGCCTACCATTTTCTT (SEQ ID NO:343)
CTAXGXGXGCCTACCATTTTCTTTTG (SEQ ID NO:344)
XGCATTGAATTAACTAXGXGXGCC (SEQ ID NO:345)
CATTGAATTAACTAXGXGXGCC (SEQ ID NO:346)
TTGAATTAACTAXGXGXGCC (SEQ ID NO:347)
GAATTAACTAXGXGXGCC (SEQ ID NO:348)
ATTAACTAXGXGXGCC (SEQ ID NO:349)
TTAACTAXGXGXGCC (SEQ ID NO:350)
GCXGCATTGAATTAACTAXGXGXG (SEQ ID NO:351)
GCXGCATTGAATTAACTAXGXG (SEQ ID NO:352)
GCXGCATTGAATTAACTAXG (SEQ ID NO:353)
GCXGCATTGAATTAACTA (SEQ ID NO:354)
GCXGCATTGAATTAAC (SEQ ID NO:355)
GCXGCATTGAATTAA (SEQ ID NO:356)
GCXGCATTGAATTCACTAXGXGXGCC (SEQ ID NO:357)
GCXGCATCGAATTAACTAXGXGXGCC (SEQ ID NO:358)
GCXGCATTGAGTTAACTAXGXGXGCC (SEQ ID NO:359)

CM3 349-371**
5'-GCXGGTGGGXGGAGAGTTAGXGAG-3' (SEQ ID NO:360)
GGCXGGTGGGXGGAGAGTTAGXGAG (SEQ ID NO:361)
GGGCXGGTGGGXGGAGAGTTAGXGAG (SEQ ID NO:362)
AGGGCXGGTGGGXGGAGAGTTAGXGAG (SEQ ID NO:363)
AAGGGCXGGTGGGXGGAGAGTTAGXGAG (SEQ ID NO:364)
AAAGGGCXGGTGGGXGGAGAGTTAGXGAG (SEQ ID NO:365)
GCXGGTGGGXGGAGAGTTAGXGAGA (SEQ ID NO:366)
GCXGGTGGGXGGAGAGTTAGXGAGAG (SEQ ID NO:367)
GCXGGTGGGXGGAGAGTTAGXGAGAGA (SEQ ID NO:368)
GCXGGTGGGXGGAGAGTTAGXGAGAGAG (SEQ ID NO:369)
GCXGGTGGGXGGAGAGTTAGXGAGAGAGG (SEQ ID NO:370)

GGCXGGTGGGXGGAGAGTTAGXGAGA (SEQ ID NO:371)
GGGCXGGTGGGXGGAGAGTTAGXGAGAG (SEQ ID NO:372)
AGGGCXGGTGGGXGGAGAGTTAGXGAGAGA (SEQ ID NO:373)
AAGGGCXGGTGGGXGGAGAGTTAGXGAGAGAG (SEQ ID NO:374)
AAAGGGCXGGTGGGXGGAGAGTTAGXGAGAGAGG (SEQ ID NO:375)
AGGGCXGGTGGGXGGAGAGTTAGX (SEQ ID NO:376)
TAAAGGGCXGGTGGGXGGAGAGTT (SEQ ID NO:377)
TTATAAAGGGCXGGTGGGXGGAGAG (SEQ ID NO:378)
GCATTATAAAGGGCXGGTGGGXGGA (SEQ ID NO:379)
CTCGCATTATAAAGGGCXGGTGGGX (SEQ ID NO:380)
GGTGGGXGGAGAG(A)TTAGXGAGAGA (SEQ ID NO:381)
GGGXGGAGAGTTAGXGAGAGAGGA (SEQ ID NO:382)
XGGAGAGTTAGXGAGAGAGGATCT (SEQ ID NO:383)
AGAGTTAGXGAGAGAGGATCTTTT (SEQ ID NO:384)
GTTAGXGAGAGAGGATCTTTTTTC (SEQ ID NO:385)
XGGTGGGXGGAGAGTTAGXGAG (SEQ ID NO:386)
GTGGGXGGAGAGTTAGXGAG (SEQ ID NO:387)
GGGXGGAGAGTTAGXGAG (SEQ ID NO:388)
GXGGAGAGTTAGXGAG (SEQ ID NO:389)
GGAGAGTTAGXGAG (SEQ ID NO:390)
GCXGGTGGGXGGAGAGTTAGXG (SEQ ID NO:391)
GCXGGTGGGXGGAGAGTTAG (SEQ ID NO:392)
GCXGGTGGGXGGAGAGTT (SEQ ID NO:393)
GCXGGTGGGXGGAGAG (SEQ ID NO:394)
GCXGGTGGGXGGAGA (SEQ ID NO:395)
GCXGGTGGGXGGTGAGTTAGXGAG (SEQ ID NO:396)
GCXGGTCGGXGGAGAGTTAGXGAG (SEQ ID NO:397)
GCXGGTGGGXGGAGAGTTAGXTAG (SEQ ID NO:398)

