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(57) Abstract: Certain embodiments are directed to compounds and compositions targeted to human androgen receptor (AR) for inhibiting androgen receptor levels in a cell, which can be useful for methods of treating cancer and inhibiting cancer cell growth or proliferation. Several embodiments provided herein relate to the discovery of compounds and compositions for inhibiting androgen receptor levels in a cell, which can be useful for methods of treating cancer and inhibiting proliferation or growth of cancer cells, such as prostate, breast, ovarian, gastric or bladder cancer or cancer cells.



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MODULATION OF ANDROGEN RECEPTOR EXPRESSION

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The
5 Sequence Listing is provided as a file entitled 200157WOSEQ.txt created October 1, 2013, which is
approximately 556 KB in size. The information in the electronic format of the sequence listing is
incorporated herein by reference in its entirety.

Field

Certain embodiments are directed to compounds and compositions targeted to human androgen
10 receptor (AR) for inhibiting androgen receptor levels in a cell, which can be useful for methods of treating
cancer and inhibiting cancer cell growth or proliferation.

Background

Androgen receptor (AR) belongs to the superfamily of nuclear receptors and is activated by
binding to its hormone ligands: androgen, testosterone, or DHT. Upon binding hormone ligand in the
15 cytoplasm, androgen receptor translocates to the nucleus where it binds DNA and functions as a
transcription factor to regulate expression of a number of target genes, such as prostate specific antigen
(PSA) and TMPRSS2. Knudsen et al. (Trends Endocrinol Metab 21: 315-24, 2010) Bennett et al. (Int J
Biochem Cell Biol. 42:813-827,201).

Androgen receptor (AR) signaling is a critical survival pathway for prostate cancer cells, and
20 androgen-deprivation therapy (ADT), also known as “chemical castration”, is a first-line treatment
strategy against hormone-sensitive, androgen-dependent prostate cancer that reduces circulating androgen
levels and thereby inhibits AR activity. Although a majority of patients initially respond to ADT, most
will eventually develop castrate resistance in which the disease progresses despite castrate levels of
testosterone. This type of cancer is known as castrate-resistant prostate cancer (CRPC). There are a
25 number of mechanisms underlying the development of castrate (castration) resistance including an
increase in the expression of AR protein which can sensitize cells to low levels of androgen, AR
mutations that can alter transactivation or sensitize AR to alternative ligands and the emergence of
alternatively spliced forms of AR, which lack the ligand binding domain but can nevertheless act to
promote tumour growth in the absence of ligand stimulation. Additionally prostate tumors may also
30 synthesize their own androgens thereby increasing the local intra-tumoral testosterone levels available to
activate the AR.

Androgen receptor (AR) signaling is a critical survival pathway for prostate cancer cells, and androgen-deprivation therapy (ADT) remains the principal treatment for patients with locally advanced and metastatic disease. Although a majority of patients initially respond to ADT, most will eventually develop castrate resistance in which the disease progresses despite castrate levels of testosterone. This type of cancer is known as castrate-resistant prostate cancer (CRPC) (Karantos et al., *Oncogene advance online*: 1-13, 2013). There are a number of mechanisms underlying the development of castration resistance including an increase in the expression of AR protein which can sensitize cells to low levels of androgen (Gregory et al., *Cancer Res* 61: 2892-2898, 2001; Linja et al., *Cancer Res* 61: 3550-3555, 2001), AR mutations that can alter transactivation or sensitize AR to alternative ligands (Scher et al., *J Clin Oncol* 23: 8253-8261, 2005) and the emergence of alternatively spliced forms of AR, which lack the ligand binding domain but can nevertheless act to promote tumour growth in the absence of ligand stimulation (Yingming et al., *Cancer Res* 73:483-489, 2013). Additionally prostate tumors may also synthesize their own androgens thereby increasing the local intra-tumoral testosterone levels available to activate the AR (Attard et al., *Cancer Cell* 16:458-462, 2009).

The fact that the androgen receptor remains active in castrate resistant prostate cancer has led to the development of new agents that inhibit the production of androgen ligands or block the actions of these ligands on the AR. These new agents include abiraterone acetate which inhibits 17- α -hydroxylase/17,20-lyase (CYP17) activity resulting in a reduction in residual androgens synthesized by the adrenals and in the prostate tumour itself deBono et al. (*N Engl J Med* 364: 1995-2006, 2011) and enzalutamide which prevents androgen ligand from binding to AR, translocating to the nucleus, and binding to DNA (Scher et al., *N Engl J Med* 367:1187-1197, 2012). A number of other androgen synthesis inhibitors or androgen receptor blockers are under development either pre-clinically or clinically and include for example, ARN509, ODM201, TOK001, VT464.

Although the activity of agents such as enzalutamide and abiraterone in CRPC is very encouraging, neither works in all patients and both are associated with the development of additional resistance through re-activation of the AR by the mechanisms described above (Yingming et al., *Cancer Res* 73:483-489, 2013). Thus, there is a continued need to identify alternative therapies for the treatment of CRPC, and in particular those that can either remove and/or inhibit the activity of all forms of AR including for example, wildtype, mutated and splice variant ARs.

The present invention provides antisense oligonucleotides which by virtue of their design and mode of action (base-pair with the AR RNA target and mediate its destruction by RNase H, an enzyme that destroys the RNA in a DNA/RNA duplex) are aimed at inhibiting the major forms of AR. By

targeting an appropriate region of the AR mRNA the antisense oligonucleotide will result in inhibition of the major forms (full length, splice variant and mutated forms) of androgen receptor proteins and therefore be suitable for the treatment of patients with CRPC.

Aside from prostate cancer, AR is also implicated as a factor in the progression of other tumours such as breast cancer. In breast cancer AR is expressed in 70-80% of tumours which are also ER positive and in 12% cases which are known as triple negative (no expression of ER, PR and HER2) (Hickey et al., Molecular Endocrinology 26: 1252-1267, 2012). In pre-clinical studies, the androgen receptor antagonist bicalutamide induces anti-proliferative responses in vitro in breast cancer cells and this is potentiated by addition of a Pi3K/mTOR inhibitor (Ni et al., Cancer Cell 20: 119-131, 2011). The 2nd generation anti-androgen, enzalutamide inhibits dihydrotestosterone (DHT) mediated proliferation in ER+/AR+ breast cancer cells and is as effective as tamoxifen at inhibiting estrogen-stimulated breast cancer tumour growth in pre-clinical models in vivo (Cochrane et al., Cancer Res 72(24 Supplement): P2-14-02, 2012). Enzalutamide also inhibits proliferation in HER2+ and triple-negative breast cancer cells. It appears that in situations where estrogen action is reduced (eg. long-term estrogen deprivation or absence of ER) AR levels increase and can become oncogenic. This would suggest that AR antagonists may be best positioned in triple negative or hormone resistant breast cancer settings (Hickey et al., Molecular Endocrinology 26: 1252-1267, 2012). AR targeted therapies are currently under investigation in clinical trials for breast cancer (NCT00468715, NCT01597193, NCT01381874, NCT00755886).

AR is also expressed in a variety of other tumours, including, but not limited to bladder, ovarian, gastric, lung and liver. Pre-clinical data support a similar role as in breast cancer, to promote tumour cell proliferation survival; thus blocking AR in these tumours could have therapeutic clinical benefit (Chang et al., Oncogene advance online: 1-10, 2013).

Summary

Several embodiments provided herein relate to the discovery of compounds and compositions for inhibiting androgen receptor levels in a cell, which can be useful for methods of treating cancer and inhibiting proliferation or growth of cancer cells, such as prostate, breast, ovarian, gastric or bladder cancer or cancer cells.

Detailed Description

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the

use of “or” means “and/or” unless stated otherwise. Furthermore, the use of the term “including” as well as other forms, such as “includes” and “included”, is not limiting. Also, terms such as “element” or “component” encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

5 The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

10 *Definitions*

 Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis. Where permitted, all patents,
15 applications, published applications and other publications, GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure herein are incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

 Unless otherwise indicated, the following terms have the following meanings:

20 “2’-O-methoxyethyl” (also 2’-MOE and 2’-O(CH₂)₂-OCH₃) refers to an O-methoxy-ethyl modification at the 2’ position of a sugar ring, e.g. a furanose ring. A 2’-O-methoxyethyl modified sugar is a modified sugar.

 “2’-MOE nucleoside” (also 2’-O-methoxyethyl nucleoside) means a nucleoside comprising a 2’-MOE modified sugar moiety.

25 “2’-substituted nucleoside” means a nucleoside comprising a substituent at the 2’-position of the furanosyl ring other than H or OH. In certain embodiments, 2’ substituted nucleosides include nucleosides with bicyclic sugar modifications.

 “3’ target site” refers to the nucleotide of a target nucleic acid which is complementary to the 3’-most nucleotide of a particular antisense compound.

30 “5’ target site” refers to the nucleotide of a target nucleic acid which is complementary to the 5’-most nucleotide of a particular antisense compound.

“5-methylcytosine” means a cytosine modified with a methyl group attached to the 5’ position. A 5-methylcytosine is a modified nucleobase.

“About” means within $\pm 7\%$ of a value. For example, if it is stated, “the compounds affected at least about 70% inhibition of Androgen Receptor”, it is implied that Androgen Receptor levels are inhibited within a range of 63% and 77%.

"Administration" or "administering" refers to routes of introducing an antisense compound provided herein to a subject to perform its intended function. An example of a route of administration that can be used includes, but is not limited to parenteral administration, such as subcutaneous, intravenous, or intramuscular injection or infusion.

“Androgen-receptor positive” with respect to breast cancer or a breast cancer cell refers to a breast cancer or a breast cancer cell that expresses androgen receptor.

“Animal” refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

“Anti-androgenic agent” refers to a therapeutic compound or drug which is an androgen synthesis inhibitor or an androgen receptor blocker.

“Antisense activity” means any detectable or measurable activity attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid.

“Antisense compound” means an oligomeric compound that is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding. Examples of antisense compounds include single-stranded and double-stranded compounds, such as, antisense oligonucleotides, siRNAs, shRNAs, ssRNAs, and occupancy-based compounds.

“Antisense inhibition” means reduction of target nucleic acid levels in the presence of an antisense compound complementary to a target nucleic acid compared to target nucleic acid levels in the absence of the antisense compound.

“Antisense mechanisms” are all those mechanisms involving hybridization of a compound with target nucleic acid, wherein the outcome or effect of the hybridization is either target degradation or target occupancy with concomitant stalling of the cellular machinery involving, for example, transcription or splicing.

“Antisense oligonucleotide” means a single-stranded oligonucleotide having a nucleobase sequence that permits hybridization to a corresponding region or segment of a target nucleic acid.

“Base complementarity” refers to the capacity for the precise base pairing of nucleobases of an antisense oligonucleotide with corresponding nucleobases in a target nucleic acid (i.e., hybridization), and is mediated by Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen binding between corresponding nucleobases.

“Bicyclic sugar moiety” means a modified sugar moiety comprising a 4 to 7 membered ring (including but not limited to a furanosyl) comprising a bridge connecting two atoms of the 4 to 7 membered ring to form a second ring, resulting in a bicyclic structure. In certain embodiments, the 4 to 7 membered ring is a sugar ring. In certain embodiments the 4 to 7 membered ring is a furanosyl. In certain such embodiments, the bridge connects the 2'-carbon and the 4'-carbon of the furanosyl.

Also included within the definition of LNA according to the invention are LNAs in which the 2'-hydroxyl group of the ribosyl sugar ring is connected to the 4' carbon atom of the sugar ring, thereby forming a methyleneoxy (4'-CH₂-O-2') bridge to form the bicyclic sugar moiety. The bridge can also be a methylene (-CH₂-) group connecting the 2' oxygen atom and the 4' carbon atom, for which the term methyleneoxy (4'-CH₂-O-2') LNA is used. Furthermore; in the case of the bicyclic sugar moiety having an ethylene bridging group in this position, the term ethyleneoxy (4'-CH₂CH₂-O-2') LNA is used. α -L-methyleneoxy (4'-CH₂-O-2'), an isomer of methyleneoxy (4'-CH₂-O-2') LNA is also encompassed within the definition of LNA, as used herein.

“Cap structure” or “terminal cap moiety” means chemical modifications, which have been incorporated at either terminus of an antisense compound.

“Castrate-resistant prostate cancer” or “Castration-resistant prostate cancer” and prostate cancer cells refer to the reduction of sensitivity of prostate cancer and prostate cancer cells to androgen deprivation therapy or an anti-androgenic agent.

“cEt” or “constrained ethyl” means a bicyclic sugar moiety comprising a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-CH(CH₃)-O-2'.

“Constrained ethyl nucleoside” (also cEt nucleoside) means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-O-2' bridge.

“Chemically distinct region” refers to a region of an antisense compound that is in some way chemically different than another region of the same antisense compound. For example, a region having

2'-O-methoxyethyl nucleotides is chemically distinct from a region having nucleotides without 2'-O-methoxyethyl modifications.

"Chimeric antisense compounds" means antisense compounds that have at least 2 chemically distinct regions, each position having a plurality of subunits.

5 "Complementarity" means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

"Comprise," "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

10 "Contiguous nucleobases" means nucleobases immediately adjacent to each other.

"Deoxyribonucleotide" means a nucleotide having a hydrogen at the 2' position of the sugar portion of the nucleotide. Deoxyribonucleotides may be modified with any of a variety of substituents.

"Designing" or "Designed to" refer to the process of designing an oligomeric compound that specifically hybridizes with a selected nucleic acid molecule.

15 "Downstream" refers to the relative direction toward the 3' end or C-terminal end of a nucleic acid.

"Efficacy" means the ability to produce a desired effect.

"Estrogen-receptor (ER) positive" with respect to breast cancer or a breast cancer cell refers to breast cancer or a breast cancer cell that expresses estrogen receptor (ER).

20 "Estrogen-receptor (ER) negative" with respect to breast cancer or a breast cancer cell refers to breast cancer or a breast cancer cell that does not express estrogen receptor (ER).

"Expression" includes all the functions by which a gene's coded information is converted into structures present and operating in a cell. Such structures include, but are not limited to the products of transcription and translation.

25 "Fully complementary" or "100% complementary" means each nucleobase of a first nucleic acid has a complementary nucleobase in a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a target nucleic acid is a second nucleic acid.

30 "Gapmer" means a chimeric antisense compound in which an internal region having a plurality of nucleosides that support RNase H cleavage is positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the

nucleoside or nucleosides comprising the external regions. The internal region may be referred to as the “gap” and the external regions may be referred to as the “wings.”

“Her2/neu negative” with respect to breast cancer or a breast cancer cell refers to breast cancer or a breast cancer cell that does not express Her2/neu.

5 “Hybridization” means the annealing of complementary nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense compound and a nucleic acid target. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense oligonucleotide and a nucleic acid target.

10 “Immediately adjacent” means there are no intervening elements between the immediately adjacent elements.

“Individual” means a human or non-human animal selected for treatment or therapy.

“Induce”, “inhibit”, “potentiate”, “elevate”, “increase”, “decrease”, “upregulate”, “downregulate”, or the like, generally denote quantitative differences between two states.

15 “Inhibiting the expression or activity” refers to a reduction, blockade of the expression or activity and does not necessarily indicate a total elimination of expression or activity.

“Internucleoside linkage” refers to the chemical bond between nucleosides.

“Lengthened” antisense oligonucleotides are those that have one or more additional nucleosides relative to an antisense oligonucleotide disclosed herein.

20 “Linked deoxynucleoside” means a nucleic acid base (A, G, C, T, U) substituted by deoxyribose linked by a phosphate ester to form a nucleotide.

“Linked nucleosides” means adjacent nucleosides linked together by an internucleoside linkage.

“Mismatch” or “non-complementary nucleobase” refers to the case when a nucleobase of a first nucleic acid is not capable of pairing with the corresponding nucleobase of a second or target nucleic acid.

25 “Modified internucleoside linkage” refers to a substitution or any change from a naturally occurring internucleoside bond (i.e. a phosphodiester internucleoside bond).

“Modified nucleobase” means any nucleobase other than adenine, cytosine, guanine, thymidine, or uracil. An “unmodified nucleobase” means the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U).

“Modified nucleoside” means a nucleoside having, independently, a modified sugar moiety and/or modified nucleobase.

“Modified nucleotide” means a nucleotide having, independently, a modified sugar moiety, modified internucleoside linkage, or modified nucleobase.

5 “Modified oligonucleotide” means an oligonucleotide comprising at least one modified internucleoside linkage, a modified sugar, and/or a modified nucleobase.

“Modified sugar” means substitution and/or any change from a natural sugar moiety.

“Monomer” refers to a single unit of an oligomer. Monomers include, but are not limited to, nucleosides and nucleotides, whether naturally occurring or modified.

10 “Motif” means the pattern of unmodified and modified nucleosides in an antisense compound.

“Natural sugar moiety” means a sugar moiety found in DNA (2'-H) or RNA (2'-OH).

“Naturally occurring internucleoside linkage” means a 3' to 5' phosphodiester linkage.

“Non-complementary nucleobase” refers to a pair of nucleobases that do not form hydrogen bonds with one another or otherwise support hybridization.

15 “Nucleic acid” refers to molecules composed of monomeric nucleotides. A nucleic acid includes, but is not limited to, ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, and double-stranded nucleic acids.

“Nucleobase” means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

20 “Nucleobase complementarity” refers to a nucleobase that is capable of base pairing with another nucleobase. For example, in DNA, adenine (A) is complementary to thymine (T). For example, in RNA, adenine (A) is complementary to uracil (U). In certain embodiments, complementary nucleobase refers to a nucleobase of an antisense compound that is capable of base pairing with a nucleobase of its target nucleic acid. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, then the position of
25 hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be complementary at that nucleobase pair.

“Nucleobase sequence” means the order of contiguous nucleobases independent of any sugar, linkage, and/or nucleobase modification.

“Nucleoside” means a nucleobase linked to a sugar.

"Nucleoside mimetic" includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo or tricyclo sugar mimetics, *e.g.*, non furanose sugar units. Nucleotide mimetic includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by -N(H)-C(=O)-O- or other non-phosphodiester linkage). Sugar surrogate overlaps with the slightly broader term nucleoside mimetic but is intended to indicate replacement of the sugar unit (furanose ring) only. The tetrahydropyranyl rings provided herein are illustrative of an example of a sugar surrogate wherein the furanose sugar group has been replaced with a tetrahydropyranyl ring system. "Mimetic" refers to groups that are substituted for a sugar, a nucleobase, and/ or internucleoside linkage. Generally, a mimetic is used in place of the sugar or sugar-internucleoside linkage combination, and the nucleobase is maintained for hybridization to a selected target.