CM6 13-39
5'-TGXGXGCCCACXGCCAXGCCAXGXGXGT-3' (SEQ ID NO:399)
CTGXGXGCCCACXGCCAXGCCAXGXGXGT (SEQ ID NO:400)
ACTGXGXGCCCACXGCCAXGCCAXGXGXGT (SEQ ID NO:401)
CACTGXGXGCCCACXGCCAXGCCAXGXGXGT (SEQ ID NO:402)
CCACTGXGXGCCCACXGCCAXGCCAXGXGXGT (SEQ ID NO:403)
ACCACTGXGXGCCCACXGCCAXGCCAXGXGXGT (SEQ ID NO:404)
TGXGXGCCCACXGCCAXGCCAXGXGXGTA (SEQ ID NO:405)
TGXGXGCCCACXGCCAXGCCAXGXGXGTAC (SEQ ID NO:406)
TGXGXGCCCACXGCCAXGCCAXGXGXGTACC (SEQ ID NO:407)
TGXGXGCCCACXGCCAXGCCAXGXGXGTACCA (SEQ ID NO:408)
TGXGXGCCCACXGCCAXGCCAXGXGXGTACCAG (SEQ ID NO:409)
CTGXGXGCCCACXGCCAXGCCAXGXGXGTA (SEQ ID NO:410)
ACTGXGXGCCCACXGCCAXGCCAXGXGXGTAC (SEQ ID NO:411)
CACTGXGXGCCCACXGCCAXGCCAXGXGXGTACC (SEQ ID NO:412)
CCACTGXGXGCCCACXGCCAXGCCAXGXGXGTACCA (SEQ ID NO:413)
ACCACTGXGXGCCCACXGCCAXGCCAXGXGXGTACCAG (SEQ ID NO:414)
CACTGXGXGCCCACXGCCAXGCCAXGXG (SEQ ID NO:415)
AACCACTGXGXGCCCACXGCCAXGCCAX (SEQ ID NO:416)
GAGAACCACTGXGXGCCCACXGCCAXGC (SEQ ID NO:417)
ACCGAGAACCACTGXGXGCCCACXGCCA (SEQ ID NO:418)
CACACCGAGAACCACTGXGXGCCCACXG (SEQ ID NO:419)
GXGCCCACXGCCAXGCCAXGXGXGTACC (SEQ ID NO:420)
CCCACXGCCAXGCCAXGXGXGTACCAGG (SEQ ID NO:421)
ACXGCCAXGCCAXGXGXGTACCAGGCTG (SEQ ID NO:422)
GCCAXGCCAXGXGXGTACCAGGCTGCAG (SEQ ID NO:423)
XGXGCCCACXGCCAXGCCAXGXGXGT (SEQ ID NO:424)
XGCCCACXGCCAXGCCAXGXGXGT (SEQ ID NO:425)
CCCACXGCCAXGCCAXGXGXGT (SEQ ID NO:426)
CACXGCCAXGCCAXGXGXGT (SEQ ID NO:427)
CXGCCAXGCCAXGXGXGT (SEQ ID NO:428)
GCCAXGCCAXGXGXGT (SEQ ID NO:429)

CCAXGCCAXGXGXGT (SEQ ID NO:430)
TGXGXGCCCACXGCCAXGCCAXGXGX (SEQ ID NO:431)
TGXGXGCCCACXGCCAXGCCAXGX (SEQ ID NO:432)
TGXGXGCCCACXGCCAXGCCAX (SEQ ID NO:433)
TGXGXGCCCACXGCCAXGCC (SEQ ID NO:434)
TGXGXGCCCACXGCCAXG (SEQ ID NO:435)
TGXGXGCCCACXGCCA (SEQ ID NO:436)
TGXGXGCCCACXGCC (SEQ ID NO:437)
TGXGXGCCCACXGCCGXGCCAXGXGXGT (SEQ ID NO:438)
TGXGXGTCCACXGCCAXGCCAXGXGXGT (SEQ ID NO:439)
TGXGXGCCCGCXGCCAXGCCAXGXGXGT (SEQ ID NO:440)

Figure 30

TG3 805-830
5'-AGGGGXGGGGAXGCTGAGXGCACXGA-3' (SEQ ID NO:1299)
AAGGGGXGGGGAXGCTGAGXGCACXGA (SEQ ID NO:1300)
TAAGGGGXGGGGAXGCTGAGXGCACXGA (SEQ ID NO:1301)
GTAAGGGGXGGGGAXGCTGAGXGCACXGA (SEQ ID NO:1302)
GGTAAGGGGXGGGGAXGCTGAGXGCACXGA (SEQ ID NO:1303)
GGGTAAGGGGXGGGGAXGCTGAGXGCACXGA (SEQ ID NO:1304)
AGGGGXGGGGAXGCTGAGXGCACXGAG (SEQ ID NO:1305)
AGGGGXGGGGAXGCTGAGXGCACXGAGC (SEQ ID NO:1306)
AGGGGXGGGGAXGCTGAGXGCACXGAGCA (SEQ ID NO:1307)
AGGGGXGGGGAXGCTGAGXGCACXGAGCAG (SEQ ID NO:1308)
AGGGGXGGGGAXGCTGAGXGCACXGAGCAGG (SEQ ID NO:1309)
AAGGGGXGGGGAXGCTGAGXGCACXGAG (SEQ ID NO:1310)
TAAGGGGXGGGGAXGCTGAGXGCACXGAGC (SEQ ID NO:1311)
GTAAGGGGXGGGGAXGCTGAGXGCACXGAGCA (SEQ ID NO:1312)
GGTAAGGGGXGGGGAXGCTGAGXGCACXGAGCAG (SEQ ID NO:1313)
GGGTAAGGGGXGGGGAXGCTGAGXGCACXGAGCAGG (SEQ ID NO:1314)
GTAAGGGGXGGGGAXGCTGAGXGCAC (SEQ ID NO:1315)
GGGGTAAGGGGXGGGGAXGCTGAGXG (SEQ ID NO:1316)
TTTGGGGTAAGGGGXGGGGAXGCTGAG (SEQ ID NO:1317)
GGGTTTGGGGTAAGGGGXGGGGAXGCT (SEQ ID NO:1318)
TGGGGGTTTGGGGTAAGGGGXGGGGAX (SEQ ID NO:1319)
GGXGGGGAXGCTGAGXGCACXGAGCA (SEQ ID NO:1320)
GGGGAXGCTGAGXGCACXGAGCAGGG (SEQ ID NO:1321)
GAXGCTGAGXGCACXGAGCAGGGCGC (SEQ ID NO:1322)
GCTGAGXGCACXGAGCAGGGCGCGGG (SEQ ID NO:1323)
GAGXGCACXGAGCAGGGCGCGGGCTG (SEQ ID NO:1324)
GGGXGGGGAXGCTGAGXGCACXGA (SEQ ID NO:1325)
GXGGGGAXGCTGAGXGCACXGA (SEQ ID NO:1326)
GGGGAXGCTGAGXGCACXGA (SEQ ID NO:1327)
GGAXGCTGAGXGCACXGA (SEQ ID NO:1328)
AXGCTGAGXGCACXGA (SEQ ID NO:1329)
GCTGAGXGCACXGA (SEQ ID NO:1330)
AGGGGXGGGGAXGCTGAGXGCACX (SEQ ID NO:1331)
AGGGGXGGGGAXGCTGAGXGCA (SEQ ID NO:1332)
AGGGGXGGGGAXGCTGAGXG (SEQ ID NO:1333)
AGGGGXGGGGAXGCTGAGX (SEQ ID NO:1334)
AGGGGXGGGGAXGCTGA (SEQ ID NO:1335)
AGGGGXGGGGAXGCT (SEQ ID NO:1336)
AGGGGXGAGGAXGCTGAGXGCACXGA (SEQ ID NO:1337)
AGGGGXGGGGAXGCTGATXGCACXGA (SEQ ID NO:1338)
AGGGGXGGGGAXGCTCAGXGCACXGA (SEQ ID NO:1339)

TG5 728-762
5'-GCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGA-3' (SEQ ID NO:1340)
AGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGA (SEQ ID NO:1341)
GAGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGA (SEQ ID NO:1342)
AGAGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGA (SEQ ID NO:1343)
CAGAGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGA (SEQ ID NO:1344)
CCAGAGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGA (SEQ ID NO:1345)
GCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAA (SEQ ID NO:1346)
GCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAAA (SEQ ID NO:1347)
GCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAAAA (SEQ ID NO:1348)
GCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAAAAG (SEQ ID NO:1349)
GCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAAAAGG (SEQ ID NO:1350)
AGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAA (SEQ ID NO:1351)
GAGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAAA (SEQ ID NO:1352)

AGAGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAAAA (SEQ ID NO:1353)
CAGAGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAAAAG (SEQ ID NO:1354)
CCAGAGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAAAAGG (SEQ ID NO:1355)
AGAGCCTGGXGGXGXGAGCXGGGCACCXGGGCXGA (SEQ ID NO:1356)
CCCAGAGCCTGGXGGXGXGAGCXGGGCACCXGGGC (SEQ ID NO:1357)
GATCCCAGAGCCTGGXGGXGXGAGCXGGGCACCXG (SEQ ID NO:1358)
TGGGATCCCAGAGCCTGGXGGXGXGAGCXGGGCAC (SEQ ID NO:1359)
ACCTGGGATCCCAGAGCCTGGXGGXGXGAGCXGGG (SEQ ID NO:1360)
TGGXGGXGXGAGCXGGGCACCXGGGCXGAXGAAAA (SEQ ID NO:1361)
XGGXGXGAGCXGGGCACCXGGGCXGAXGAAAAGGC (SEQ ID NO:1362)
XGXGAGCXGGGCACCXGGGCXGAXGAAAAGGCAGG (SEQ ID NO:1363)
GAGCXGGGCACCXGGGCXGAXGAAAAGGCAGGAAG (SEQ ID NO:1364)
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CTGGXGGXGXGAGCXGGGCACCXGGGCXGAXGA (SEQ ID NO:1366)
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CACCXGGGCXGAXGA (SEQ ID NO:1375)
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GCCTGGXGGXGXGAGCXGGGCACCXGGGCXG (SEQ ID NO:1377)
GCCTGGXGGXGXGAGCXGGGCACCXGGGC (SEQ ID NO:1378)
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GCCTGGXGGXGXGAGCXGGGC (SEQ ID NO:1382)
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GCCTGGXGGXGXGAG (SEQ ID NO:1385)
GCCTGGXGGXGXGAGCXAGGCACCXGGGCXGAXGA (SEQ ID NO:1386)
GCCTGGXGCXGXGAGCXGGGCACCXGGGCXGAXGA (SEQ ID NO:1387)
GCCTGGXGGXGXGAGCXGGGCACCXGGGCXTAXGA (SEQ ID NO:1388)

TG8 754-787**
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TGGGXGGGGXGACCTGGGATCCCAGGCCTGGXGG (SEQ ID NO:1390)
CTGGGXGGGGXGACCTGGGATCCCAGGCCTGGXGG (SEQ ID NO:1391)
GCTGGGXGGGGXGACCTGGGATCCCAGGCCTGGXGG (SEQ ID NO:1392)
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CTGCTGGGXGGGGXGACCTGGGATCCCAGGCCTGGXGG (SEQ ID NO:1394)
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Figure 31