"Nucleotide" means a nucleoside having a phosphate group covalently linked to the sugar portion of the nucleoside.

"Oligomeric compound" means a polymer of linked monomeric subunits which is capable of hybridizing to at least a region of a nucleic acid molecule.

"Oligonucleoside" means an oligonucleotide in which the internucleoside linkages do not contain a phosphorus atom.

"Oligonucleotide" means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another.

"Phosphorothioate linkage" means a linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom. A phosphorothioate linkage is a modified internucleoside linkage.

"Portion" means a defined number of contiguous (*i.e.*, linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound

"Progesterone receptor (PR) negative" with respect to breast cancer or a breast cancer cell refers to breast cancer or a breast cancer cell that does not express progesterone receptor (PR).

"Region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic.

“Ribonucleotide” means a nucleotide having a hydroxy at the 2’ position of the sugar portion of the nucleotide. Ribonucleotides may be modified with any of a variety of substituents.

“Segments” are defined as smaller or sub-portions of regions within a target nucleic acid.

“Sites,” as used herein, are defined as unique nucleobase positions within a target nucleic acid.

5 “Specifically hybridizable” refers to an antisense compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays and therapeutic treatments. “Stringent hybridization conditions” or “stringent conditions” refer to conditions under which
10 an oligomeric compound will hybridize to its target sequence, but to a minimal number of other sequences.

“Subject” means a human or non-human animal selected for treatment or therapy.

“Synergy” or “synergize” refers to an effect of a combination that is greater than additive of the effects of each component alone.

15 “Target” refers to a protein, the modulation of which is desired.

“Target gene” refers to a gene encoding a target.

“Targeting” means the process of design and selection of an antisense compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

20 “Target nucleic acid,” “target RNA,” “target RNA transcript” and “nucleic acid target” all mean a nucleic acid capable of being targeted by antisense compounds.

“Target region” means a portion of a target nucleic acid to which one or more antisense compounds is targeted.

25 “Target segment” means the sequence of nucleotides of a target nucleic acid to which an antisense compound is targeted. “5’ target site” refers to the 5’-most nucleotide of a target segment. “3’ target site” refers to the 3’-most nucleotide of a target segment.

30 “Treating cancer” refers to performing actions that lead to amelioration of cancer or of the symptoms accompanied therewith. The combination of said actions is encompassed by the term “treatment.” Amelioration of cancer includes, but is not limited to, reducing the number of cancer cells in a subject or reducing the number of cancer cells in the subject. Said treatment as used herein also includes an entire restoration of the health with respect to cancer. It is to be understood that treatment as used in

accordance with embodiments provided herein may not be effective in all subjects to be treated. However, a population of subjects suffering from cancer referred to herein can be successfully treated. In certain embodiments, “treating cancer” can be described by a number of different parameters including, but not limited to, reduction in the size of a tumor in a subject having cancer, reduction in the growth or proliferation of a tumor in a subject having cancer, preventing metastasis or reducing the extent of metastasis, and/or extending the survival of a subject having cancer compared to control. The cancer referred to in this definition can be any cancer including one selected from prostate cancer, breast cancer, ovarian cancer, gastric cancer and bladder cancer.

“Unmodified” nucleobases mean the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U).

“Unmodified nucleotide” means a nucleotide composed of naturally occurring nucleobases, sugar moieties, and internucleoside linkages. In certain embodiments, an unmodified nucleotide is an RNA nucleotide (i.e. β -D-ribonucleosides) or a DNA nucleotide (i.e. β -D-deoxyribonucleoside).

“Upstream” refers to the relative direction toward the 5’ end or N-terminal end of a nucleic acid.

Certain Embodiments

Certain embodiments provide methods, compounds, and compositions for inhibiting androgen receptor (AR) mRNA expression.

Certain embodiments provide antisense compounds or compositions targeted to an androgen receptor nucleic acid. In certain embodiments, the androgen receptor nucleic acid is the sequences set forth in GENBANK Accession No. NT_011669.17_TRUNC_5079000_5270000 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NM_000044.3 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No. NM_001011645.2 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. FJ235916.1 (incorporated herein as SEQ ID NO: 4), GENBANK Accession No. FJ235917.1 (incorporated herein as SEQ ID NO: 5), GENBANK Accession No. FJ235918.1 (incorporated herein as SEQ ID NO: 6), GENBANK Accession No. FJ235919.1 (incorporated herein as SEQ ID NO: 7), or GENBANK Accession No. FJ235920.1 (incorporated herein as SEQ ID NO: 8).

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide 10 to 30 linked nucleosides in length targeted to AR. The AR target can have a sequence recited in any one of SEQ ID NOs: 1-8 or a portion thereof or a variant thereof. In certain embodiments, the AR target can have a sequence of known AR splicing variants including, but are not limited to, AR-V1, AR-V2, AR-V3, AR-V4, AR-V5, AR-V6, and AR-V7 (also referred to as AR3), which contain exons 1-3 but lack exons 4-8. AR-V1, AR-V2, AR-V3, AR-V4, AR-V5, AR-V6, AR-V7, and additional splicing variants

targetable by compounds provided herein are described in Hu et al., *Cancer Res* 2009; 69:16-22 and US Patent Application Publication No. US 2010/0068802, each of which is incorporated herein by reference in its entirety.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 12-179. In certain embodiments, one or more modified nucleosides in the wing segment have a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified nucleoside is an LNA nucleoside. In certain embodiments, the modified nucleoside is a 2'-substituted nucleoside. In certain embodiments, 2' substituted nucleosides include nucleosides with bicyclic sugar modifications. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside. In certain embodiments, the modified nucleoside is a constrained ethyl (cEt) nucleoside.

In certain embodiments, the compounds or compositions comprise a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence consisting of a nucleobase sequence of any of SEQ ID NOs: 12-179. In certain embodiments, one or more modified nucleosides in the wing segment have a modified sugar. In certain embodiments, the modified sugar is a bicyclic sugar. In certain embodiments, the modified nucleoside is an LNA nucleoside. In certain embodiments, the modified nucleoside is a 2'-substituted nucleoside. In certain embodiments, 2' substituted nucleosides include nucleosides with bicyclic sugar modifications. In certain embodiments, the modified nucleoside is a 2'-MOE nucleoside. In certain embodiments, the modified nucleoside is a constrained ethyl (cEt) nucleoside.

In certain embodiments, the compounds or compositions targeted to androgen receptor comprise a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175, or a pharmaceutically acceptable salt thereof. In certain embodiments, the antisense compound targeted to human AR is ISIS 560131, ISIS 569213, ISIS 569216, ISIS 569221, ISIS 569236, ISIS 579671, ISIS 586124, ISIS 583918, ISIS 584149, ISIS 584163, ISIS 584269, or ISIS 584468.

In certain embodiments, the modified oligonucleotide comprises: a) a gap segment consisting of linked deoxynucleosides; b) a 5' wing segment consisting of linked nucleosides; and c) a 3' wing segment consisting of linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment and each nucleoside of each wing segment comprises a modified sugar.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of 10 linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, a gap segment consisting of 10 linked deoxynucleosides, a 5' wing segment consisting of three linked nucleosides, a 3' wing segment consisting of three linked nucleosides, each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, a gap segment consisting of 9 linked deoxynucleosides, a 5' wing segment consisting of three linked nucleosides, a 3' wing segment consisting of four linked nucleosides; the three linked nucleosides of the 5' wing segment are each a constrained ethyl (cEt) sugar; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, a gap segment consisting of 8 linked deoxynucleosides, a 5' wing segment consisting of five linked nucleosides, a 3' wing segment consisting of three linked nucleosides; the five linked nucleosides of the 5' wing segment are each a constrained ethyl (cEt) sugar; the three linked nucleosides of the 3' wing segment are each a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, a gap segment consisting of 8 linked deoxynucleosides, a 5' wing segment consisting of four linked nucleosides, a 3' wing segment consisting of four linked nucleosides; the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, a gap segment consisting of 8 linked deoxynucleosides, a 5' wing segment consisting of five linked nucleosides, a 3' wing segment consisting of three linked nucleosides; the five linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the three linked nucleosides of the 3' wing segment are each a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, a gap segment consisting of 7 linked deoxynucleosides, a 5' wing segment consisting of seven linked nucleosides, a 3' wing segment consisting of two linked nucleosides; the seven linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the two linked nucleosides of the 3' wing segment are each a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, a gap segment consisting of 7 linked deoxynucleosides, a 5' wing segment consisting of six linked nucleosides, a 3' wing segment consisting of three linked nucleosides; the six linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the three linked nucleosides of the 3' wing segment are each a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, a gap segment consisting of 7 linked deoxynucleosides, a 5' wing segment consisting of five linked nucleosides, a 3' wing segment consisting of four linked nucleosides; the five linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides, a gap segment consisting of 7 linked deoxynucleosides, a 5' wing segment consisting of four linked nucleosides, a 3' wing segment consisting of five linked nucleosides; the four linked nucleosides of the 5'

wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the five linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions targeted to androgen receptor comprise a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises a gap segment consisting of deoxynucleosides; a 5' wing segment; and a 3' wing segment, wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and each nucleoside of each wing segment comprises a modified sugar. In certain embodiments, each internucleoside linkage of the modified oligonucleotide is a phosphorothioate linkage. In certain embodiments, each cytosine of the modified oligonucleotide is a 5'-methylcytosine.

In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 35, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 9 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the three linked nucleosides of the 5' wing segment are each a constrained ethyl (cEt) sugar; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 39, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 9 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the three linked nucleosides of the 5' wing segment are each a constrained ethyl (cEt) sugar; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 39, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 8 linked deoxynucleosides;

a 5' wing segment consisting of four linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 39, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 8 linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the five linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar

in the 5' to 3' direction; the three linked nucleosides of the 3' wing segment are each a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 39, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 7 linked deoxynucleosides;

a 5' wing segment consisting of four linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the five linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 35, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 7 linked deoxynucleosides;

a 5' wing segment consisting of six linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the six linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the three linked nucleosides of the 3' wing segment are each a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 43, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

- 5 a gap segment consisting of 10 linked deoxynucleosides;
 a 5' wing segment consisting of three linked nucleosides; and
 a 3' wing segment consisting of three linked nucleosides;

 wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside
10 linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 124, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

- 15 a gap segment consisting of 10 linked deoxynucleosides;
 a 5' wing segment consisting of three linked nucleosides; and
 a 3' wing segment consisting of three linked nucleosides;

 wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside
20 linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 150, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

- 25 a gap segment consisting of 10 linked deoxynucleosides;
 a 5' wing segment consisting of three linked nucleosides; and
 a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

5 In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 155, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

10 a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

15 In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 169, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

20 a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

25 In certain embodiments, a compound targeted to androgen receptor comprises a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 175, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

5 In certain embodiments, an antisense compound or antisense oligonucleotide targeted to an androgen receptor nucleic acid is complementary within the following nucleotide regions of SEQ ID NO: 1: 2957-2972, 3079-3094, 3099-3114, 3109-3124, 3113-3128, 3120-3135, 3133-3148, 3224-3239, 3226-3241, 3351-3366, 3353-3368, 3361-3376, 3388-3403, 3513-3528, 3517-3532, 3519-3534, 3641-3656, 3735-3750, 3764-3779, 3768-3783, 3798-3813, 3799-3814, 3851-3866, 3870-3885, 3874-3889, 3876-3891, 3878-3893, 3884-3899, 3886-3901, 3888-3903, 3901-3916, 3956-3971, 3962-3977, 3964-3979, 10 3967-3982, 4019-4034, 4038-4053, 4049-4064, 4056-4071, 4059-4074, 4062-4077, 4066-4081, 4070-4085, 4101-4116, 4103-4118, 4105-4120, 4109-4124, 4305-4320, 4405-4420, 4532-4547, 4534-4549, 4537-4552, 4539-4554, 4555-4570, 4571-4586, 4573-4588, 4578-4593, 4597-4612, 4632-4647, 4655-4670, 4656-4671, 4662-4677, 4699-4714, 4747-4762, 4750-4765, 4752-4767, 4754-4769, 4755-4770, 15 4769-4784, 4798-4813, 4804-4819, 4807-4822, 4833-4848, 4837-4852, 4839-4854, 4865-4880, 4868-4883, 4872-4887, 4874-4889, 4876-4891, 4887-4902, 4889-4904, 4916-4931, 4918-4933, 4938-4953, 4942-4957, 4944-4959, 4951-4966, 5050-5065, 5052-5067, 5054-5069, 5056-5071, 5060-5075, 5061-5076, 5062-5077, 5133-5148, 5141-5156, 5155-5170, 5265-5280, 5293-5308, 5308-5323, 5392-5407, 5448-5463, 5469-5484, 5481-5496, 5483-5498, 5486-5501, 5488-5503, 5494-5509, 5521-5536, 5666-20 5681, 6222-6237, 6701-6716, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58739, 58720-58735, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58724-58739, 58725-58740, 58725-25 58740, 58725-58740, 58750-58769, 58750-58765, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-60917, 67454-67469, 102156-102171, 114874-114889, 115272-115287, 115365-115380, 134971-134986, 139682-139697, 139762-139777, 139782-139797, 144856-144871, 144938-144953, 148406-148421, 148443-148458, 148520-148535, 181695-181710, 182958-182973, or 183049-183064.

30 In certain embodiments, an antisense compound or antisense oligonucleotide targeted to an androgen receptor nucleic acid target the following nucleotide regions of SEQ ID NO: 1: 2957-2972, 3079-3094, 3099-3114, 3109-3124, 3113-3128, 3120-3135, 3133-3148, 3224-3239, 3226-3241, 3351-3366, 3353-3368, 3361-3376, 3388-3403, 3513-3528, 3517-3532, 3519-3534, 3641-3656, 3735-3750,

3764-3779, 3768-3783, 3798-3813, 3799-3814, 3851-3866, 3870-3885, 3874-3889, 3876-3891, 3878-3893, 3884-3899, 3886-3901, 3888-3903, 3901-3916, 3956-3971, 3962-3977, 3964-3979, 3967-3982, 4019-4034, 4038-4053, 4049-4064, 4056-4071, 4059-4074, 4062-4077, 4066-4081, 4070-4085, 4101-4116, 4103-4118, 4105-4120, 4109-4124, 4305-4320, 4405-4420, 4532-4547, 4534-4549, 4537-4552, 4539-4554, 4555-4570, 4571-4586, 4573-4588, 4578-4593, 4597-4612, 4632-4647, 4655-4670, 4656-4671, 4662-4677, 4699-4714, 4747-4762, 4750-4765, 4752-4767, 4754-4769, 4755-4770, 4769-4784, 4798-4813, 4804-4819, 4807-4822, 4833-4848, 4837-4852, 4839-4854, 4865-4880, 4868-4883, 4872-4887, 4874-4889, 4876-4891, 4887-4902, 4889-4904, 4916-4931, 4918-4933, 4938-4953, 4942-4957, 4944-4959, 4951-4966, 5050-5065, 5052-5067, 5054-5069, 5056-5071, 5060-5075, 5061-5076, 5062-5077, 5133-5148, 5141-5156, 5155-5170, 5265-5280, 5293-5308, 5308-5323, 5392-5407, 5448-5463, 5469-5484, 5481-5496, 5483-5498, 5486-5501, 5488-5503, 5494-5509, 5521-5536, 5666-5681, 6222-6237, 6701-6716, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58739, 58720-58735, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58724-58739, 58725-58740, 58725-58740, 58725-58740, 58750-58769, 58750-58765, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-60917, 67454-67469, 102156-102171, 114874-114889, 115272-115287, 115365-115380, 134971-134986, 139682-139697, 139762-139777, 139782-139797, 144856-144871, 144938-144953, 148406-148421, 148443-148458, 148520-148535, 181695-181710, 182958-182973, or 183049-183064.

In certain embodiments, antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of an androgen receptor nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the region. For example, the portion can be at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobases portion complementary to an equal length portion of a region recited herein. In certain embodiments, such compounds or oligonucleotide target the following nucleotide regions of SEQ ID NO: 1: 2957-2972, 3079-3094, 3099-3114, 3109-3124, 3113-3128, 3120-3135, 3133-3148, 3224-3239, 3226-3241, 3351-3366, 3353-3368, 3361-3376, 3388-3403, 3513-3528, 3517-3532, 3519-3534, 3641-3656, 3735-3750, 3764-3779, 3768-3783, 3798-3813, 3799-3814, 3851-3866, 3870-3885, 3874-3889, 3876-3891, 3878-3893, 3884-3899, 3886-3901, 3888-3903, 3901-3916, 3956-3971, 3962-3977, 3964-3979, 3967-3982, 4019-4034, 4038-4053, 4049-4064, 4056-4071, 4059-4074, 4062-4077, 4066-4081, 4070-4085, 4101-4116, 4103-4118, 4105-4120, 4109-4124, 4305-4320, 4405-4420, 4532-4547, 4534-4549, 4537-4552, 4539-4554, 4555-4570, 4571-4586, 4573-4588, 4578-

4593, 4597-4612, 4632-4647, 4655-4670, 4656-4671, 4662-4677, 4699-4714, 4747-4762, 4750-4765, 4752-4767, 4754-4769, 4755-4770, 4769-4784, 4798-4813, 4804-4819, 4807-4822, 4833-4848, 4837-4852, 4839-4854, 4865-4880, 4868-4883, 4872-4887, 4874-4889, 4876-4891, 4887-4902, 4889-4904, 4916-4931, 4918-4933, 4938-4953, 4942-4957, 4944-4959, 4951-4966, 5050-5065, 5052-5067, 5054-5069, 5056-5071, 5060-5075, 5061-5076, 5062-5077, 5133-5148, 5141-5156, 5155-5170, 5265-5280, 5293-5308, 5308-5323, 5392-5407, 5448-5463, 5469-5484, 5481-5496, 5483-5498, 5486-5501, 5488-5503, 5494-5509, 5521-5536, 5666-5681, 6222-6237, 6701-6716, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58739, 58720-58735, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58724-58739, 58725-58740, 58725-58740, 58725-58740, 58750-58769, 58750-58765, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-60917, 67454-67469, 102156-102171, 114874-114889, 115272-115287, 115365-115380, 134971-134986, 139682-139697, 139762-139777, 139782-139797, 144856-144871, 144938-144953, 148406-148421, 148443-148458, 148520-148535, 181695-181710, 182958-182973, or 183049-183064.