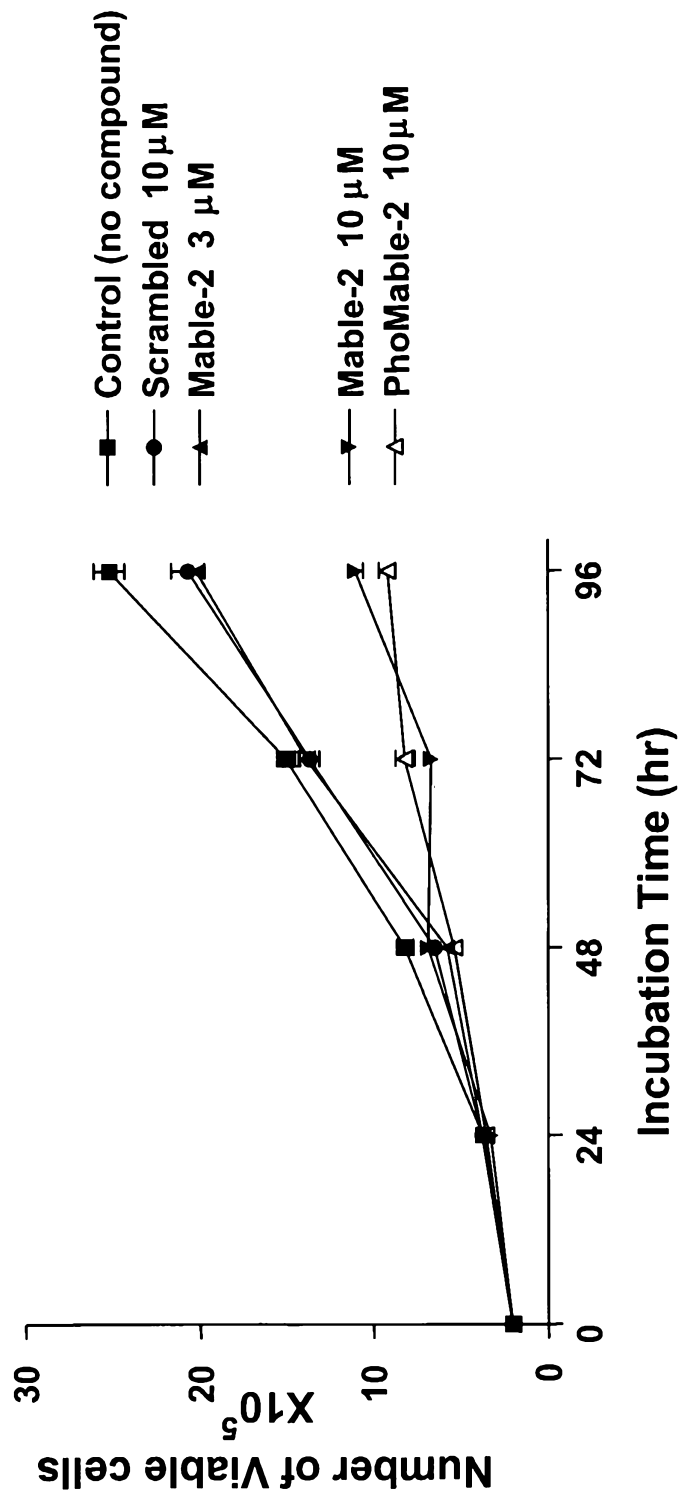

Number of Viable cells
X10^5
30
20
10
0
0
24
48
72
96
Incubation Time (hr)
Control (no compound)
Scrambled 10 μM
Mable-2 3 μM
Mable-2 10 μM
PhoMable-2 10μM

Figure 32

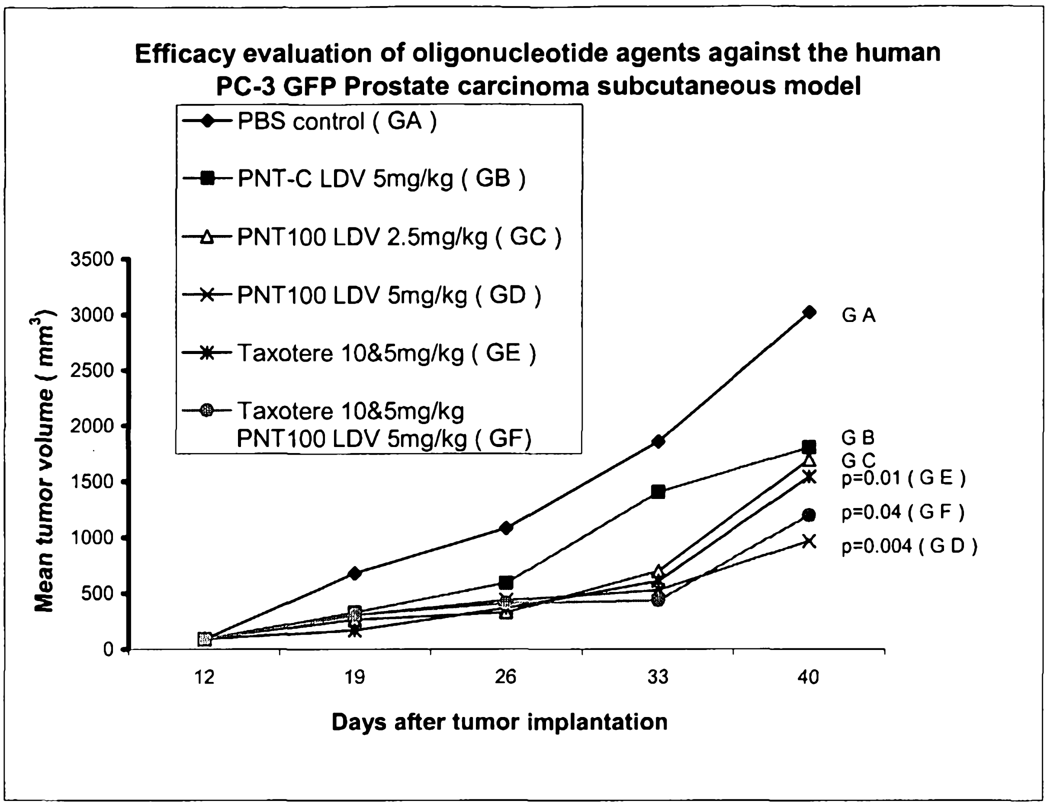

Efficacy evaluation of oligonucleotide agents against the human PC-3 GFP Prostate carcinoma subcutaneous model
PBS control (GA)
PNT-C LDV 5mg/kg (GB)
PNT100 LDV 2.5mg/kg (GC)
PNT100 LDV 5mg/kg (GD)
Taxotere 10&5mg/kg (GE)
Taxotere 10&5mg/kg PNT100 LDV 5mg/kg (GF)
Mean tumor volume (mm3)
3500
3000
2500
2000
1500
1000
500
0
12
19
26
33
40
Days after tumor implantation
G A
G B
G C
p=0.01 (G E)
p=0.04 (G F)
p=0.004 (G D)

Figure 33

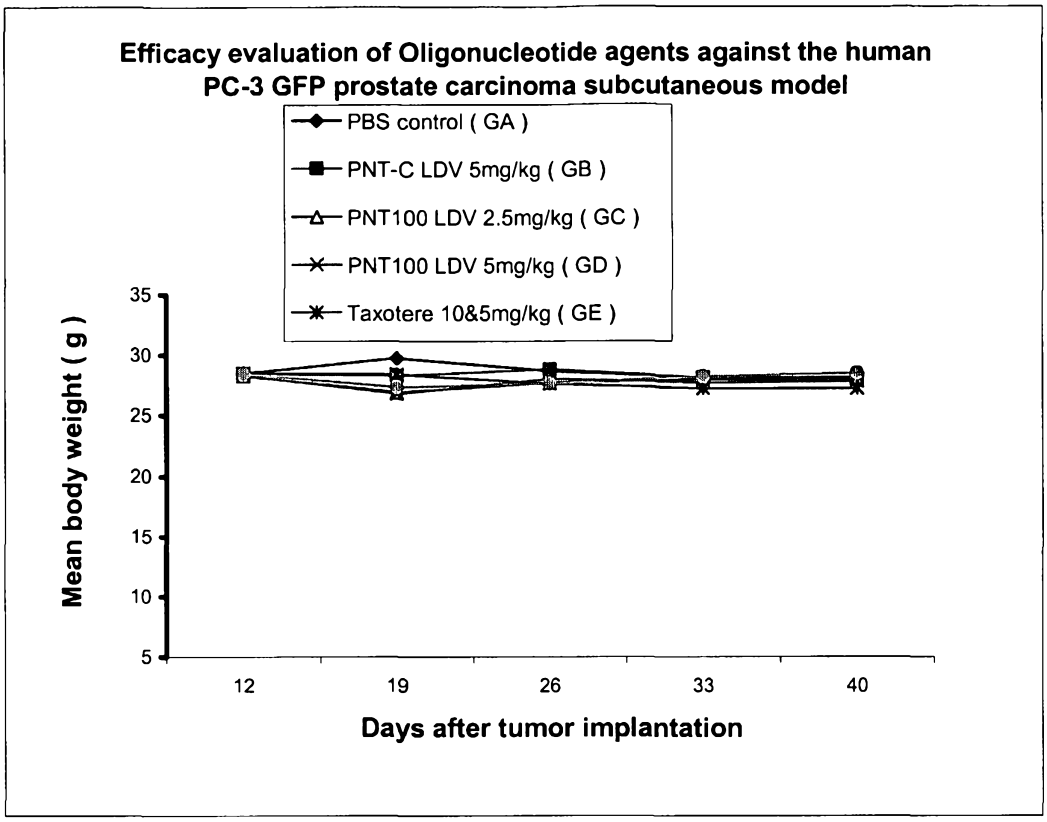

Efficacy evaluation of Oligonucleotide agents against the human PC-3 GFP prostate carcinoma subcutaneous model
PBS control (GA)
PNT-C LDV 5mg/kg (GB)
PNT100 LDV 2.5mg/kg (GC)
PNT100 LDV 5mg/kg (GD)
Taxotere 10&5mg/kg (GE)
Mean body weight (g)
35
30
25
20
15
10
5
12
19
26
33
40
Days after tumor implantation