In certain embodiments, an antisense compound or antisense oligonucleotide provided herein targets AR within exon 1, for example within nucleotide regions 2863-5593 (exon 1) or 27672-27853 (exon 1B) of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to exon 1 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 2957-2972, 3079-3094, 3099-3114, 3109-3124, 3113-3128, 3120-3135, 3133-3148, 3224-3239, 3226-3241, 3351-3366, 3353-3368, 3361-3376, 3388-3403, 3513-3528, 3517-3532, 3519-3534, 3641-3656, 3735-3750, 3764-3779, 3768-3783, 3798-3813, 3799-3814, 3851-3866, 3870-3885, 3874-3889, 3876-3891, 3878-3893, 3884-3899, 3886-3901, 3888-3903, 3901-3916, 3956-3971, 3962-3977, 3964-3979, 3967-3982, 4019-4034, 4038-4053, 4047-4062, 4049-4064, 4056-4071, 4059-4074, 4062-4077, 4066-4081, 4070-4085, 4101-4116, 4103-4118, 4105-4120, 4109-4124, 4305-4320, 4405-4420, 4532-4547, 4534-4549, 4537-4552, 4539-4554, 4555-4570, 4571-4586, 4573-4588, 4578-4593, 4597-4612, 4632-4647, 4655-4670, 4656-4671, 4662-4677, 4699-4714, 4747-4762, 4750-4765, 4752-4767, 4754-4769, 4755-4770, 4769-4784, 4798-4813, 4804-4819, 4807-4822, 4833-4848, 4837-4852, 4839-4854, 4865-4880, 4868-4883, 4872-4887, 4874-4889, 4876-4891, 4887-4902, 4889-4904, 4916-4931, 4918-4933, 4938-4953, 4942-4957, 4944-4959, 4951-4966, 5050-5065, 5052-5067, 5054-5069, 5056-5071, 5060-5075, 5061-5076, 5062-5077, 5133-5148, 5141-5156, 5155-5170, 5265-5280, 5293-5308, 5308-5323, 5392-5407, 5448-5463, 5469-5484, 5481-5496, 5483-5498, 5486-5501, 5488-5503, 5494-5509, or 5521-5536.

In certain embodiments, an antisense compound or antisense oligonucleotide provided herein targets AR within exon 2, for example within nucleotide regions 102087-102238 (exon 2) or 139551-139834 (exon 2c) of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to exon 2 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 102155-102170, 102156-102171, 139682-139697, 139762-139777, or 139782-139797.

In certain embodiments, an antisense compound or antisense oligonucleotide provided herein targets AR within exon 3, for example within nucleotide regions 144841-144957 (exon 3), 148380-148594 (exon 3b), or 153504-154908 (exon 3d) of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to exon 3 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 144856-144871, 144938-144953, 148406-148421, 148443-148458, or 148520-148535.

In certain embodiments, an antisense compound or antisense oligonucleotide provided herein targets AR within exon 7, for example within nucleotide region 181658-181815 of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to exon 7 of AR is complementary within nucleotide region 181695-181710 of SEQ ID NO: 1.

In certain embodiments, an antisense compound or antisense oligonucleotide provided herein targets AR within exon 8, for example within nucleotide region 182517-189455 of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to exon 8 of AR is complementary within nucleotide regions 182958-182973 or 183049-183064 of SEQ ID NO: 1.

In certain embodiments, an antisense compound or antisense oligonucleotide provided herein targets AR within intron 1, for example within nucleotide regions 5594-27671 or 27854-102086 of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to intron 1 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 5666-5681, 6222-6237, 6701-6716, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58739, 58720-58735, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58724-58739, 58725-58740, 58725-58740, 58725-58740, 58750-58769, 58750-58765, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-60917, or 67454-67469.

In certain embodiments, an antisense compound or antisense oligonucleotide provided herein targets AR within intron 2, for example within nucleotide regions 102239-139550 or 139835-144840 of

SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to intron 2 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 114874-114889, 115272-115287, 115365-115380, or 134971-134986.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or antisense oligonucleotides, display at least 50% inhibition: 3099-3114, 3120-3135, 3351-3366, 3353-3368, 3361-3376, 3513-3528, 3519-3534, 3768-3783, 3799-3814, 3851-3866, 3888-3903, 4059-4074, 4534-4549, 4555-4570, 4571-4586, 4578-4593, 4655-4670, 4699-4714, 4750-4765, 4755-4770, 4865-4880, 5054-5069, 5060-5075, 5061-5076, 5062-5077, 5155-5170, 5265-5280, 5392-5407, 5448-5463, 5483-5498, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58735, 58720-58739, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58725-58740, 58750-58765, 58750-58769, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-60917, 67454-67469, 102156-102171, 114874-114889, 114874-114889, 115272-115287, 115365-115380, 134971-134986, 144856-144871, 181695-181710, 182958-182973, and 183049-183064.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or antisense oligonucleotides, display at least 60% inhibition: 3799-3814, 3851-3866, 3888-3903, 4059-4074, 4534-4549, 4555-4570, 4571-4586, 4578-4593, 4655-4670, 4699-4714, 4755-4770, 4865-4880, 5060-5075, 5061-5076, 5062-5077, 5155-5170, 5265-5280, 5392-5407, 5448-5463, 5483-5498, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 42017-42032, 56050-56065, 58719-58734, 58720-58735, 58720-58739, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58725-58740, 58750-58765, 58750-58769, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 67454-67469, 102156-102171, 115272-115287, 115365-115380, 144856-144871, 181695-181710, 182958-182973, and 183049-183064.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or antisense oligonucleotides, display at least 70% inhibition: 3799-3814, 3851-3866, 3888-3903, 4059-4074, 4534-4549, 4655-4670, 4699-4714, 4755-4770, 4865-4880, 5060-5075, 5062-5077, 5155-5170, 5265-5280, 5392-5407, 5448-5463, 5483-5498, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 33315-33330, 42017-42032,

58719-58734, 58720-58739, 58720-58735, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58725-58740, 58750-58769, 58750-58765, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 102156-102171, 115365-115380, 144856-144871, 181695-181710, 182958-182973, and 183049-183064.

5 In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or antisense oligonucleotides, display at least 80% inhibition: 3799-3814, 3851-3866, 3888-3903, 4059-4074, 4534-4549, 4655-4670, 4699-4714, 4755-4770, 4865-4880, 5060-5075, 5062-5077, 5155-5170, 5265-5280, 5392-5407, 5448-5463, 5483-5498, 8471-8486, 8638-8653, 9464-9479, 10865-10880, 11197-11212, 13189-13204, 16793-16808, 58719-58734, 58720-58735, 58721-10 58736, 58722-58737, 58723-58738, 58724-58739, 58725-58740, 58750-58765, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 102156-102171, 144856-144871, 181695-181710, 182958-182973, and 183049-183064.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or antisense oligonucleotides, display at least 90% inhibition: 4534-4549, 5060-15 5075, 5062-5077, 5155-5170, 5265-5280, 5448-5463, 58720-58735, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58725-58740, 58750-58765, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 182958-182973, and 183049-183064.

In certain embodiments, the following antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid and effect at least a 50% inhibition of an androgen receptor mRNA, ISIS IDs: 549332, 549334, 549338, 549347, 549358, 549360, 549361, 549362, 549366, 549371, 20 549372, 549374, 549377, 549379, 549380, 549381, 549387, 549390, 549414, 549432, 549434, 549457, 549458, 549459, 560071, 560098, 560099, 560100, 560131, 560132, 560133, 560137, 569213, 569215, 569216, 569220, 569222, 569223, 569227, 569228, 569229, 569236, 569238, 583559, 583567, 583608, 583609, 583613, 583635, 583638, 583662, 583795, 583796, 583799, 583834, 583919, 584145, 584148, 25 584149, 584152, 584157, 584158, 584162, 584163, 584165, 584166, 584167, 584168, 584179, 584180, 584183, 584184, 584192, 584233, 584242, 584245, 584263, 584269, 584274, 584312, 584329, 584361, 584465, 584465, 584468, 584469, 584469, 584495, 584495, 585233, 585259, 585262, 585263, 585264, 585265, 585268, 585269, 585271, 585274, 586124, 586224, 586224, 586225, 586225, 586227, and 586227.

30 In certain embodiments, the following antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid and effect at least a 50% inhibition of an androgen receptor mRNA, SEQ ID NOs: 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 46, 49, 53, 54, 55, 57, 59, 63, 92, 93, 95, 101, 125, 148, 149, 150, 151, 152,

153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, and 177.

In certain embodiments, the following antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid and effect at least a 60% inhibition of an androgen receptor mRNA, ISIS IDs: 549332, 549334, 549338, 549347, 549358, 549360, 549361, 549362, 549366, 549371, 549372, 549374, 549377, 549379, 549380, 549381, 549387, 549390, 549414, 549432, 549434, 549457, 549458, 549459, 560071, 560098, 560099, 560100, 560131, 560137, 569213, 569216, 569222, 569228, 569236, 583795, 583796, 583799, 584145, 584148, 584149, 584152, 584157, 584158, 584162, 584163, 584165, 584166, 584167, 584168, 584179, 584180, 584183, 584184, 584192, 584233, 584242, 584245, 584274, 584312, 584361, 584468, 584469, 585233, 585259, 585262, 585263, 585264, 585265, 585268, 585269, 585274, 586124, 586224, 586225, and 586227.

In certain embodiments, the following antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid and effect at least a 60% inhibition of an androgen receptor mRNA, SEQ ID NOs: 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 92, 93, 95, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 170, 171, 173, 175, and 176.

In certain embodiments, the following antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid and effect at least a 70% inhibition of an androgen receptor mRNA, ISIS IDs: 549332, 549334, 549338, 549347, 549358, 549360, 549361, 549362, 549366, 549371, 549372, 549374, 549377, 549379, 549380, 549381, 549387, 549390, 549414, 549432, 549434, 549457, 549458, 549459, 560071, 560098, 560099, 560100, 560131, 560137, 569222, 584145, 584148, 584149, 584152, 584162, 584163, 584165, 584166, 584167, 584168, 584179, 584180, 584183, 584184, 584192, 584245, 584274, 584469, 585259, 585262, 585268, 585269, 586124, 586224, 586225, and 586227.

In certain embodiments, the following antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid and effect at least a 70% inhibition of an androgen receptor mRNA, SEQ ID NOs: 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 43, 148, 149, 150, 151, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 167, 170, and 176.

In certain embodiments, the following antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid and effect at least a 80% inhibition of an androgen receptor mRNA, ISIS IDs: 549332, 549334, 549338, 549347, 549358, 549360, 549361, 549362, 549366, 549371, 549372, 549374, 549377, 549379, 549380, 549381, 549387, 549390, 549414, 549432, 549434, 549457,

549458, 549459, 560098, 560099, 560100, 560137, 584148, 584149, 584152, 584162, 584163, 584166, 584180, 586124, 586224, 586225, and 586227.

In certain embodiments, the following antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid and effect at least a 80% inhibition of an androgen receptor mRNA, SEQ ID NOs: 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41, 43, 149, 150, 151, 154, 155, 157, and 161.

In certain embodiments, the following antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid and effect at least a 90% inhibition of an androgen receptor mRNA, ISIS IDs: 549358, 549371, 549372, 549374, 549377, 549380, 549432, 549434, 549457, 549458, 549459, 560098, 560099, 560100, 560137, and 586224.

In certain embodiments, the following antisense compounds or antisense oligonucleotides target a region of an androgen receptor nucleic acid and effect at least a 90% inhibition of an androgen receptor mRNA, SEQ ID NOs: 16, 21, 22, 23, 24, 26, 33, 34, 35, 36, 37, 39, 40, and 41.

Percent inhibition of androgen receptor mRNA can be determined using standard methods known to those of skill in the art, such as described in Example 1.

It is understood that the sequence set forth in each SEQ ID NO in the examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Antisense compounds described by ISIS number (ISIS #) indicate a combination of nucleobase sequence, chemical modification, and motif.

In certain embodiments, the compounds or compositions as described herein are efficacious by virtue of having at least one of an *in vitro* IC₅₀ of less than 250 nM, less than 200 nM, less than 150 nM, less than 100 nM, less than 90 nM, less than 80 nM, less than 70 nM, less than 65 nM, less than 60 nM, less than 55 nM, less than 50 nM, less than 45 nM, less than 40 nM, less than 35 nM, less than 30 nM, less than 25 nM, or less than 20 nM when delivered to HuVEC cells. In certain embodiments inhibition is measured with primer probe set RTS3559, as described herein.

In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having at least one of an increase an ALT or AST value of no more than 4 fold, 3 fold, or 2 fold over saline treated animals or an increase in liver, spleen, or kidney weight of no more than 30%, 20%, 15%, 12%, 10%, 5%, or 2%. In certain embodiments, the compounds or compositions as

described herein are highly tolerable as demonstrated by having no increase of ALT or AST over saline treated animals. In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having no increase in liver, spleen, or kidney weight over saline treated animals.

5 In certain embodiments, an antisense compound provided herein targets an AR splicing variant that includes exon 1 encoding the N-terminal domain and exons 2 and 3 encoding the DNA binding domain, but does not include at least a portion of exon 4 encoding the short hinge region or at least a portion of exons 4-8 encoding the ligand binding domain. An example of such an AR splicing variant includes, but is not limited to, AR-V7, which contains exons 1-3 but lacks exons 4-8. Additional
10 examples of such AR splicing variants include, for example, AR3, AR4, AR4b, AR5, and AR6 (SEQ ID NOs: 4-8, respectively). In certain embodiments, an antisense compound targeted to AR upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain is capable of inhibiting androgen receptor levels to a greater extent than an antisense compound targeted to the ligand binding domain, such as EZN-4176, which is targeted to exon 4 and corresponds to SEQ ID NO: 58 described in US 7,737,125.

15 In certain embodiments, an antisense compound targets an AR splicing variant that has a functional DNA binding domain, but not a functional ligand binding domain. It will be understood that in certain embodiments an antisense compound can target an AR splicing variant that includes the entire or at least a functional portion of exon 1 encoding the N-terminal domain and the entire or at least a functional portion of exons 2 and 3 encoding the DNA binding domain, but does not include at least a
20 functional portion of exon 4 encoding the short hinge region or at least a functional portion of exons 4-8 encoding the ligand binding domain. It is contemplated that certain AR splicing variants targeted by the antisense compounds provided herein substantially consisting of exons 1-3 may also include a non-functional portion of nucleic acid sequence from a genomic region or exons 4-8. It is contemplated that the splicing process may give rise to such AR splicing variants that retain DNA binding function but not
25 ligand binding function. In certain embodiments, an antisense compound targeted to an AR splicing variant that has a functional DNA binding domain, but not a functional ligand binding domain, is capable of inhibiting growth or proliferation of prostate cancer cells that are castrate-resistant. In certain embodiments, an antisense compound targeted to an AR splicing variant that has a functional DNA binding domain, but not a functional ligand binding domain, is capable of inhibiting growth or
30 proliferation of a prostate cancer cell resistant to a diarylhydantoin AR inhibitor of Formula XI to a greater extent than an antisense compound targeted to the ligand binding domain, such as EZN-4176, which is targeted to exon 4 and corresponds to SEQ ID NO: 58 described in US 7,737,125. In certain embodiments, an antisense compound provided herein targets AR within exon 1, which is upstream of the

3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound provided herein targets AR within exon 2, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound provided herein targets AR within intron 1, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain.

In certain embodiments, an antisense compound provided herein is capable of reducing expression of both full-length AR and an AR splicing variant that includes exon 1 encoding the N-terminal domain and exons 2 and 3 encoding the DNA binding domain, but does not include at least a portion of exon 4 encoding the short hinge region or at least a portion of any one of exons 4-8 encoding the ligand binding domain. In certain embodiments, such an antisense compound targets human androgen receptor upstream of the ligand binding domain. In certain embodiments, such antisense compounds target human androgen receptor upstream of the 3' end of exon 3. In certain embodiments, an antisense compound provided herein targets AR within exon 1, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound provided herein targets AR within exon 2, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound provided herein targets AR within intron 1, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain.

In certain embodiments, an antisense compound provided herein targets an AR splicing variant that includes exon 1 encoding the N-terminal domain and exons 2 and 3 encoding the DNA binding domain, but does not include at least a portion of exon 4 encoding the short hinge region or at least a portion of exons 4-8 encoding the ligand binding domain. An example of such an AR splicing variant includes, but is not limited to, AR-V7, which contains exons 1-3 but lacks exons 4-8.

Certain embodiments are drawn to an antisense compound targeted to human androgen receptor (AR) upstream of the ligand binding domain that is capable of inhibiting growth or proliferation of the resistant prostate cancer cell to a greater extent than an antisense compound targeted to the ligand binding domain, such as EZN-4176, which is targeted to exon 4 and corresponds to SEQ ID NO: 58 described in US 7,737,125. In certain embodiments, an antisense compound targeted to human androgen receptor (AR) upstream of the ligand binding domain is targeted to a region of AR upstream of the 3' end of exon 3. In certain embodiments, an antisense compound provided herein targets AR within exon 1, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound provided herein targets AR within exon 2, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound

provided herein targets AR within intron 1, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain.

In certain embodiments, the nucleobase sequence of a modified oligonucleotide provided herein is at least 70%, 75%, 80%, 85%, 90%, 95% or 100% complementary to any one of SEQ ID NOs: 1-8, as measured over the entirety of the modified oligonucleotide.

In certain embodiments, an antisense compound is a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence at least 90% complementary to any of SEQ ID NOs: 1-8 as measured over the entirety of said modified oligonucleotide.

In certain embodiments, an antisense compound is a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence 100% complementary to any of SEQ ID NOs: 1-8 as measured over the entirety of said modified oligonucleotide. In certain embodiments, a compound or modified oligonucleotide provided herein is single-stranded.

In certain embodiments, a modified oligonucleotide provided herein consists of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In certain embodiments, at least one internucleoside linkage of a modified oligonucleotide provided herein is a modified internucleoside linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

In certain embodiments, at least one nucleoside of the modified oligonucleotide comprises a modified sugar. In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl group ($2'\text{-O}(\text{CH}_2)_2\text{-OCH}_3$). In certain embodiments, the modified sugar comprises a 2'-O-CH₃ group.

In certain embodiments, at least one modified sugar is a bicyclic sugar. In certain embodiments, the bicyclic sugar comprises a 4'- (CH₂)_n -O-2' bridge, wherein n is 1 or 2. In certain embodiments, the bicyclic sugar comprises a 4'- CH₂-O-2' bridge. In certain embodiments, the bicyclic sugar comprises a 4'-CH(CH₃)-O-2' bridge.

In certain embodiments, at least one nucleoside of a modified oligonucleotide provided herein comprises a modified nucleobase. In certain embodiments, the modified nucleobase is a 5-methylcytosine.