Figure 34

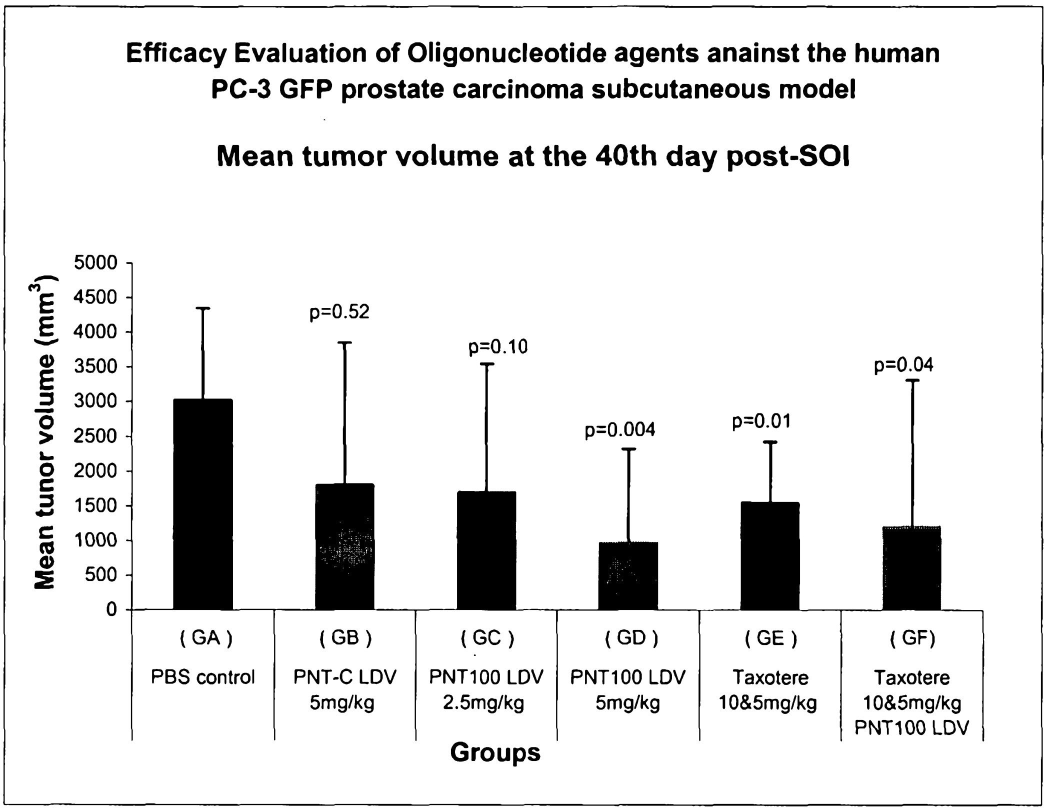
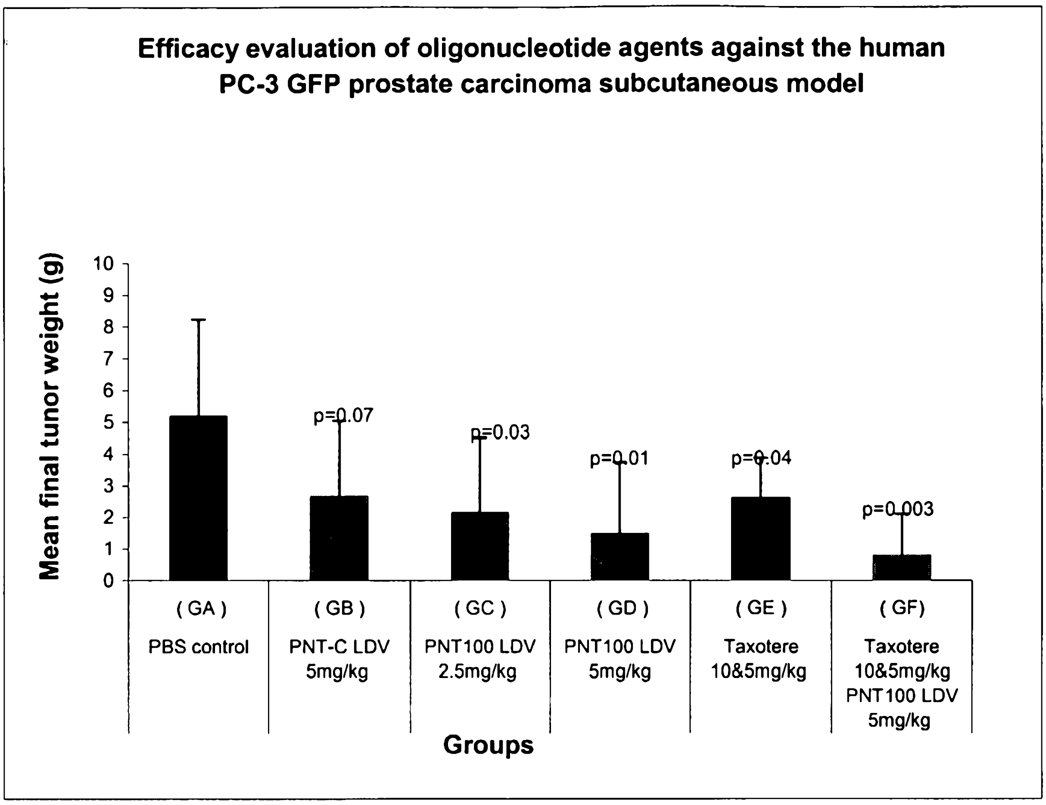

Efficacy Evaluation of Oligonucleotide agents anainst the human PC-3 GFP prostate carcinoma subcutaneous model
Mean tumor volume at the 40th day post-SOI
Mean tunor volume (mm3)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
p=0.52
p=0.10
p=0.004
p=0.01
p=0.04
(GA) PBS control
(GB) PNT-C LDV 5mg/kg
(GC) PNT100 LDV 2.5mg/kg
(GD) PNT100 LDV 5mg/kg
(GE) Taxotere 10&5mg/kg
(GF) Taxotere 10&5mg/kg PNT100 LDV
Groups

Figure 35


Efficacy evaluation of oligonucleotide agents against the human PC-3 GFP prostate carcinoma subcutaneous model
Mean final tunor weight (g)
10
9
8
7
6
5
4
3
2
1
0
p=0.07
p=0.03
p=0.01
p=0.04
p=0.003
(GA)
PBS control
(GB)
PNT-C LDV 5mg/kg
(GC)
PNT100 LDV 2.5mg/kg
(GD)
PNT100 LDV 5mg/kg
(GE)
Taxotere 10&5mg/kg
(GF)
Taxotere 10&5mg/kg PNT100 LDV 5mg/kg
Groups

SEQUENCE LISTING

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       Sooch, Mina Patel
       Goodwin, Neal
       Olson, David