In certain embodiments, a modified oligonucleotide provided herein consists of a single-stranded modified oligonucleotide.

In certain embodiments, compounds or compositions provided herein comprise a salt of the modified oligonucleotide.

Compositions and Methods for Formulating Pharmaceutical Compositions

Antisense oligonucleotides may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

An antisense compound targeted to an androgen receptor nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutically acceptable diluent is water, such as sterile water suitable for injection. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to an androgen receptor nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is water. In certain embodiments, the antisense compound is an antisense oligonucleotide provided herein.

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense compound.

In certain embodiments, the compounds or compositions further comprise a pharmaceutically acceptable carrier or diluent.

Certain Indications

Certain aspects of the invention are directed to methods of treating cancer which comprises administering an antisense compound targeted to androgen receptor as provided herein. In certain

embodiments, the cancer is AR positive. In certain embodiments, the cancer is prostate cancer, breast cancer, ovarian cancer, bladder cancer or gastric cancer. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of the nucleobase sequence of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175. In certain embodiments, the antisense compound is single-stranded. In certain embodiments, the antisense compound targeted to androgen receptor is ISIS 560131, ISIS 569213, ISIS 569216, ISIS 569221, ISIS 569236, ISIS 579671, ISIS 586124, ISIS 583918, ISIS 584149, ISIS 584163, ISIS 584269, or ISIS 584468.

Certain aspects are directed to an antisense compound targeted to androgen receptor provided herein for use in treating cancer. In certain embodiments, the cancer is AR positive. In certain embodiments, the cancer is prostate cancer, breast cancer, ovarian cancer, bladder cancer or gastric cancer. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of the nucleobase sequence of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175. In certain embodiments, the antisense compound is single-stranded. In certain embodiments, the antisense compound targeted to androgen receptor is ISIS 560131, ISIS 569213, ISIS 569216, ISIS 569221, ISIS 569236, ISIS 579671, ISIS 586124, ISIS 583918, ISIS 584149, ISIS 584163, ISIS 584269, or ISIS 584468.

Certain aspects are directed to use of an antisense compound targeted to androgen receptor provided herein for the manufacture of a medicament for treating cancer. In certain embodiments, the cancer is AR positive. In certain embodiments, the cancer is prostate cancer, breast cancer, ovarian cancer, bladder cancer or gastric cancer. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of the nucleobase sequence of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175. In certain embodiments, the antisense compound is single-stranded. In certain embodiments, the antisense compound targeted to androgen receptor is ISIS 560131, ISIS 569213, ISIS 569216, ISIS 569221, ISIS 569236, ISIS 579671, ISIS 586124, ISIS 583918, ISIS 584149, ISIS 584163, ISIS 584269, or ISIS 584468.

Certain aspects of the invention are directed to the use of an antisense compound targeted to human androgen receptor (AR) as described herein, for treating a cancer patient whose cancer has become resistant to treatment with an anti-androgenic agent (e.g. compound or drug). In certain embodiments, said cancer is prostate cancer. In certain embodiments, said patient is one that has, or whose cancer has, developed resistance to treatment with an agent selected from: MDV3100, ARN-059, ODM-201, abiraterone acetate, TOK001, TAK700 and VT464. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of the nucleobase sequence of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175. In certain embodiments, the antisense compound targets

AR within exon 1, for example within nucleotide regions 2863-5593 (exon 1) or 27672-27853 (exon 1B) of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to exon 1 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 2957-2972, 3079-3094, 3099-3114, 3109-3124, 3113-3128, 3120-3135, 3133-3148, 3224-3239, 3226-3241, 3351-3366, 3353-3368, 3361-3376, 3388-3403, 3513-3528, 3517-3532, 3519-3534, 3641-3656, 3735-3750, 3764-3779, 3768-3783, 3798-3813, 3799-3814, 3851-3866, 3870-3885, 3874-3889, 3876-3891, 3878-3893, 3884-3899, 3886-3901, 3888-3903, 3901-3916, 3956-3971, 3962-3977, 3964-3979, 3967-3982, 4019-4034, 4038-4053, 4049-4064, 4056-4071, 4059-4074, 4062-4077, 4066-4081, 4070-4085, 4101-4116, 4103-4118, 4105-4120, 4109-4124, 4305-4320, 4405-4420, 4532-4547, 4534-4549, 4537-4552, 4539-4554, 4555-4570, 4571-4586, 4573-4588, 4578-4593, 4597-4612, 4632-4647, 4655-4670, 4656-4671, 4662-4677, 4699-4714, 4747-4762, 4750-4765, 4752-4767, 4754-4769, 4755-4770, 4769-4784, 4798-4813, 4804-4819, 4807-4822, 4833-4848, 4837-4852, 4839-4854, 4865-4880, 4868-4883, 4872-4887, 4874-4889, 4876-4891, 4887-4902, 4889-4904, 4916-4931, 4918-4933, 4938-4953, 4942-4957, 4944-4959, 4951-4966, 5050-5065, 5052-5067, 5054-5069, 5056-5071, 5060-5075, 5061-5076, 5062-5077, 5133-5148, 5141-5156, 5155-5170, 5265-5280, 5293-5308, 5308-5323, 5392-5407, 5448-5463, 5469-5484, 5481-5496, 5483-5498, 5486-5501, 5488-5503, 5494-5509, or 5521-5536. In certain embodiments, an antisense compound provided herein targets AR within exon 2, for example within nucleotide regions 102087-102238 (exon 2) or 139551-139834 (exon 2c) of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to exon 2 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 102155-102170, 102156-102171, 139682-139697, 139762-139777, or 139782-139797. In certain embodiments, an antisense compound provided herein targets AR within exon 3, for example within nucleotide regions 144841-144957 (exon 3), 148380-148594 (exon 3b), or 153504-154908 (exon 3d) of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to exon 3 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 144856-144871, 144938-144953, 148406-148421, 148443-148458, or 148520-148535. In certain embodiments, an antisense compound provided herein targets AR within intron 1, for example within nucleotide regions 5594-27671 or 27854-102086 of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to intron 1 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 5666-5681, 6222-6237, 6701-6716, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58739, 58720-58735, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58724-58739, 58725-58740, 58725-58740, 58725-58740,

58750-58769, 58750-58765, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-60917, or 67454-67469. In certain embodiments, an antisense compound provided herein targets AR within intron 2, for example within nucleotide regions 102239-139550 or 139835-144840 of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to intron 2 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 114874-114889, 115272-115287, 115365-115380, or 134971-134986. In certain embodiments, the antisense compound is single-stranded. In certain embodiments, the antisense compound targeted to androgen receptor is ISIS 560131, ISIS 569213, ISIS 569216, ISIS 569221, ISIS 569236, ISIS 579671, ISIS 586124, ISIS 583918, ISIS 584149, ISIS 584163, ISIS 584269, or ISIS 584468.

By resistant to treatment with a particular agent (e.g. compound or drug) is meant that the agent is less or no longer effective in halting the growth or spread of the cancer and so the patient, or their cancer, has become less responsive or sensitive to it over time. Typically such patient would be classed as resistant to said agent and would no longer be treated with such agent. A subject having prostate cancer resistant to an agent selected from: MDV3100, ARN-059, ODM-201, abiraterone acetate, TOK001, TAK700 and VT464 can include, for example, a patient who previously received said agent but whose prostate cancer has become less sensitive or responsive to a agent. For example, prostate cancer resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone acetate, TOK001, TAK700 and VT464, can include prostate cancer that has increased in tumor volume, metastasis, or progression despite treatment with the agent. In certain embodiments, prostate cancer resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone acetate, TOK001, TAK700 and VT464, can include prostate cancer that is refractory to the agent and is not decreasing in tumor volume, metastasis, or progression despite treatment. Several embodiments relate to a method of treating prostate cancer resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone acetate, TOK001, TAK700 and VT464, in a subject comprising identifying the subject as having prostate cancer resistant to the agent and administering to the subject an antisense compound targeted to human androgen receptor (AR) upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain, as described herein. Several embodiments relate to a method of treating prostate cancer resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone acetate, TOK001, TAK700 and VT464, in a subject comprising administering to a subject identified or diagnosed as having prostate cancer resistant to said anti-androgenic agent an antisense compound targeted to human androgen receptor (AR) upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain, as described herein. In certain embodiments, prostate cancer cells

resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone acetate, TOK001, TAK700 and VT464, preferentially expresses an AR splicing variant over full-length AR.

Certain aspects of the invention are directed to a method of treating a patient suffering from prostate cancer wherein the patient has, or their cancer has, developed or become resistant to treatment with an anti-androgenic agent (compound or drug) comprising administering to said patient an antisense compound targeted to human androgen receptor (AR) as described herein. In certain embodiments, said patient is one that has developed resistance to treatment with an agent selected from: MDV3100, ARN-059, ODM-201, abiraterone acetate, TOK001, TAK700 and VT464. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of the nucleobase sequence of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175. In certain embodiments, the antisense compound is single-stranded. In certain embodiments, the antisense compound targeted to androgen receptor is ISIS 560131, ISIS 569213, ISIS 569216, ISIS 569221, ISIS 569236, ISIS 579671, ISIS 586124, ISIS 583918, ISIS 584149, ISIS 584163, ISIS 584269, or ISIS 584468.

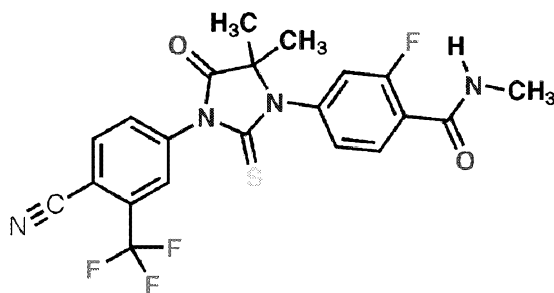
A prostate cancer that has developed or become resistant to treatment with an anti-androgenic agent is referred to as castrate-resistant prostate cancer (CRPC). Thus, in several embodiments, a prostate cancer cell resistant to an anti-androgenic agent, such as MDV3100, was previously exposed to the inhibitor and has become less responsive or sensitive to it over time. For example, MDV3100 might initially inhibit prostate cancer cell growth or proliferation in the patient, but over time such inhibitory effect may be diminished when the cells become resistant to the inhibitor.

Certain aspects of the invention are directed to the use of an antisense compound targeted to androgen receptor provided herein for the manufacture of a medicament for treating cancer in a patient whose cancer has become become resistant to treatment with an anti-androgenic agent (compound or drug). In certain embodiments the cancer is prostate cancer. In certain embodiments, said patient is one that has, or whose cancer has, developed resistance to treatment with an agent selected from: MDV3100,

ARN-059, ODM-201, abiraterone acetate, TOK001, TAK700 and VT464. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of the nucleobase sequence of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175. In certain embodiments, the antisense compound is single-stranded. In certain embodiments, the antisense compound targeted to androgen receptor is ISIS 560131, ISIS 569213, ISIS 569216, ISIS 569221, ISIS 569236, ISIS 579671, ISIS 586124, ISIS 583918, ISIS 584149, ISIS 584163, ISIS 584269, or ISIS 584468.

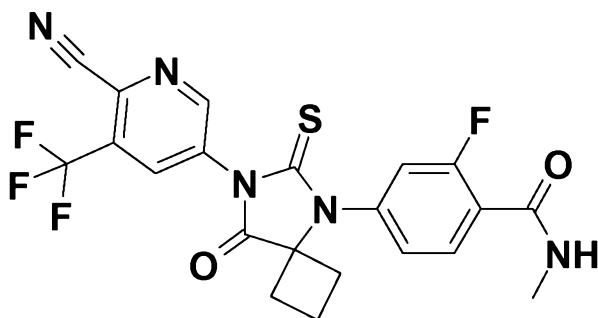
Enzalutamide:

MDV3100, also known as enzalutamide (Xtandi™) and by the IUPAC name 4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide, is an androgen receptor ligand binding inhibitor belonging to the diarylhydantoin class of androgen receptor inhibitors represented by Formula XI. MDV3100 has the following chemical formula:



MDV3100 and additional diarylhydantoin androgen receptor inhibitors suitable for use in certain embodiments provided herein are described in US Patent No. 7,709,517, US Patent Application Publication No. US20100172975 and US Patent Application Publication No. US20100210665, which are incorporated herein by reference in their entireties.

ARN-509:

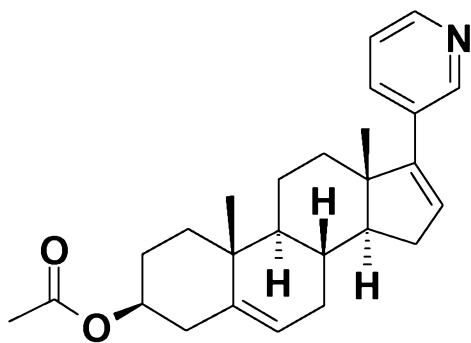


(XII)

The compound of Formula XII, also known as ARN-509 and by the IUPAC name 4-(7-(6-Cyano-5-(trifluoromethyl)pyridin-3-yl)-6,8-dioxo-5,7-diazaspiro[3.4]octan-5-yl)-2-fluoro-N-methylbenzamide, is an androgen receptor ligand binding inhibitor. ARN-509 and additional androgen receptor inhibitors suitable for use in certain embodiments provided herein are described in WO 2007126765, WO 2008119015 and US Patent Application Publication No. 2013/0116258, which are incorporated herein by reference in their entirety.

Abiraterone acetate

The compound of Formula XIII, which is also known as Abiraterone acetate and ZYTIGA® and by the IUPAC name [(3S,8R,9S,10R,13S,14S)-10,13-dimethyl-17-(3-pyridyl)-2,3,4,7,8,9,11,12,14,15-decahydro-1H-cyclopenta[a]phenanthren-3-yl] acetate, is an androgen biosynthesis inhibitor and has the following chemical formula:

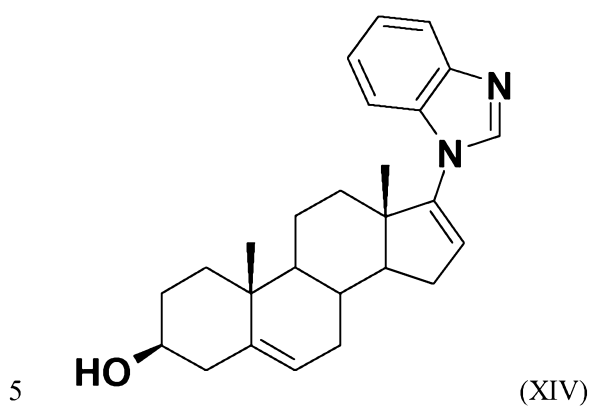


(XIII)

The structure and synthesis of Abiraterone acetate is described in Potter et al., Journal of Medicinal Chemistry (38(13), 2463-71, 1995), which is incorporated herein by reference in its entirety.

Galeterone:

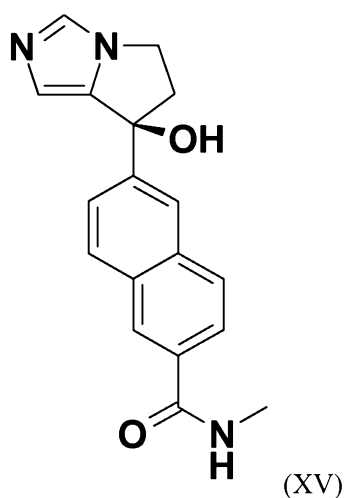
The compound of Formula XIV, which is also known as TOK-001 and Galeterone, and by the IUPAC name (3S,10R,13S)-17-(1H-benzo[d]imidazol-1-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol, has the following chemical formula:



The structure and synthesis of TOK-001 is described in Handratta et al., (Journal of Medicinal Chemistry (2005), 48(8), 2972-84, 2005), which is incorporated herein by reference in its entirety:

Orteronel:

10 The compound of Formula XV, which is also known as TAK-700 and Orteronel and by the IUPAC name 6-[7(S)-hydroxy-6,7-dihydro-5H-pyrrolo[1,2-c]imidazol-7-yl]-N-methylnaphthalene-2-carboxamide, is an androgen biosynthesis inhibitor and has the following chemical formula:



The structure and synthesis of TAK-700 is described in Kaku et al., Bioorganic and Medicinal Chemistry (19(21), 6383-99, 2011).

Yin et al., (*Int. J. Mol. Sci.*,14(7):13958-13978, 2013) discusses recent progress with various pharmaceutical therapies, including ODM-21, VT464, ARN509, TAK700 and TOK-001, for castration-resistant prostate cancer.

Certain Combinations and Combination Therapies

5 In certain embodiments, a first agent comprising the compound described herein is co-administered with one or more secondary agents. In certain embodiments, such second agents are designed to treat the same disease, disorder, or condition as the first agent described herein. In certain
10 embodiments, such second agents are designed to treat a different disease, disorder, or condition as the first agent described herein. In certain embodiments, a first agent is designed to treat an undesired side effect of a second agent. In certain embodiments, second agents are co-administered with the first agent to
15 treat an undesired effect of the first agent. In certain embodiments, such second agents are designed to treat an undesired side effect of one or more pharmaceutical compositions as described herein. In certain embodiments, second agents are co-administered with the first agent to produce a combinational effect. In certain
20 embodiments, second agents are co-administered with the first agent to produce a synergistic effect. In certain embodiments, the co-administration of the first and second agents permits use of lower dosages than would be required to achieve a therapeutic or prophylactic effect if the agents were
25 administered as independent therapy.

In certain embodiments, one or more compounds or compositions provided herein are co-administered with one or more anti-androgenic agents. In certain embodiments, one or more compounds
20 or compositions provided herein and one or more anti-androgenic agents, are administered at different times. In certain embodiments, one or more compounds or compositions provided herein and one or more anti-androgenic agents, are prepared together in a single formulation. In certain embodiments, one or more compounds or compositions provided herein and one or more anti-androgenic agents, are prepared
25 separately. In certain embodiments, an anti-androgenic agent is selected from MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464.

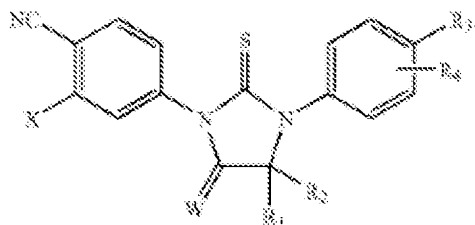
Certain aspects of the invention are directed to the use of an antisense compound targeted to human androgen receptor (AR) as described herein in combination with an anti-androgenic agent. In
30 particular embodiments such use is in a method of treating a patient suffering from cancer or in the manufacture of a medicament for treating cancer. In certain embodiments the cancer is selected from: prostate cancer, breast cancer, ovarian cancer, bladder cancer or gastric cancer. Particular classes of anti-androgenic agents are the second generation anti-hormonal agents such as: enzalutamide (MDV3100),

ARN-059, ODM-201, abiraterone acetate, Galeterone (TOK001), orteronel (TAK700) and VT464 (see Yin et al. *supra*).