<120> Methods and Compositions for the Inhibition of Gene Expression

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<140> PCT/US2005/018993
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gtctgcgagt gtgaatgtgt atgtgtgtat ctatgtacct catttgagaa agtgcggcca   240

actaggattg gctacgaggc aaaggtggag acctttagga gcccacccac cccagcgtta   300

ggacggtggg cctgaaagtt actatatgga agtcctcatc gtgtagcact aaaccagtgt   360

aaaaggtgtt agggacagag ggaaaacatt gacttaaact gtcgtaaagc ccttgataaa   420

ccccttttccc tggagctgct gagttctgca tggcctgggc acggactagg tgttcaggtg   480

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gtaaacacca cgctcggggc gtccctagac attgcttaaa acgtgcagag tcacctgtct   600

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aaggtccagg cgagacacac acacacacac acacacacac actccacaca cactcacacg   840

gccagaaagg gtccaggcgg ttggcggcgc ttttccagcc cttccgcccg gcccggcgcg   900

tcttttcatg gcgcaccctc ccgccagccg ccccccctccg cactccgtcg tgcgattccc   960

cgggagcccc caccccgtcg cggaccccag cgaccaccaa gtcgcaccgg cctccgcagg   1020

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ggcgccctcc ccggcccggc cccgcgcgcc atgtgccccc ggcgggacgg ccactcccgg   1200

gcctgccgcg gcgcctttaa cccgggccag ggagcggggc ggagggggcg gtcggggtgg   1260

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<400> 44
gcgtccctcc tagcgccgg   19

<210> 45
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<400> 45
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tggccgctcg cggcgtctcc ctgtggcatc cgcacagccc gccgcggtcc ggtcccgctc   120

cgggtcagaa ttggcggctg cggggacagc cttgcggcta ggcagggggc gggccgccgc   180

gtgggtccgg cagtccctcc tcccgccaag gcgccgccca gacccgctct ccagccggcc   240

cggctcgcca ccctagaccg ccccagccac cccttcctcc gccggcccgg cccccgctcc   300

tcccccgccg gcccggcccg gccccctcct tctccccgcc ggcgctcgct gcctccccct   360

cttccctctt cccacaccgc cctcagccgc tccctctcgt acgcccgtct gaagaagaat   420

cgagcgcgga acgcatcgat agctctgccc tctgcggccg cccggccccg aactcatcgg   480

tgtgctcgga gctcgatttt cctaggcggc ggccgcggcg gcggaggcag cagcggcggc   540

ggcagtggcg gcggcgaagg tggcggcggc tcggccagta ctcccggccc ccgccatttc   600

ggactgggag cgagcgcggc gcaggcactg aaggcggcgg cggggccaga ggctcagcgg   660

ctcccaggtg cgggagagag gtacggagcg gaccacccct cctgggcccc tgcccggg   718

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<400> 47
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<210> 48

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<400> 48
gccggaccca cgcggcggcc cgcc   24

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<210> 50
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ttcgccgccg ccactgccgc cgccgct   27

<210> 51
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<400> 52
tgcgcgcgct cgctcccagt ccgaa   25

<210> 53
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<400> 53
gcggccacgc cgccccgctg accggtct   28

<210> 54
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<400> 54
ccacagagaa gctgcgaaga gcaccccgc   29

<210> 55
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<400> 55
gcgggctgtg cggatgccac agggagacgc cgcg   34

<210> 56
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<400> 56
gccgcaaggc tgtccccgca gccgcc   26

<210> 57
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<400> 57
gcgggtctgg gcggcgcctt ggcgg   25

<210> 58
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<400> 58
cggtctaggg tggcgagccg ggccggc   27

<210> 59
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<400> 59
gccggcgggg gaggagcggg ggccgggccg gcg   33

<210> 60
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<400> 60
gcgagcgccg gcggggagaa ggagggggcc gggccgg   37

<210> 61
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<400> 61
gctatcgatg cgttccgcgc tcg   23

<210> 62
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<400> 62
cgagctccga gcacaccgat gagttcgggg ccgggcggcc gc   42

<210> 63
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<400> 63
gacgggcgta cgagagggag cggctgaggg cgg   33

<210> 64
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<400> 64
ccgctgagcc tctggccccg ccgccgcctt c   31

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<400> 65
cgctccgtac ctctctcccg c   21

<210> 66
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gccggcgcag ggagggcctc tggtgcaccg gcaccgctga gtcgggttct ctcgccggcc   120

tgttcccggg agagcccggg gccctgctcg gagatgccgc cccgggcccc cagacaccgg   180

ctccctggcc ttcctcgagc aaccccgagc tcggctccgg tctccagcca agcccaaccc   240

cgagaggccg cggccctact ggctccgcct cccgcgttgc tcccggaagc cccgcccgac   300

cgcggctcct gacagacggg ccgctcagcc aaccggggtg gggcggggcc cgatggcgcg   360

cagccaatgg taggccgcgc ctggcagacg gacgggcgcg gggcggggcg tgcgcaggcc   420

cgcccgagtc tccgccgccc gtgccctgcg cccgcaaccc gagccgcacc cgccgcggac   480

ggagcccatg cgcggggcga accgcgcgcc cccgcccccg ccccgccccg gcctcggccc   540

cggccctggc cccgggggca gtcgcgcctg tgaacggtga gtgcgggcag ggatcggccg   600

ggccgcgcgc cctcctcgcc cccaggcggc agcaatacgc gcggcgcggg ccgggggcgc   660

ggggccggcg ggcgtaagcg gcggcggcgg cggcgggtgg gtggggccgg gcggggcccg   720

cgggcacagg tgagcgggcg tcgggggctg cggcgggcgg gggccccttc ctccctgggg   780

cctgcgggaa tccgggcccc acccgtggcc tcgcgctggg cacggtcccc acgccggcgt   840

acccgggagc ctcgggcccg gcgccctcac acccgggggc gtctgggagg aggcggccgc   900

ggccacggca cgcccgggca cccccgattc agcatcacag gtcgcggacc aggccggggg   960

cctcagcccc agtgcctttt ccctctccgg gtctcccgcg ccgcttctcg gccccttcct   1020

gtcgctcagt ccctgcttcc caggagctcc tctgtcttct ccagctttct gtggctgaaa   1080

gatgccccg gttccccgcc gggggtgcgg ggcgctgccc gggtctgccc tcccctcggc   1140

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agcagcttct aatttgggtg cgtggttgag agcgctcagc tgtcagccct gcctttgagg 1380