Certain aspects are drawn to a combination of an antisense compound targeted to human androgen receptor (AR) as described herein and an anti-androgenic agent, such as a second generation anti-hormonal agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464.

In certain embodiments, such a combination of an antisense compound targeted to androgen receptor (AR) as described herein and an anti-androgenic agent, such as a second generation anti-hormonal agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, is useful for inhibiting cancer cell growth or proliferation and/or treating cancer. In certain embodiments the cancer is selected from: prostate cancer, breast cancer, ovarian cancer, bladder cancer or gastric cancer. In certain embodiments the cancer is prostate cancer. In certain embodiments the cancer is breast cancer. In certain embodiments, an antisense compound targeted to AR as described herein and an anti-androgenic agent, such as a second generation anti-hormonal agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, synergize in combination to inhibit growth or proliferation of a cancer cell. In several embodiments, the cancer cell is a prostate cancer cell which is or has become castration-resistant. In various embodiments, the cancer cell is a prostate cancer cell which is or has become resistant to a second generation anti-hormonal agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of the nucleobase sequence of any of SEQ ID NOs: 12-179. In certain embodiments, the antisense compound targeted to androgen receptor comprises a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175. In certain embodiments, the antisense compound targeted to androgen receptor is ISIS 560131, ISIS 569213, ISIS 569216, ISIS 569221, ISIS 569236, ISIS 579671, ISIS 586124, ISIS 583918, ISIS 584149, ISIS 584163, ISIS 584269, or ISIS 584468.

Several embodiments are drawn to a combination of an antisense compound targeted to human androgen receptor (AR) and a diarylhydantoin AR inhibitor of Formula XI, such as MDV3100. In several embodiments, a diarylhydantoin Androgen Receptor (AR) inhibitor is a compound of Formula XVI:



(XVI)

wherein X is selected from the group consisting of trifluoromethyl and iodo, wherein W is selected from the group consisting of O and NR₅, wherein R₅ is selected from the group consisting of H, methyl, and



wherein D is S or O and E is N or O and G is alkyl, aryl, substituted alkyl or substituted aryl; or D is S or O and E-G together are C1-C4 lower alkyl,

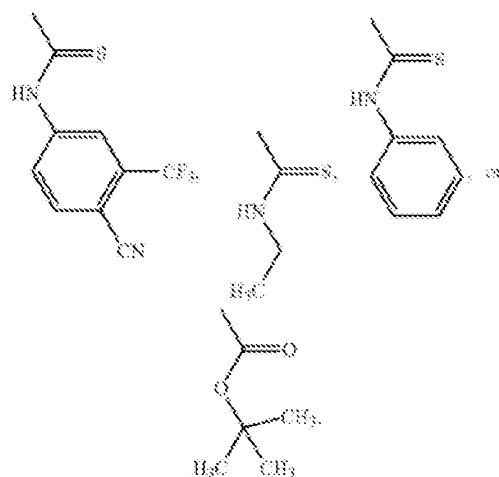
wherein R₁ and R₂ together comprise eight or fewer carbon atoms and are selected from the group consisting of alkyl, substituted alkyl including haloalkyl, and, together with the carbon to which they are linked, a cycloalkyl or substituted cycloalkyl group,

wherein R₃ is selected from the group consisting of hydrogen, halogen, methyl, C1 -C4 alkoxy, formyl, haloacetoxy, trifluoromethyl, cyano, nitro, hydroxyl, phenyl, amino, methylcarbamoyl, methoxycarbonyl, acetamido, methanesulfonamino, methanesulfonyl, 4-methanesulfonyl-1 -piperazinyl, piperazinyl, and C1-C6 alkyl or alkenyl optionally substituted with hydroxyl, methoxycarbonyl, cyano, amino, amido, nitro, carbamoyl, or substituted carbamoyl including methylcarbamoyl, dimethylcarbamoyl, and hydroxyethylcarbamoyl,

wherein R₄ is selected from the group consisting of hydrogen, halogen, alkyl, and haloalkyl, and

wherein R₃ is not methylaminomethyl or dimethylaminomethyl.

R5 may be



In certain embodiments, such a combination of an antisense compound targeted to androgen receptor (AR) and a diarylhydantoin AR inhibitor of Formula XVI, such as MDV3100, is useful for inhibiting prostate cancer cell growth or proliferation and/or treating prostate cancer. In certain embodiments, an antisense compound targeted to AR and a diarylhydantoin AR inhibitor of Formula XVI, such as MDV3100, synergize in combination to inhibit growth or proliferation of a prostate cancer cell. In several embodiments, the prostate cancer cell is castration-resistant. In various embodiments, the prostate cancer cell is resistant to a diarylhydantoin AR inhibitor of Formula XVI, such as MDV3100. In certain embodiments, the prostate cancer cell or castration-resistant prostate cancer cell preferentially expresses an AR splicing variant over full-length AR. In certain embodiments the antisense compound targeted to AR as described herein and the other anti-androgenic agent are used in combination treatment by administering the two agents simultaneously, separately or sequentially. In certain embodiments the two agents are formulated as a fixed dose combination product. In other embodiments the two agents are provided to the patient as separate units which can then either be taken simultaneously or serially (sequentially).

In certain embodiments, antisense compounds useful for inhibiting prostate cancer cell and/or castration-resistant prostate cancer cell growth or proliferation in combination with another anti-androgenic agent, such as a second generation anti-hormonal agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, target human androgen receptor upstream of the

3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound provided herein targets AR within exon 1, exon 2, exon 3, intron 1, or intron 2 as described herein.

In certain embodiments, an antisense compound provided herein targets an AR splicing variant that includes exon 1 encoding the N-terminal domain and exons 2 and 3 encoding the DNA binding domain, but does not include at least a portion of exon 4 encoding the short hinge region or at least a portion of exons 4-8 encoding the ligand binding domain. An example of such an AR splicing variant includes, but is not limited to, AR-V7, which contains exons 1-3 but lacks exons 4-8. Additional examples of such AR splicing variants include, for example, AR3, AR4, AR4b, AR5, and AR6 (SEQ ID NOs: 4-8, respectively). In certain embodiments, the prostate cancer cell, which may be castration-resistant, preferentially expresses an AR splicing variant over full-length AR. In particular embodiments the prostate cancer cell is castration-resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464. In certain embodiments, an antisense compound targeted to AR upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain is capable of inhibiting growth or proliferation of a prostate cancer cell, including a castration-resistant prostate cancer cell, in combination with an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, to a greater extent than an antisense compound targeted to the ligand binding domain, such as EZN-4176, which is targeted to exon 4 and corresponds to SEQ ID NO: 58 described in US 7,737,125, in combination with the same anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464. In certain embodiments, the combination of an antisense compound as described herein and the anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, provides a synergistic (*e.g.* greater-than-additive) effect in inhibiting the growth or proliferation of a prostate cancer cell, such as a castration-resistant prostate cancer cell, compared to the antisense compound alone or the anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464 alone. Accordingly, in certain embodiments the amounts of either or both of the antisense compound and/or anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, when used in combination can be less than the corresponding amount of either the antisense compound alone or the anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, alone necessary to achieve an equivalent level of prostate cancer cell growth or proliferation inhibition.

In certain embodiments, an antisense compound provided herein useful for inhibiting prostate cancer cell and/or castration-resistant prostate cancer cell growth or proliferation in combination with an

anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, targets an AR splicing variant that has a functional DNA binding domain, but not a functional ligand binding domain. It will be understood that in certain embodiments an antisense compound can target an AR splicing variant that includes the entire or at least a functional portion of exon 1 encoding the N-terminal domain and the entire or at least a functional portion of exons 2 and 3 encoding the DNA binding domain, but does not include at least a functional portion of exon 4 encoding the short hinge region or at least a functional portion of exons 4-8 encoding the ligand binding domain. It is contemplated that certain AR splicing variants targeted by the antisense compounds provided herein substantially consisting of exons 1-3 may also include a non-functional portion of nucleic acid sequence from a genomic region or exons 4-8. It is contemplated that the splicing process may give rise to such AR splicing variants that retain DNA binding function but not ligand binding function. In certain embodiments, the prostate cancer cell, which may be castrate-resistant, preferentially expresses an AR splicing variant over full-length AR. In certain embodiments the prostate cancer cell is castrate-resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464. In certain embodiments, an antisense compound provided herein targets AR within exon 1, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound provided herein targets AR within exon 1, exon 2, exon 3, intron 1, or intron 2 as described herein.

In certain embodiments, an antisense compound targeted to an AR splicing variant that has a functional DNA binding domain, but not a functional ligand binding domain, is capable of inhibiting growth or proliferation of a prostate cancer cell, including a castration-resistant prostate cancer cell, in combination with an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, to a greater extent than an antisense compound targeted to the ligand binding domain, such as EZN-4176, which is targeted to exon 4 and corresponds to SEQ ID NO: 58 described in US 7,737,125, in combination with the same anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464. In certain embodiments, the combination of an antisense compound and anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, provides a synergistic (*e.g.* greater-than-additive) effect in inhibiting the growth or proliferation of a prostate cancer cell, such as a castration-resistant prostate cancer cell, compared to the antisense compound alone or the anti-androgenic agent alone. Accordingly, in certain embodiments the amounts of either or both of the antisense compound and/or anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, when used in combination can be less than the corresponding amount of either the

antisense compound alone or anti-androgenic agent, alone necessary to achieve an equivalent level of prostate cancer cell growth or proliferation inhibition.

In certain embodiments, an antisense compound provided herein useful for inhibiting prostate cancer cell and/or castration-resistant prostate cancer cell growth or proliferation in combination with a
5 anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464 is capable of reducing expression of both full-length AR and an AR splicing variant that includes exon 1 encoding the N-terminal domain and exons 2 and 3 encoding the DNA binding domain, but does not include at least a portion of exon 4 encoding the short hinge region or at least a portion of
10 any one of exons 4-8 encoding the ligand binding domain. In certain embodiments, such an antisense compound targets human androgen receptor upstream of the ligand binding domain. In certain embodiments, such antisense compounds target human androgen receptor upstream of the 3' end of exon 3. In certain embodiments, an antisense compound provided herein targets AR within exon 1, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain.

In certain embodiments, there is provided a combination of an antisense compound targeted to
15 human androgen receptor (AR) as described herein and an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, wherein the antisense compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 12-179. In certain embodiments, there is provided a combination of
20 an antisense compound targeted to human androgen receptor (AR) and an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, wherein the antisense compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising any of SEQ ID NOs: 12-179. In certain embodiments, there is provided a combination of an antisense compound targeted to human androgen receptor (AR) and an anti-
25 androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, wherein the antisense compound comprises a modified oligonucleotide consisting of the nucleobase sequence of any of SEQ ID NOs: 12-179. In certain embodiments, there is provided a combination of an antisense compound targeted to human androgen receptor (AR) and a diarylhydantoin AR inhibitor of Formula XI, such as MDV3100, wherein the antisense compound comprises a modified
30 oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175. In certain embodiments, there is provided a combination of an antisense compound targeted to human androgen receptor (AR) and a diarylhydantoin AR inhibitor of Formula XI, such as MDV3100, wherein the antisense compound targeted to androgen receptor is ISIS

560131, ISIS 569213, ISIS 569216, ISIS 569221, ISIS 569236, ISIS 579671, ISIS 586124, ISIS 583918, ISIS 584149, ISIS 584163, ISIS 584269, or ISIS 584468.

Several embodiments are drawn to a method of inhibiting prostate cancer cell growth or proliferation comprising contacting the prostate cancer cell with an antisense compound targeted to human androgen receptor (AR) and an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464. In certain embodiments, the antisense compound and an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, synergize in combination to inhibit the growth or proliferation of the prostate cancer cell. In several embodiments, the prostate cancer cell is castration-resistant. In various embodiments, the prostate cancer cell is castration-resistant by being resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464. In certain embodiments, the prostate cancer cell or castration-resistant prostate cancer cell preferentially expresses an AR splicing variant over full-length AR.

In certain aspects of any of the foregoing embodiments, antisense compounds useful for inhibiting prostate cancer cell growth or proliferation in combination with a diarylhydantoin AR inhibitor of Formula XVI, such as MDV3100, can target (i) human androgen receptor upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain or (ii) an AR splicing variant that has a functional DNA binding domain, but not a functional ligand binding domain; and/or is capable of (i) reducing expression of both full-length AR and an AR splicing variant that includes exon 1 encoding the N-terminal domain and exons 2 and 3 encoding the DNA binding domain, but does not include at least a portion of exon 4 encoding the short hinge region or at least a portion of any one of exons 4-8 encoding the ligand binding domain; with the proviso that the antisense compounds do not have a nucleobase sequence consisting of any of SEQ ID NOs: 194-215 identified in Table A below.

Table A

SEQ ID NO:	Sequence
194	GAGAACCATCCTCACC
195	GGACCAGGTAGCCTGT
196	CCCCTGGACTCAGATG
197	GCACAAGGAGTGGGAC
198	GCTGTGAAGAGAGTGT
199	TTTGACACAAGTGGGA

200	GTGACACCCAGAAGCT
201	CATCCCTGCTTCATAA
202	TGGGGAGAACCATCCTCACCTGC
203	TCCAGGACCAGGTAGCCTGTGGGG
204	TGTTCCCCTGGACTCAGATGCTCC
205	TGGGGCACAAGGAGTGGGACGCAC
206	TTCGGCTGTGAAGAGAGTGTGCCA
207	CGCTTTTGACACAAGTGGGACTGG
208	CATAGTGACACCCAGAAGCTTCAT
209	GAGTCATCCCTGCTTCATAACATT
210	CTGTGAAGAGAGTG
211	TGTGAAGAGAGT
212	TTGACACAAGTGGG
213	TGACACAAGTGG
214	TGACACCCAGAAGC
215	GACACCCAGAAG

In certain aspects of any of the foregoing embodiments, antisense compounds useful for inhibiting growth or proliferation of a prostate cancer cell resistant to anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, can target (i) human androgen receptor upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain or (ii) an AR splicing variant that has a functional DNA binding domain, but not a functional ligand binding domain; and/or is capable of (i) reducing expression of both full-length AR and an AR splicing variant that includes exon 1 encoding the N-terminal domain and exons 2 and 3 encoding the DNA binding domain, but does not include at least a portion of exon 4 encoding the short hinge region or at least a portion of any one of exons 4-8 encoding the ligand binding domain; or (ii) inhibiting growth or proliferation of the resistant prostate cancer cell to a greater extent than an antisense compound targeted to the ligand binding domain, such as EZN-4176; with the proviso that the antisense compounds do not have a nucleobase sequence consisting of any of SEQ ID NOs: 194-215 described in US 7,737,125 as SEQ ID NOs: 2-9, 49-50, 52-53, 55-56, and 86-93 (herein incorporated by reference), and identified in Table A.

Certain aspects are directed to methods of treating breast cancer and methods of inhibiting breast cancer cell growth or proliferation with an antisense oligonucleotide targeted to human androgen receptor (AR) as described herein. In certain embodiments, the breast cancer has one or more of the following characteristics: Androgen Receptor positive, dependent on androgen for growth, Estrogen Receptor (ER) negative, independent of estrogen for growth, Progesterone Receptor (PR) negative, independent of progesterone for growth, or Her2/neu negative. In certain embodiments, the breast cancer or breast cancer cell is apocrine.

Certain embodiments are drawn to a method of treating breast cancer in a subject comprising administering to the subject an antisense compound targeted to human androgen receptor (AR). Certain embodiments are drawn to a method of treating breast cancer in a subject comprising identifying a subject having breast cancer and administering to the subject an antisense compound targeted to human androgen receptor (AR), thereby treating the subject's breast cancer. Certain embodiments are directed to a method of inhibiting growth or proliferation of a breast cancer cell comprising contacting the breast cancer cell with an antisense compound targeted to human androgen receptor (AR). Certain embodiments relate to a method of inhibiting AR expression in a subject having or at risk of having breast cancer comprising identifying a subject breast cancer, and administering to the subject an antisense compound targeted to human AR, wherein the antisense compound inhibits AR expression in the subject.

In certain embodiments, the breast cancer or breast cancer cell has one or more of the following characteristics: Androgen Receptor positive, dependent on androgen for growth, Estrogen Receptor (ER) negative, independent of estrogen for growth, Progesterone Receptor (PR) negative, independent of progesterone for growth, or Her2/neu negative. In certain embodiments, the breast cancer or breast cancer cell is ER, PR, and HER2 triple negative and AR positive (ER-, PR-, HER2-, AR+). In certain embodiments, the breast cancer or breast cancer cell is ER negative and AR positive (ER-, AR+). In certain embodiments, the breast cancer or breast cancer cell is ER positive and AR positive (ER+, AR+).

In certain embodiments, the breast cancer or breast cancer cell is apocrine. Apocrine breast cancers are often "triple negative", meaning that the cells do not express ER, PR, or HER2 receptors, and usually, but not necessarily, AR positive. In certain embodiments, an apocrine breast cancer or breast cancer cell is ER, PR, and HER2 triple negative and AR positive (ER-, PR-, HER2-, AR+). In certain embodiments, an apocrine breast cancer or breast cancer cell is ER negative and AR positive (ER-, AR+). In certain embodiments, an apocrine breast cancer or breast cancer cell originates from the sweat gland of the breast. In certain embodiments, an apocrine breast cancer or breast cancer cell is a ductal cancer or cancer cell of the breast. In certain embodiments, an apocrine breast cancer can have any one or more of the following features: a large amount of eosinophilic granular cytoplasm, well-defined margins, large

vesicular nuclei, a nuclear to cytoplasmic ratio of about 1:2, and/or accumulations of secreted granules in the apical cytoplasm known as apical snouts.

In certain embodiments, the breast cancer or breast cancer cell is an ER negative and AR positive (ER-, AR+) molecular apocrine breast cancer or breast cancer cell. In certain aspects, an ER negative and AR positive (ER-, AR+) molecular apocrine breast cancer or breast cancer cell can further be PR positive, PR negative, HER2 negative, or HER2 positive.

Breast cancer can be identified as positive or negative with respect to hormone receptors, such as ER, PR, or HER2 by standard histological techniques. For example, histological breast cancer samples can be classified as “triple negative” (ER-, PR-, HER2-) when less than 1% of cells demonstrate nuclear staining for estrogen and progesterone receptors, and immunohistochemical staining for HER2 shows a 0, 1-fold, or a 2-fold positive score and a FISH ratio (HER2 gene signals to chromosome 17 signals) of less than 1.8 according to the relevant ASCO and CAP guidelines. (Meyer, P. et al., PLoS ONE 7(5): e38361 (2012)).

In certain embodiments, an antisense compound useful for treating breast cancer or inhibiting growth or proliferation of a breast cancer cell target provided herein targets AR within exon 1, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound provided herein targets AR within exon 1, for example within nucleotide regions 2863-5593 (exon 1) or 27672-27853 (exon 1B) of SEQ ID NO: 1. In certain embodiments, an antisense compound provided herein targeted to exon 1 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 3353-3368, 3361-3376, 3519-3534, 3735-3750, 3768-3783, 3798-3813, 3799-3814, 3851-3866, 3870-3885, 3874-3889, 3888-3903, 4047-4062, 4062-4077, 4109-4124, 4534-4549, 4537-4552, 4555-4570, 4571-4586, 4573-4588, 4578-4593, 4655-4670, 4750-4765, 4752-4767, 4833-4848, 4837-4852, 4839-4854, 4865-4880, 4872-4887, 4874-4889, 4876-4891, 4916-4931, 4918-4933, 5052-5067, 5054-5069, 5060-5075, 5061-5076, 5061-5076, 5062-5077, 5155-5170, 5265-5280, 5293-5308, 5392-5407, 5448-5463, 5483-5498, 5486-5501, or 5494-5509.

In certain embodiments, an antisense compound provided herein targets AR within exon 2, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound useful for treating breast cancer or inhibiting growth or proliferation of a breast cancer cell target provided herein targets AR within exon 2, for example within nucleotide regions 102087-102238 (exon 2) or 139551-139834 (exon 2c) of SEQ ID NO: 1. In certain

embodiments, an antisense compound provided herein targeted to exon 2 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 102155-102170 or 102156-107171.

In certain aspects, an antisense compound useful for treating breast cancer or inhibiting growth or proliferation of a breast cancer cell provided herein targets AR within intron 1, which is upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, an antisense compound provided herein targets AR within intron 1, for example within nucleotide regions 5594-27671 or 27854-102086 of SEQ ID NO: 1. In certain aspects, an antisense compound provided herein targeted to intron 1 of AR is complementary within any of the following nucleotide regions of SEQ ID NO: 1: 5666-5681, 6701-6716, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58735, 58721-58736, 58722-58737, 58723-58738, 58725-58740, 58750-58765, 58751-58766, 58752-58767, 58753-58768, 58754-58769, or 58755-58770.

In certain aspects of any of the foregoing embodiments, antisense compounds useful for treating breast cancer or inhibiting growth or proliferation of a breast cancer cell target human androgen receptor upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain. In certain embodiments, antisense compounds provided herein, including but not limited to those that target human androgen receptor upstream of the 3' end of exon 3 and/or upstream of the ligand binding domain, can treat breast cancer or inhibiting growth or proliferation of a breast cancer cell to a greater extent than an antisense compound targeted to the ligand binding domain, such as EZN-4176; with the proviso that the antisense compounds do not have a nucleobase sequence consisting of any of SEQ ID NOs: 194-215 described in US 7,737,125 as SEQ ID NOs: 2-9, 49-50, 52-53, 55-56, and 86-93 (herein incorporated by reference), and identified in Table A.

Antisense compounds

Oligomeric compounds include, but are not limited to, oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics, antisense compounds, antisense oligonucleotides, and siRNAs. An oligomeric compound may be "antisense" to a target nucleic acid, meaning that it is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

In certain embodiments, an antisense compound has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted. In certain such embodiments, an antisense oligonucleotide has a nucleobase

sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted.

In certain embodiments, an antisense compound is 10-30 subunits in length. In certain embodiments, an antisense compound is 12 to 30 subunits in length. In certain embodiments, an antisense compound is 12 to 22 subunits in length. In certain embodiments, an antisense compound is 14 to 30 subunits in length. In certain embodiments, an antisense compound is 14 to 20 subunits in length. In certain embodiments, an antisense compound is 15 to 30 subunits in length. In certain embodiments, an antisense compound is 15 to 20 subunits in length. In certain embodiments, an antisense compound is 16 to 30 subunits in length. In certain embodiments, an antisense compound is 16 to 20 subunits in length. In certain embodiments, an antisense compound is 17 to 30 subunits in length. In certain embodiments, an antisense compound is 17 to 20 subunits in length. In certain embodiments, an antisense compound is 18 to 30 subunits in length. In certain embodiments, an antisense compound is 18 to 21 subunits in length. In certain embodiments, an antisense compound is 18 to 20 subunits in length. In certain embodiments, an antisense compound is 20 to 30 subunits in length. In other words, such antisense compounds are from 12 to 30 linked subunits, 14 to 30 linked subunits, 14 to 20 subunits, 15 to 30 subunits, 15 to 20 subunits, 16 to 30 subunits, 16 to 20 subunits, 17 to 30 subunits, 17 to 20 subunits, 18 to 30 subunits, 18 to 20 subunits, 18 to 21 subunits, 20 to 30 subunits, or 12 to 22 linked subunits, respectively. In certain embodiments, an antisense compound is 14 subunits in length. In certain embodiments, an antisense compound is 16 subunits in length. In certain embodiments, an antisense compound is 17 subunits in length. In certain embodiments, an antisense compound is 18 subunits in length. In certain embodiments, an antisense compound is 20 subunits in length. In other embodiments, the antisense compound is 8 to 80, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked subunits. In certain such embodiments, the antisense compounds are 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked subunits in length, or a range defined by any two of the above values. In some embodiments the antisense compound is an antisense oligonucleotide, and the linked subunits are nucleotides.

In certain embodiments antisense oligonucleotides may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to an Androgen Receptor nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the

3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

When a single additional subunit is present in a lengthened antisense compound, the additional subunit may be located at the 5' or 3' end of the antisense compound. When two or more additional subunits are present, the added subunits may be adjacent to each other, for example, in an antisense compound having two subunits added to the 5' end (5' addition), or alternatively to the 3' end (3' addition), of the antisense compound. Alternatively, the added subunits may be dispersed throughout the antisense compound, for example, in an antisense compound having one subunit added to the 5' end and one subunit added to the 3' end.

It is possible to increase or decrease the length of an antisense compound, such as an antisense oligonucleotide, and/or introduce mismatch bases without eliminating activity. For example, in Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7305-7309, 1992), a series of antisense oligonucleotides 13-25 nucleobases in length were tested for their ability to induce cleavage of a target RNA in an oocyte injection model. Antisense oligonucleotides 25 nucleobases in length with 8 or 11 mismatch bases near the ends of the antisense oligonucleotides were able to direct specific cleavage of the target mRNA, albeit to a lesser extent than the antisense oligonucleotides that contained no mismatches. Similarly, target specific cleavage was achieved using 13 nucleobase antisense oligonucleotides, including those with 1 or 3 mismatches.

Gautschi et al. (*J. Natl. Cancer Inst.* 93:463-471, March 2001) demonstrated the ability of an oligonucleotide having 100% complementarity to the bcl-2 mRNA and having 3 mismatches to the bcl-xL mRNA to reduce the expression of both bcl-2 and bcl-xL *in vitro* and *in vivo*. Furthermore, this oligonucleotide demonstrated potent anti-tumor activity *in vivo*.

Maher and Dolnick (*Nuc. Acid. Res.* 16:3341-3358, 1988) tested a series of tandem 14 nucleobase antisense oligonucleotides, and a 28 and 42 nucleobase antisense oligonucleotides comprised of the sequence of two or three of the tandem antisense oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase antisense oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

Certain Antisense Compound Motifs and Mechanisms

In certain embodiments, antisense compounds have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

5 Chimeric antisense compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of a chimeric antisense compound may confer another desired property e.g., serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

10 Antisense activity may result from any mechanism involving the hybridization of the antisense compound (e.g., oligonucleotide) with a target nucleic acid, wherein the hybridization ultimately results in a biological effect. In certain embodiments, the amount and/or activity of the target nucleic acid is modulated. In certain embodiments, the amount and/or activity of the target nucleic acid is reduced. In certain embodiments, hybridization of the antisense compound to the target nucleic acid ultimately results
15 in target nucleic acid degradation. In certain embodiments, hybridization of the antisense compound to the target nucleic acid does not result in target nucleic acid degradation. In certain such embodiments, the presence of the antisense compound hybridized with the target nucleic acid (occupancy) results in a modulation of antisense activity. In certain embodiments, antisense compounds having a particular chemical motif or pattern of chemical modifications are particularly suited to exploit one or more
20 mechanisms. In certain embodiments, antisense compounds function through more than one mechanism and/or through mechanisms that have not been elucidated. Accordingly, the antisense compounds described herein are not limited by particular mechanism.

Antisense mechanisms include, without limitation, RNase H mediated antisense; RNAi mechanisms, which utilize the RISC pathway and include, without limitation, siRNA, ssRNA and
25 microRNA mechanisms; and occupancy based mechanisms. Certain antisense compounds may act through more than one such mechanism and/or through additional mechanisms.

RNase H-Mediated Antisense

In certain embodiments, antisense activity results at least in part from degradation of target RNA by RNase H. RNase H is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex.
30 It is known in the art that single-stranded antisense compounds which are “DNA-like” elicit RNase H activity in mammalian cells. Accordingly, antisense compounds comprising at least a portion of DNA or DNA-like nucleosides may activate RNase H, resulting in cleavage of the target nucleic acid. In certain

embodiments, antisense compounds that utilize RNase H comprise one or more modified nucleosides. In certain embodiments, such antisense compounds comprise at least one block of 1-8 modified nucleosides. In certain such embodiments, the modified nucleosides do not support RNase H activity. In certain embodiments, such antisense compounds are gapmers, as described herein. In certain such embodiments, the gap of the gapmer comprises DNA nucleosides. In certain such embodiments, the gap of the gapmer comprises DNA-like nucleosides. In certain such embodiments, the gap of the gapmer comprises DNA nucleosides and DNA-like nucleosides.

Certain antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β -D-ribonucleosides, β -D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE and 2'-O-CH₃, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a constrained ethyl). In certain embodiments, nucleosides in the wings may include several modified sugar moieties, including, for example 2'-MOE and bicyclic sugar moieties such as constrained ethyl or LNA. In certain embodiments, wings may include several modified and unmodified sugar moieties. In certain embodiments, wings may include various combinations of 2'-MOE nucleosides, bicyclic sugar moieties such as constrained ethyl nucleosides or LNA nucleosides, and 2'-deoxynucleosides.

Each distinct region may comprise uniform sugar moieties, variant, or alternating sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5'-wing, "Y" represents the length of the gap, and "Z" represents the length of the 3'-wing. "X" and "Z" may comprise uniform, variant, or alternating sugar moieties. In certain embodiments, "X" and "Y" may include one or more 2'-deoxynucleosides. "Y" may comprise 2'-deoxynucleosides. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap is positioned immediately adjacent to each of the 5'-wing and the 3' wing. Thus, no intervening nucleotides exist between the 5'-wing and gap, or the gap and the 3'-wing. Any of the antisense compounds described herein can have a gapmer motif. In certain embodiments, "X" and "Z" are the same; in other embodiments they are

different. In certain embodiments, “Y” is between 8 and 15 nucleosides. X, Y, or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleosides.

In certain embodiments, the antisense compound targeted to an Androgen Receptor nucleic acid has a gapmer motif in which the gap consists of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 linked nucleosides.

5 In certain embodiments, the antisense oligonucleotide has a sugar motif described by Formula A as follows: $(J)_m-(B)_n-(J)_p-(B)_r-(A)_t-(D)_g-(A)_v-(B)_w-(J)_x-(B)_y-(J)_z$

wherein:

each A is independently a 2'-substituted nucleoside;

each B is independently a bicyclic nucleoside;

10 each J is independently either a 2'-substituted nucleoside or a 2'-deoxynucleoside;

each D is a 2'-deoxynucleoside;

m is 0-4; n is 0-2; p is 0-2; r is 0-2; t is 0-2; v is 0-2; w is 0-4; x is 0-2; y is 0-2; z is 0-4; g is 6-14;

provided that:

at least one of m, n, and r is other than 0;

15 at least one of w and y is other than 0;

the sum of m, n, p, r, and t is from 2 to 5; and

the sum of v, w, x, y, and z is from 2 to 5.

RNAi Compounds

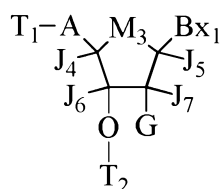
In certain embodiments, antisense compounds are interfering RNA compounds (RNAi), which
20 include double-stranded RNA compounds (also referred to as short-interfering RNA or siRNA) and single-stranded RNAi compounds (or ssRNA). Such compounds work at least in part through the RISC pathway to degrade and/or sequester a target nucleic acid (thus, include microRNA/microRNA-mimic compounds). In certain embodiments, antisense compounds comprise modifications that make them particularly suited for such mechanisms.

25 *i. ssRNA compounds*

In certain embodiments, antisense compounds including those particularly suited for use as single-stranded RNAi compounds (ssRNA) comprise a modified 5'-terminal end. In certain such embodiments, the 5'-terminal end comprises a modified phosphate moiety. In certain embodiments, such

modified phosphate is stabilized (e.g., resistant to degradation/cleavage compared to unmodified 5'-phosphate). In certain embodiments, such 5'-terminal nucleosides stabilize the 5'-phosphorous moiety. Certain modified 5'-terminal nucleosides may be found in the art, for example in WO/2011/139702.

In certain embodiments, the 5'-nucleoside of an ssRNA compound has Formula IIc:



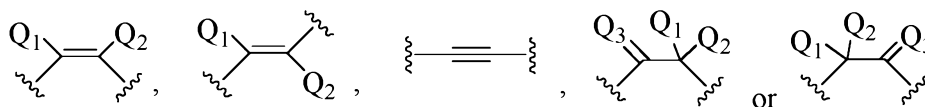
IIc

wherein:

T₁ is an optionally protected phosphorus moiety;

T₂ is an internucleoside linking group linking the compound of Formula IIc to the oligomeric compound;

A has one of the formulas:



Q₁ and Q₂ are each, independently, H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, substituted C₂-C₆ alkynyl or N(R₃)(R₄);

Q₃ is O, S, N(R₅) or C(R₆)(R₇);

each R₃, R₄, R₅, R₆ and R₇ is, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl or C₁-C₆ alkoxy;

M₃ is O, S, NR₁₄, C(R₁₅)(R₁₆), C(R₁₅)(R₁₆)C(R₁₇)(R₁₈), C(R₁₅)=C(R₁₇), OC(R₁₅)(R₁₆) or OC(R₁₅)(Bx₂);

R₁₄ is H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl;

R₁₅, R₁₆, R₁₇ and R₁₈ are each, independently, H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl;

Bx₁ is a heterocyclic base moiety;

or if Bx₂ is present then Bx₂ is a heterocyclic base moiety and Bx₁ is H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl;

5 J₄, J₅, J₆ and J₇ are each, independently, H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl;

or J₄ forms a bridge with one of J₅ or J₇ wherein said bridge comprises from 1 to 3 linked biradical groups selected from O, S, NR₁₉, C(R₂₀)(R₂₁), C(R₂₀)=C(R₂₁), C[=C(R₂₀)(R₂₁)] and C(=O) and
10 the other two of J₅, J₆ and J₇ are each, independently, H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl;

each R₁₉, R₂₀ and R₂₁ is, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆
15 alkynyl;

G is H, OH, halogen or O-[C(R₈)(R₉)]_n[(C=O)_m-X₁]_j-Z;

each R₈ and R₉ is, independently, H, halogen, C₁-C₆ alkyl or substituted C₁-C₆ alkyl;

X₁ is O, S or N(E₁);

Z is H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-
20 C₆ alkynyl, substituted C₂-C₆ alkynyl or N(E₂)(E₃);

E₁, E₂ and E₃ are each, independently, H, C₁-C₆ alkyl or substituted C₁-C₆ alkyl;

n is from 1 to about 6;

m is 0 or 1;

j is 0 or 1;

25 each substituted group comprises one or more optionally protected substituent groups independently selected from halogen, OJ₁, N(J₁)(J₂), =NJ₁, SJ₁, N₃, CN, OC(=X₂)J₁, OC(=X₂)N(J₁)(J₂) and C(=X₂)N(J₁)(J₂);

X₂ is O, S or NJ₃;

each J₁, J₂ and J₃ is, independently, H or C₁-C₆ alkyl;

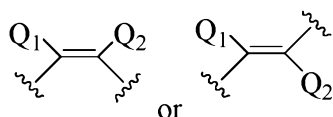
when j is 1 then Z is other than halogen or N(E₂)(E₃); and

wherein said oligomeric compound comprises from 8 to 40 monomeric subunits and is hybridizable to at least a portion of a target nucleic acid.

In certain embodiments, M₃ is O, CH=CH, OCH₂ or OC(H)(Bx₂). In certain embodiments, M₃ is O.

In certain embodiments, J₄, J₅, J₆ and J₇ are each H. In certain embodiments, J₄ forms a bridge with one of J₅ or J₇.

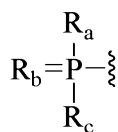
In certain embodiments, A has one of the formulas:



wherein:

Q₁ and Q₂ are each, independently, H, halogen, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkoxy or substituted C₁-C₆ alkoxy. In certain embodiments, Q₁ and Q₂ are each H. In certain embodiments, Q₁ and Q₂ are each, independently, H or halogen. In certain embodiments, Q₁ and Q₂ is H and the other of Q₁ and Q₂ is F, CH₃ or OCH₃.

In certain embodiments, T₁ has the formula:



wherein:

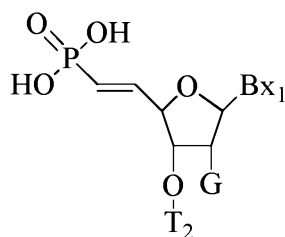
R_a and R_c are each, independently, protected hydroxyl, protected thiol, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, protected amino or substituted amino; and

R_b is O or S. In certain embodiments, R_b is O and R_a and R_c are each, independently, OCH₃, OCH₂CH₃ or CH(CH₃)₂.