gctgggtccc ttttcccatc actgggtcat taagagcaag tgggggcgag gcgacagccc 1440

tcccgcacgc tgggttgcag ctgcacaggt aggcacgctg cagtccttgc tgcctggcgt 1500

tggggcccag ggaccgctgt gggtttgccc ttcagatggc cctgccagca gctgccctgt 1560

ggggcctggg gctgggcctg ggcctggctg agcagggccc tccttggcag gtggggcagg 1620

agaccctgta ggaggacccc gggccgcagg cccctgagga gcgatgacgg aatataagct 1680

ggtggtggtg ggcgccggcg gtgtgggcaa gagtgcgctg accatccagc tgatccagaa 1740

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ggcgcgcggt tcgccccgcg catggg 26

<210> 68
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ggcgcgcggt tcgccccgcg catggg   26

<210> 69
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ttccgtcatc gctcctcagg ggcctgcggc cc   32

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gcgcacgccc cgccccgcgc ccgtccgt   28

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ggcggggcgg gggcgggggc gcgcggt   27

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gcgcgccatc gggccccgcc ccaccccgg 29

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agcgtgcggg agggctgtcg cctcgccccc a   31

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gcggctcggg ttgcgggcgc agggca   26

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<400> 85
gcgccggcag cgcggcgccg cccaccgcgg aggtcccg   38

<210> 86
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<400> 86
cggcgagaga acccgactca gcggtgccgg tgc   33

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<400> 87
gcggcatctc cgagcagggc cccgggctct cccg   34

<210> 88
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<400> 88
ccggagccga gctcggggtt gctcg   25

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<400> 89
gcggagccag tagggccgcg gcctctcg   28

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cggcccgtct gtcaggagcc gcggtcg   27

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<400> 92
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cgggtacgcc ggcgtgggga ccgtgc   26

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<400> 102
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ctgtgcccgc gggccccgcc cggc   24

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cggcctggtc cgcgacctgt gatgctgaat cgggggtgc   39

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agctgttccc cctgcgatga tttatactca caggacaagg atgcgatttg tcaaacacta   120

cgaggagtag cagagaaagg gagagggttt gaaagggagc aaaagaaaat ggtaggcgcg   180

cgtagttaat tcatgcggct ctcttactct gtttacatcc tagaggtaga gtgctcggct   240

gcccggctga gtctcctccc caccttcccc accctcccca ccctccccat aagcgccctc   300

ccgggttccc aaagcagagg gcgtgggggga aaagaaaaaa gatcctctct cgctaatctc   360

cgcccaccgg ccctttataa tgcgagggtc tggacggctg aggacccccg agctgtgctg   420

ctcgcggccg ccaccgcggg ccccggccgt ctttggctcc cctcctgcct cgagaagggc   480

agggcttctc agaggcttgg cgggaaaaag aacggaggga gggatcgctc tgagtataaa   540

agccgatttt cggggcttta tctaactcgc tgtagtaatt ttaccgagag gcagagggag   600

cgagcgggcg gccgcctagg gtggaagagc cgggcgagta gagttgcact tgggaaggga   660

gatccggagc gaatcggggg cttcgcctct ggcccagccc tcccgctgat cccccaggca   720

gtggtccgca atccttgccg cattcacgaa aatttggcca taacacgggg cggtcacttt   780

acactggaac gtccaacacc cgagcaagga cgcgagtctc ccgancgcga ggagactatt   840

gagatgtgtc gattggctac atagggtgtc ttttctccca ctcctgcgcc atcgacagtt   900

ttctcagaat acctttggta agtggggctg gggtgggcgt tatttcagaa ctgtatcggg   960

caaattgtat tcctcaccgc cacctcccgc ggcttcttaa gggcgccagg gccgctttcg   1020

attcctctgc cgctgcgggg ccgacgttcg ggactgagca ctgcgcgctg cgccaggttt   1080

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<400> 116
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<400> 117
ctcagccggg cagccgagca ctctacc   27

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ccccaaattc cagcaccagg ggccacctca gagccacaaa tcctgaaagg accacccagg 180

tgactcgggc ccacacaccc ctctttcggg gtacacttgg tggcaggcta ggagcttttg 240

gcctcagctg gtggtgccac acaccagatt cgtcacagag acccattttt tatcagtccc 300

aggccagaag tacgaagact gatcttaacg atgtggcctg cctgggaggt aaggcggcag 360

gcgttgcaga attgatggga actgtggcac aggtgggaaa cctggtttaa caaattcttc 420

attgattcag gggaccactt tccttgagcc aagtcttggc aagcggccgg cgaaactcac 480

aggtcccttt cctggctgcg tccccagcct ccagccttcc ccgccccaga gatgccccag 540

gagcggcccc tcggtgtagg taacgggtgc ccgggcggct ccgtccgccg cctagagcct 600

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tggagtccgc tgccgcacgg cgctggcagt cgggggtggt gtctgaagtc aggcgcttcc 720

tgccttttcg tcggcccggg tgcccggctc gcgccgccag gctctgggat cccaggtcgc 780

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ccggcgcggg agggcggagg gaggaccgt   29

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