In certain embodiments, G is halogen, OCH₃, OCH₂F, OCHF₂, OCF₃, OCH₂CH₃, O(CH₂)₂F, OCH₂CHF₂, OCH₂CF₃, OCH₂-CH=CH₂, O(CH₂)₂-OCH₃, O(CH₂)₂-SCH₃, O(CH₂)₂-OCF₃, O(CH₂)₃-N(R₁₀)(R₁₁), O(CH₂)₂-ON(R₁₀)(R₁₁), O(CH₂)₂-O(CH₂)₂-N(R₁₀)(R₁₁), OCH₂C(=O)-N(R₁₀)(R₁₁), OCH₂C(=O)-N(R₁₂)-(CH₂)₂-N(R₁₀)(R₁₁) or O(CH₂)₂-N(R₁₂)-C(=NR₁₃)[N(R₁₀)(R₁₁)] wherein R₁₀, R₁₁, R₁₂ and R₁₃ are each, independently, H or C₁-C₆ alkyl. In certain embodiments, G is halogen, OCH₃, OCF₃,

OCH₂CH₃, OCH₂CF₃, OCH₂-CH=CH₂, O(CH₂)₂-OCH₃, O(CH₂)₂-O(CH₂)₂-N(CH₃)₂, OCH₂C(=O)-N(H)CH₃, OCH₂C(=O)-N(H)-(CH₂)₂-N(CH₃)₂ or OCH₂-N(H)-C(=NH)NH₂. In certain embodiments, G is F, OCH₃ or O(CH₂)₂-OCH₃. In certain embodiments, G is O(CH₂)₂-OCH₃.

In certain embodiments, the 5'-terminal nucleoside has Formula IIc:



IIc

In certain embodiments, antisense compounds, including those particularly suitable for ssRNA comprise one or more type of modified sugar moieties and/or naturally occurring sugar moieties arranged along an oligonucleotide or region thereof in a defined pattern or sugar modification motif. Such motifs may include any of the sugar modifications discussed herein and/or other known sugar modifications.

In certain embodiments, the oligonucleotides comprise or consist of a region having uniform sugar modifications. In certain such embodiments, each nucleoside of the region comprises the same RNA-like sugar modification. In certain embodiments, each nucleoside of the region is a 2'-F nucleoside. In certain embodiments, each nucleoside of the region is a 2'-OMe nucleoside. In certain embodiments, each nucleoside of the region is a 2'-MOE nucleoside. In certain embodiments, each nucleoside of the region is a cEt nucleoside. In certain embodiments, each nucleoside of the region is an LNA nucleoside. In certain embodiments, the uniform region constitutes all or essentially all of the oligonucleotide. In certain embodiments, the region constitutes the entire oligonucleotide except for 1-4 terminal nucleosides.

In certain embodiments, oligonucleotides comprise one or more regions of alternating sugar modifications, wherein the nucleosides alternate between nucleotides having a sugar modification of a first type and nucleotides having a sugar modification of a second type. In certain embodiments, nucleosides of both types are RNA-like nucleosides. In certain embodiments the alternating nucleosides are selected from: 2'-OMe, 2'-F, 2'-MOE, LNA, and cEt. In certain embodiments, the alternating modifications are 2'-F and 2'-OMe. Such regions may be contiguous or may be interrupted by differently modified nucleosides or conjugated nucleosides.

In certain embodiments, the alternating region of alternating modifications each consist of a single nucleoside (i.e., the pattern is (AB)_xA_y wherein A is a nucleoside having a sugar modification of a

first type and B is a nucleoside having a sugar modification of a second type; x is 1-20 and y is 0 or 1). In certain embodiments, one or more alternating regions in an alternating motif includes more than a single nucleoside of a type. For example, oligonucleotides may include one or more regions of any of the following nucleoside motifs:

5 AABBA;

ABBAB;

AABAAB;

ABBABAAB;

ABABAA;

10 AABABAB;

ABABAA;

ABBAABBABABAA;

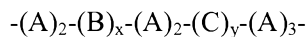
BABBAABBABABAA; or

ABABBAABBABABAA;

15 wherein A is a nucleoside of a first type and B is a nucleoside of a second type. In certain embodiments, A and B are each selected from 2'-F, 2'-OMe, BNA, and MOE.

In certain embodiments, oligonucleotides having such an alternating motif also comprise a modified 5' terminal nucleoside, such as those of formula IIc or IIe.

20 In certain embodiments, oligonucleotides comprise a region having a 2-2-3 motif. Such regions comprises the following motif:



wherein: A is a first type of modified nucleoside;

B and C, are nucleosides that are differently modified than A, however, B and C may have the same or different modifications as one another;

25 x and y are from 1 to 15.

In certain embodiments, A is a 2'-OMe modified nucleoside. In certain embodiments, B and C are both 2'-F modified nucleosides. In certain embodiments, A is a 2'-OMe modified nucleoside and B and C are both 2'-F modified nucleosides.

In certain embodiments, oligonucleosides have the following sugar motif:



wherein:

Q is a nucleoside comprising a stabilized phosphate moiety. In certain embodiments, Q is a nucleoside having Formula IIc or IIe;

A is a first type of modified nucleoside;

B is a second type of modified nucleoside;

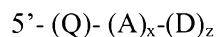
D is a modified nucleoside comprising a modification different from the nucleoside adjacent to it. Thus, if y is 0, then D must be differently modified than B and if y is 1, then D must be differently modified than A. In certain embodiments, D differs from both A and B.

X is 5-15;

Y is 0 or 1;

Z is 0-4.

In certain embodiments, oligonucleosides have the following sugar motif:



wherein:

Q is a nucleoside comprising a stabilized phosphate moiety. In certain embodiments, Q is a nucleoside having Formula IIc or IIe;

A is a first type of modified nucleoside;

D is a modified nucleoside comprising a modification different from A.

X is 11-30;

Z is 0-4.

In certain embodiments A, B, C, and D in the above motifs are selected from: 2'-OMe, 2'-F, 2'-MOE, LNA, and cEt. In certain embodiments, D represents terminal nucleosides. In certain embodiments, such terminal nucleosides are not designed to hybridize to the target nucleic acid (though one or more might hybridize by chance). In certain embodiments, the nucleobase of each D nucleoside is adenine, regardless of the identity of the nucleobase at the corresponding position of the target nucleic acid. In certain embodiments the nucleobase of each D nucleoside is thymine.

In certain embodiments, antisense compounds, including those particularly suited for use as ssRNA comprise modified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or modified internucleoside linkage motif. In certain embodiments, oligonucleotides comprise a region having an alternating internucleoside linkage motif. In certain embodiments, 5 oligonucleotides comprise a region of uniformly modified internucleoside linkages. In certain such embodiments, the oligonucleotide comprises a region that is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate. In certain embodiments, each 10 internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate and at least one internucleoside linkage is phosphorothioate.

In certain embodiments, the oligonucleotide comprises at least 6 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 8 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at 15 least 10 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 6 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 8 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 10 consecutive phosphorothioate internucleoside linkages. In certain 20 embodiments, the oligonucleotide comprises at least one block of at least 12 consecutive phosphorothioate internucleoside linkages. In certain such embodiments, at least one such block is located at the 3' end of the oligonucleotide. In certain such embodiments, at least one such block is located within 3 nucleosides of the 3' end of the oligonucleotide.

Oligonucleotides having any of the various sugar motifs described herein, may have any linkage 25 motif. For example, the oligonucleotides, including but not limited to those described above, may have a linkage motif selected from non-limiting the table below:

5' most linkage	Central region	3'-region
PS	Alternating PO/PS	6 PS
PS	Alternating PO/PS	7 PS
PS	Alternating PO/PS	8 PS

ii. siRNA compounds

In certain embodiments, antisense compounds are double-stranded RNAi compounds (siRNA). In such embodiments, one or both strands may comprise any modification motif described above for ssRNA. In certain embodiments, ssRNA compounds may be unmodified RNA. In certain embodiments, siRNA compounds may comprise unmodified RNA nucleosides, but modified internucleoside linkages.

Several embodiments relate to double-stranded compositions wherein each strand comprises a motif defined by the location of one or more modified or unmodified nucleosides. In certain embodiments, compositions are provided comprising a first and a second oligomeric compound that are fully or at least partially hybridized to form a duplex region and further comprising a region that is complementary to and hybridizes to a nucleic acid target. It is suitable that such a composition comprise a first oligomeric compound that is an antisense strand having full or partial complementarity to a nucleic acid target and a second oligomeric compound that is a sense strand having one or more regions of complementarity to and forming at least one duplex region with the first oligomeric compound.

The compositions of several embodiments modulate gene expression by hybridizing to a nucleic acid target resulting in loss of its normal function. In some embodiments, the target nucleic acid is Androgen Receptor. In certain embodiment, the degradation of the targeted Androgen Receptor is facilitated by an activated RISC complex that is formed with compositions of the invention.

Several embodiments are directed to double-stranded compositions wherein one of the strands is useful in, for example, influencing the preferential loading of the opposite strand into the RISC (or cleavage) complex. The compositions are useful for targeting selected nucleic acid molecules and modulating the expression of one or more genes. In some embodiments, the compositions of the present invention hybridize to a portion of a target RNA resulting in loss of normal function of the target RNA.

Certain embodiments are drawn to double-stranded compositions wherein both the strands comprises a hemimer motif, a fully modified motif, a positionally modified motif or an alternating motif. Each strand of the compositions of the present invention can be modified to fulfil a particular role in for example the siRNA pathway. Using a different motif in each strand or the same motif with different chemical modifications in each strand permits targeting the antisense strand for the RISC complex while inhibiting the incorporation of the sense strand. Within this model, each strand can be independently modified such that it is enhanced for its particular role. The antisense strand can be modified at the 5'-end to enhance its role in one region of the RISC while the 3'-end can be modified differentially to enhance its role in a different region of the RISC.

The double-stranded oligonucleotide molecules can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The double-stranded oligonucleotide molecules can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double-stranded structure, for example wherein the double-stranded region is about 15 to about 30, e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 base pairs; the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof (e.g., about 15 to about 25 or more nucleotides of the double-stranded oligonucleotide molecule are complementary to the target nucleic acid or a portion thereof). Alternatively, the double-stranded oligonucleotide is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siRNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s).

The double-stranded oligonucleotide can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The double-stranded oligonucleotide can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either in vivo or in vitro to generate an active siRNA molecule capable of mediating RNAi.

In certain embodiments, the double-stranded oligonucleotide comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linker molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der Waals interactions, hydrophobic interactions,

and/or stacking interactions. In certain embodiments, the double-stranded oligonucleotide comprises nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the double-stranded oligonucleotide interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene.

5 As used herein, double-stranded oligonucleotides need not be limited to those molecules containing only RNA, but further encompasses chemically modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules lack 2'-hydroxy (2'-OH) containing nucleotides. In certain embodiments short interfering nucleic acids optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such double-stranded oligonucleotides that do
10 not require the presence of ribonucleotides within the molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, double-stranded oligonucleotides can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. As used herein, the term siRNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of
15 mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post
20 transcriptional gene silencing, translational inhibition, or epigenetics. For example, double-stranded oligonucleotides can be used to epigenetically silence genes at both the post-transcriptional level and the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siRNA molecules of the invention can result from siRNA mediated modification of chromatin structure or methylation pattern to alter gene expression (see, for example, Verdel et al., 2004, Science, 303, 672-676;
25 Pal-Bhadra et al., 2004, Science, 303, 669-672; Allshire, 2002, Science, 297, 1818-1819; Volpe et al., 2002, Science, 297, 1833-1837; Jenuwein, 2002, Science, 297, 2215-2218; and Hall et al., 2002, Science, 297, 2232-2237).

 It is contemplated that compounds and compositions of several embodiments provided herein can target Androgen Receptor by a dsRNA-mediated gene silencing or RNAi mechanism, including, e.g.,
30 "hairpin" or stem-loop double-stranded RNA effector molecules in which a single RNA strand with self-complementary sequences is capable of assuming a double-stranded conformation, or duplex dsRNA effector molecules comprising two separate strands of RNA. In various embodiments, the dsRNA consists entirely of ribonucleotides or consists of a mixture of ribonucleotides and deoxynucleotides, such as the

RNA/DNA hybrids disclosed, for example, by WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999. The dsRNA or dsRNA effector molecule may be a single molecule with a region of self-complementarity such that nucleotides in one segment of the molecule base pair with nucleotides in another segment of the molecule. In various embodiments, a dsRNA that consists of a single molecule consists entirely of ribonucleotides or includes a region of ribonucleotides that is complementary to a region of deoxyribonucleotides. Alternatively, the dsRNA may include two different strands that have a region of complementarity to each other.

In various embodiments, both strands consist entirely of ribonucleotides, one strand consists entirely of ribonucleotides and one strand consists entirely of deoxyribonucleotides, or one or both strands contain a mixture of ribonucleotides and deoxyribonucleotides. In certain embodiments, the regions of complementarity are at least 70, 80, 90, 95, 98, or 100% complementary to each other and to a target nucleic acid sequence. In certain embodiments, the region of the dsRNA that is present in a double-stranded conformation includes at least 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 50, 75, 100, 200, 500, 1000, 2000 or 5000 nucleotides or includes all of the nucleotides in a cDNA or other target nucleic acid sequence being represented in the dsRNA. In some embodiments, the dsRNA does not contain any single stranded regions, such as single stranded ends, or the dsRNA is a hairpin. In other embodiments, the dsRNA has one or more single stranded regions or overhangs. In certain embodiments, RNA/DNA hybrids include a DNA strand or region that is an antisense strand or region (e.g., has at least 70, 80, 90, 95, 98, or 100% complementarity to a target nucleic acid) and an RNA strand or region that is a sense strand or region (e.g., has at least 70, 80, 90, 95, 98, or 100% identity to a target nucleic acid), and vice versa.

In various embodiments, the RNA/DNA hybrid is made in vitro using enzymatic or chemical synthetic methods such as those described herein or those described in WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999. In other embodiments, a DNA strand synthesized in vitro is complexed with an RNA strand made in vivo or in vitro before, after, or concurrent with the transformation of the DNA strand into the cell. In yet other embodiments, the dsRNA is a single circular nucleic acid containing a sense and an antisense region, or the dsRNA includes a circular nucleic acid and either a second circular nucleic acid or a linear nucleic acid (see, for example, WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999.) Exemplary circular nucleic acids include lariat structures in which the free 5' phosphoryl group of a nucleotide becomes linked to the 2' hydroxyl group of another nucleotide in a loop back fashion.

In other embodiments, the dsRNA includes one or more modified nucleotides in which the 2' position in the sugar contains a halogen (such as fluorine group) or contains an alkoxy group (such as a

methoxy group) which increases the half-life of the dsRNA in vitro or in vivo compared to the corresponding dsRNA in which the corresponding 2' position contains a hydrogen or an hydroxyl group. In yet other embodiments, the dsRNA includes one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramidate, phosphorothioate, and phosphorodithioate linkages. The dsRNAs may also be chemically modified nucleic acid molecules as taught in U.S. Pat. No. 6,673,661. In other embodiments, the dsRNA contains one or two capped strands, as disclosed, for example, by WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999.

In other embodiments, the dsRNA can be any of the at least partially dsRNA molecules disclosed in WO 00/63364, as well as any of the dsRNA molecules described in U.S. Provisional Application 60/399,998; and U.S. Provisional Application 60/419,532, and PCT/US2003/033466, the teaching of which is hereby incorporated by reference. Any of the dsRNAs may be expressed in vitro or in vivo using the methods described herein or standard methods, such as those described in WO 00/63364.

Occupancy

In certain embodiments, antisense compounds are not expected to result in cleavage or the target nucleic acid via RNase H or to result in cleavage or sequestration through the RISC pathway. In certain such embodiments, antisense activity may result from occupancy, wherein the presence of the hybridized antisense compound disrupts the activity of the target nucleic acid. In certain such embodiments, the antisense compound may be uniformly modified or may comprise a mix of modifications and/or modified and unmodified nucleosides.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode human Androgen Receptor include, without limitation, the following: GENBANK Accession No. NT_011669.17_TRUNC_5079000_5270000 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NM_000044.3 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No. NM_001011645.2 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. FJ235916.1 (incorporated herein as SEQ ID NO: 4), GENBANK Accession No. FJ235917.1 (incorporated herein as SEQ ID NO: 5), GENBANK Accession No. FJ235918.1 (incorporated herein as SEQ ID NO: 6), GENBANK Accession No. FJ235919.1 (incorporated herein as SEQ ID NO: 7), and GENBANK Accession No. FJ235920.1 (incorporated herein as SEQ ID NO: 8).

Androgen Receptor mRNA encodes several functional domains. In certain embodiments, full-length Androgen Receptor mRNA includes exon 1 encoding the N-terminal domain, exons 2 and 3

encoding the DNA binding domain, exon 4 encoding the short hinge region, and exons 4-8 encoding the ligand binding domain.

In certain embodiments, Androgen Receptor splicing variants targetable by the antisense compounds provided herein include exon 1 encoding the N-terminal domain and exons 2 and 3 encoding the DNA binding domain, or functional portions thereof, but does not include at least a portion of exon 4 encoding the short hinge region or at least a portion of exons 4-8 encoding the ligand binding domain. Examples of such AR splicing variants include, but are not limited to, AR-V1, AR-V2, AR-V3, AR-V4, AR-V5, AR-V6, and AR-V7 (also referred to as AR3), which contain exons 1-3 but lack exons 4-8. AR-V1, AR-V2, AR-V3, AR-V4, AR-V5, AR-V6, AR-V7, and additional splicing variants targetable by the antisense compounds provided herein are described in Hu et al., *Cancer Res* 2009; 69:16-22 and US Patent Application Publication No. US 2010/0068802, each of which is incorporated herein by reference in its entirety. Further examples of such AR splicing variants targetable by the antisense compounds provided herein include, but are not limited to, AR3, AR4, AR4b, AR5, and AR6 (SEQ ID NOs: 4-8, respectively) as described in Guo et al., *Cancer Res.* 2009; 69: 2305-13, which is incorporated herein by reference in its entirety.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and an Androgen Receptor. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

Hybridization can occur under varying conditions. Stringent conditions are sequence-dependent and are determined by the nature and composition of the nucleic acid molecules to be hybridized.

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with Androgen Receptor.

Complementarity

An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the corresponding nucleobases of the target nucleic acid, such that a desired effect will occur (e.g., antisense inhibition of a target nucleic acid, such as an Androgen Receptor nucleic acid).

Non-complementary nucleobases between an antisense compound and an Androgen Receptor nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or more segments of an Androgen Receptor nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to an Androgen Receptor nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having four noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403-410; Zhang and Madden, *Genome Res.*, 1997, 7, 649-656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.*, 1981, 2, 482-489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to an Androgen Receptor nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound.

Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be “fully complementary” to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

The location of a non-complementary nucleobase may be at the 5' end or 3' end of the antisense compound. Alternatively, the non-complementary nucleobase or nucleobases may be at an internal position of the antisense compound. When two or more non-complementary nucleobases are present, they may be contiguous (i.e. linked) or non-contiguous. In one embodiment, a non-complementary nucleobase is located in the wing segment of a gapmer antisense oligonucleotide.

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as an Androgen Receptor nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as an Androgen Receptor nucleic acid, or specified portion thereof.

The antisense compounds provided also include those which are complementary to a portion of a target nucleic acid. As used herein, “portion” refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A “portion” can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 9 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 10 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least an 11 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 13 nucleobase portion of a

target segment. In certain embodiments, the antisense compounds are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 15 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof. As used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

In certain embodiments, the antisense compounds, or portions thereof, are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

In certain embodiments, a portion of the antisense compound is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

In certain embodiments, a portion of the antisense oligonucleotide is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

Modifications

A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that

include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

5 Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

10 Chemically modified nucleosides may also be employed to increase the binding affinity of a shortened or truncated antisense oligonucleotide for its target nucleic acid. Consequently, comparable results can often be obtained with shorter antisense compounds that have such chemically modified nucleosides.

Modified Internucleoside Linkages

15 The naturally occurring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

20 Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

25 In certain embodiments, antisense compounds targeted to an Androgen Receptor nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate internucleoside linkage.

Modified Sugar Moieties

30 Antisense compounds can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased

binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include without limitation, addition of substituent groups (including 5' and 2' substituent groups, bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or C(R₁)(R₂) (R, R₁ and R₂ are each independently H, C₁-C₁₂ alkyl or a protecting group) and combinations thereof. Examples of chemically modified sugars include 2'-F-5'-methyl substituted nucleoside (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5',2'-bis substituted nucleosides) or replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a BNA (see PCT International Application WO 2007/134181 Published on 11/22/07 wherein 4'-(CH₂)-O-2' (LNA) is substituted with for example a 5'-methyl or a 5'-vinyl group).

Examples of nucleosides having modified sugar moieties include without limitation nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH₃, 2'-OCH₂CH₃, 2'-OCH₂CH₂F and 2'-O(CH₂)₂OCH₃ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C₁-C₁₀ alkyl, OCF₃, OCH₂F, O(CH₂)₂SCH₃, O(CH₂)₂-O-N(R_m)(R_n), O-CH₂-C(=O)-N(R_m)(R_n), and O-CH₂-C(=O)-N(R₁)-(CH₂)₂-N(R_m)(R_n), where each R₁, R_m and R_n is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl.

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to one of the formulae: 4'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-2' (ENA); 4'-CH(CH₃)-O-2' (also referred to as constrained ethyl or cEt) and 4'-CH(CH₂OCH₃)-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH₃)(CH₃)-O-2' (and analogs thereof see published International Application WO/2009/006478, published January 8, 2009); 4'-CH₂-N(OCH₃)-2' (and analogs thereof see published International Application WO/2008/150729, published December 11, 2008); 4'-CH₂-O-N(CH₃)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH₂-N(R)-O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH₂-C(H)(CH₃)-2' (see Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂-C(=CH₂)-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008).

Further reports related to bicyclic nucleosides can also be found in published literature (see for example: Singh *et al.*, *Chem. Commun.*, 1998, 4, 455-456; Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630; Wahlestedt *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 5633-5638; Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222; Singh *et al.*, *J. Org. Chem.*, 1998, 63, 10035-10039; Srivastava *et al.*, *J. Am. Chem. Soc.*, 2007, 129(26) 8362-8379; Elayadi *et al.*, *Curr. Opinion Invest. Drugs*, 2001, 2, 558-561; Braasch *et al.*, *Chem. Biol.*, 2001, 8, 1-7; and Orum *et al.*, *Curr. Opinion Mol. Ther.*, 2001, 3, 239-243; U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 7,034,133; 7,053,207; 7,399,845; 7,547,684; and 7,696,345; U.S. Patent Publication No. US2008-0039618; US2009-0012281; U.S. Patent Serial Nos. 60/989,574; 61/026,995; 61/026,998; 61/056,564; 61/086,231; 61/097,787; and 61/099,844; Published PCT International applications WO 1994/014226; WO 2004/106356; WO 2005/021570; WO 2007/134181; WO 2008/150729; WO 2008/154401; and WO 2009/006478. Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α -L-ribofuranose and β -D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

In certain embodiments, bicyclic sugar moieties of BNA nucleosides include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the pentofuranosyl sugar moiety wherein such bridges independently comprises 1 or from 2 to 4 linked groups independently selected from $-\text{C}(\text{R}_a)(\text{R}_b)_n-$, $-\text{C}(\text{R}_a)=\text{C}(\text{R}_b)-$, $-\text{C}(\text{R}_a)=\text{N}-$, $-\text{C}(=\text{O})-$, $-\text{C}(=\text{NR}_a)-$, $-\text{C}(=\text{S})-$, $-\text{O}-$, $-\text{Si}(\text{R}_a)_2-$, $-\text{S}(=\text{O})_x-$, and $-\text{N}(\text{R}_a)-$;

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5 - C_7 alicyclic radical, substituted C_5 - C_7 alicyclic radical, halogen, OJ_1 , NJ_1J_2 , SJ_1 , N_3 , COOJ_1 , acyl ($\text{C}(=\text{O})-\text{H}$), substituted acyl, CN, sulfonyl ($\text{S}(=\text{O})_2-\text{J}_1$), or sulfoxyl ($\text{S}(=\text{O})-\text{J}_1$); and

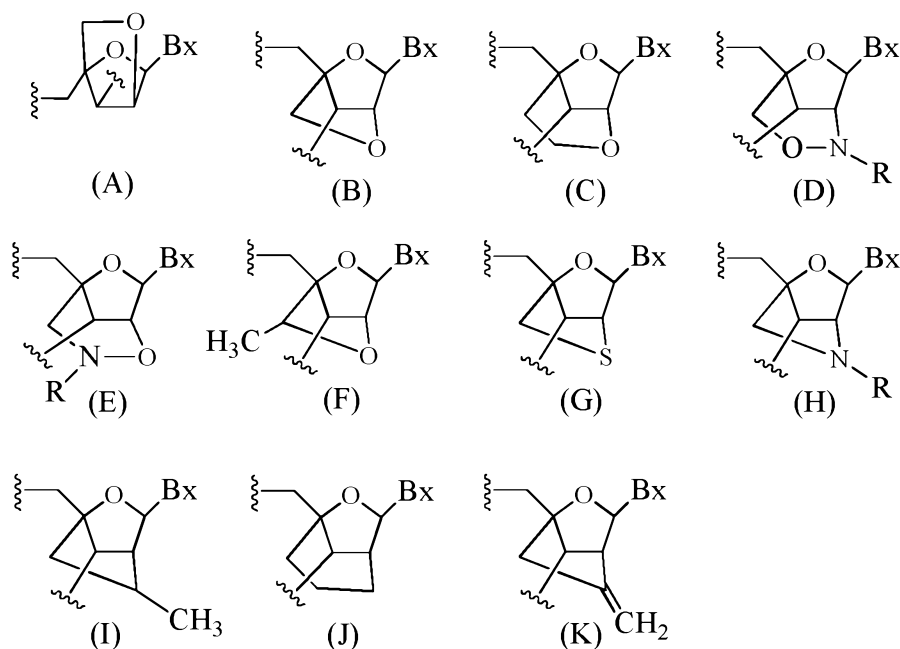
each J_1 and J_2 is, independently, H, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_5 - C_{20} aryl, substituted C_5 - C_{20} aryl, acyl ($\text{C}(=\text{O})-\text{H}$), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1 - C_{12} aminoalkyl, substituted C_1 - C_{12} aminoalkyl or a protecting group.

In certain embodiments, the bridge of a bicyclic sugar moiety is $-[C(R_a)(R_b)]_n-$, $-[C(R_a)(R_b)]_n-O-$, $-C(R_aR_b)-N(R)-O-$ or $-C(R_aR_b)-O-N(R)-$. In certain embodiments, the bridge is 4'-CH₂-2', 4'-(CH₂)₂-2', 4'-(CH₂)₃-2', 4'-CH₂-O-2', 4'-(CH₂)₂-O-2', 4'-CH₂-O-N(R)-2' and 4'-CH₂-N(R)-O-2' wherein each R is, independently, H, a protecting group or C₁-C₁₂ alkyl.

5 In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α -L configuration or in the β -D configuration. Previously, α -L-methyleneoxy (4'-CH₂-O-2') BNA's have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden *et al.*, *Nucleic Acids Research*, 2003, 21, 6365-6372).

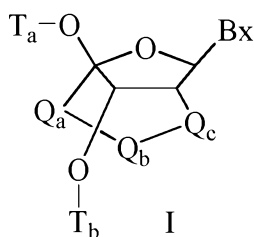
10 In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α -L-methyleneoxy (4'-CH₂-O-2') BNA, (B) β -D-methyleneoxy (4'-CH₂-O-2') BNA, (C) ethyleneoxy (4'-(CH₂)₂-O-2') BNA, (D) aminooxy (4'-CH₂-O-N(R)-2') BNA, (E) oxyamino (4'-CH₂-N(R)-O-2') BNA, and (F) methyl(methyleneoxy) (4'-CH(CH₃)-O-2') BNA, (G) methylene-thio (4'-CH₂-S-2') BNA, (H) methylene-amino (4'-CH₂-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH₂-CH(CH₃)-2') BNA, (J) propylene carbocyclic (4'-(CH₂)₃-2') BNA and (K) vinyl BNA as depicted below:

15



wherein Bx is the base moiety and R is independently H, a protecting group, C₁-C₁₂ alkyl or C₁-C₁₂ alkoxy.

In certain embodiments, bicyclic nucleosides are provided having Formula I:



wherein:

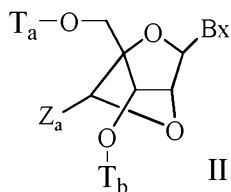
5 Bx is a heterocyclic base moiety;

-Q_a-Q_b-Q_c- is -CH₂-N(R_c)-CH₂-, -C(=O)-N(R_c)-CH₂-, -CH₂-O-N(R_c)-, -CH₂-N(R_c)-O- or -N(R_c)-O-CH₂;

R_c is C₁-C₁₂ alkyl or an amino protecting group; and

10 T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium.

In certain embodiments, bicyclic nucleosides are provided having Formula II:



15 wherein:

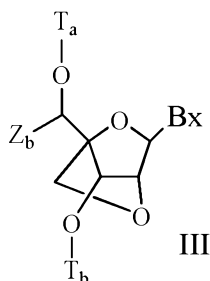
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

20 Z_a is C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, substituted C₁-C₆ alkyl, substituted C₂-C₆ alkenyl, substituted C₂-C₆ alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thio.

In one embodiment, each of the substituted groups is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ_c , NJ_cJ_d , SJ_c , N_3 , $\text{OC}(=\text{X})\text{J}_c$, and $\text{NJ}_c\text{C}(=\text{X})\text{NJ}_c\text{J}_d$, wherein each J_c , J_d and J_e is, independently, H, $\text{C}_1\text{-C}_6$ alkyl, or substituted $\text{C}_1\text{-C}_6$ alkyl and X is O or NJ_c .

5 In certain embodiments, bicyclic nucleosides are provided having Formula III:



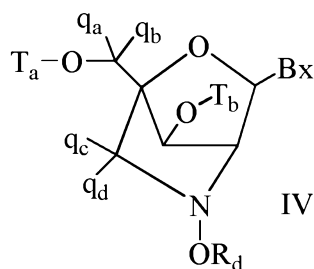
wherein:

Bx is a heterocyclic base moiety;

10 T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

Z_b is $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl, substituted $\text{C}_1\text{-C}_6$ alkyl, substituted $\text{C}_2\text{-C}_6$ alkenyl, substituted $\text{C}_2\text{-C}_6$ alkynyl or substituted acyl ($\text{C}(=\text{O})\text{-}$).

In certain embodiments, bicyclic nucleosides are provided having Formula IV:



15 wherein:

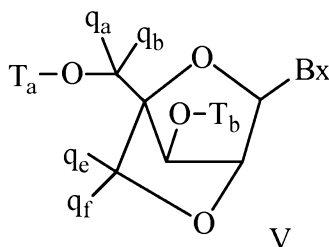
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

20 R_d is $\text{C}_1\text{-C}_6$ alkyl, substituted $\text{C}_1\text{-C}_6$ alkyl, $\text{C}_2\text{-C}_6$ alkenyl, substituted $\text{C}_2\text{-C}_6$ alkenyl, $\text{C}_2\text{-C}_6$ alkynyl or substituted $\text{C}_2\text{-C}_6$ alkynyl;

each q_a , q_b , q_c and q_d is, independently, H, halogen, C_1 - C_6 alkyl, substituted C_1 - C_6 alkyl, C_2 - C_6 alkenyl, substituted C_2 - C_6 alkenyl, C_2 - C_6 alkynyl or substituted C_2 - C_6 alkynyl, C_1 - C_6 alkoxyl, substituted C_1 - C_6 alkoxyl, acyl, substituted acyl, C_1 - C_6 aminoalkyl or substituted C_1 - C_6 aminoalkyl;

In certain embodiments, bicyclic nucleosides are provided having Formula V:



wherein:

B_x is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

q_a , q_b , q_c and q_f are each, independently, hydrogen, halogen, C_1 - C_{12} alkyl, substituted C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, substituted C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, substituted C_2 - C_{12} alkynyl, C_1 - C_{12} alkoxy, substituted C_1 - C_{12} alkoxy, OJ_j , SJ_j , SOJ_j , SO_2J_j , NJ_jJ_k , N_3 , CN , $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O-C(=O)-NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$;

or q_c and q_f together are $=C(q_g)(q_h)$;

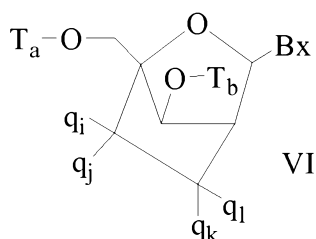
q_g and q_h are each, independently, H, halogen, C_1 - C_{12} alkyl or substituted C_1 - C_{12} alkyl.

The synthesis and preparation of the methyleneoxy (4'- CH_2 -O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin *et al.*, *Tetrahedron*, 1998, 54, 3607-3630). Bicyclic nucleic acids (BNAs) and preparation thereof are also described in WO 98/39352 and WO 99/14226.

Analogues of methyleneoxy (4'- CH_2 -O-2') BNA and 2'-thio-BNAs, have also been prepared (Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, 8, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (Wengel *et al.*, WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel conformationally restricted high-affinity oligonucleotide analog has been described in the art (Singh *et al.*, *J. Org. Chem.*, 1998, 63, 10035-10039). In addition, 2'-amino- and 2'-methylamino-BNA's have been

prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

In certain embodiments, bicyclic nucleosides are provided having Formula VI:



5 wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

10 each q_i, q_j, q_k and q_l is, independently, H, halogen, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₁-C₁₂ alkoxy, substituted C₁-C₁₂ alkoxy, OJ_j, SJ_j, SOJ_j, SO₂J_j, NJ_jJ_k, N₃, CN, C(=O)OJ_j, C(=O)NJ_jJ_k, C(=O)J_j, O-C(=O)NJ_jJ_k, N(H)C(=NH)NJ_jJ_k, N(H)C(=O)NJ_jJ_k or N(H)C(=S)NJ_jJ_k; and

q_i and q_j or q_l and q_k together are =C(q_g)(q_h), wherein q_g and q_h are each, independently, H, halogen, C₁-C₁₂ alkyl or substituted C₁-C₁₂ alkyl.

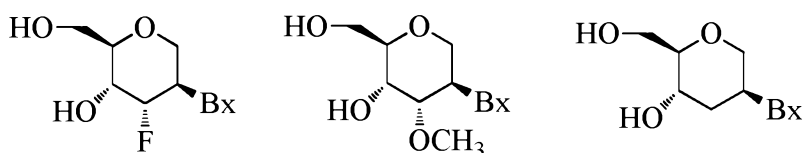
15 One carbocyclic bicyclic nucleoside having a 4'-(CH₂)₃-2' bridge and the alkenyl analog bridge 4'-CH=CH-CH₂-2' have been described (Freier *et al.*, *Nucleic Acids Research*, 1997, 25(22), 4429-4443 and Albaek *et al.*, *J. Org. Chem.*, 2006, 71, 7731-7740). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (Srivastava *et al.*, *J. Am. Chem. Soc.*, 2007, 129(26), 8362-8379).

20 As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting two carbon atoms of the furanose ring connects the 2' carbon atom and the 4' carbon atom of the sugar ring.

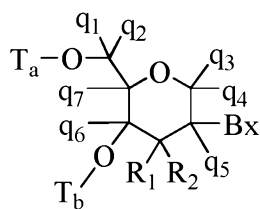
As used herein, "monocyclic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, 25 of a nucleoside may be modified or substituted at any position.

As used herein, “2’-modified sugar” means a furanosyl sugar modified at the 2’ position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2’ modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nF$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2’- substituent groups can also be selected from: C_1 - C_{12} alkyl, substituted alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, F, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving pharmacokinetic properties, or a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modified nucleosides comprise a 2’-MOE side chain (Baker *et al.*, *J. Biol. Chem.*, 1997, 272, 11944-12000). Such 2’-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2’- O-methyl, O-propyl, and O-aminopropyl. Oligonucleotides having the 2’-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (Martin, *Helv. Chim. Acta*, 1995, 78, 486-504; Altmann *et al.*, *Chimia*, 1996, 50, 168-176; Altmann *et al.*, *Biochem. Soc. Trans.*, 1996, 24, 630-637; and Altmann *et al.*, *Nucleosides Nucleotides*, 1997, 16, 917-926).

As used herein, a “modified tetrahydropyran nucleoside” or “modified THP nucleoside” means a nucleoside having a six-membered tetrahydropyran “sugar” substituted in for the pentofuranosyl residue in normal nucleosides (a sugar surrogate). Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (see Leumann, *Bioorg. Med. Chem.*, 2002, 10, 841-854) or fluoro HNA (F-HNA) having a tetrahydropyran ring system as illustrated below:



In certain embodiments, sugar surrogates are selected having Formula VII:



VII

wherein independently for each of said at least one tetrahydropyran nucleoside analog of Formula VII:

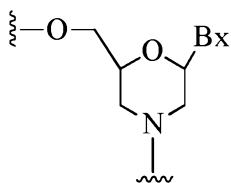
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound or one of T_a and T_b is an internucleoside linking group linking the tetrahydropyran nucleoside analog to the antisense compound and the other of T_a and T_b is H,
 5 a hydroxyl protecting group, a linked conjugate group or a 5' or 3'-terminal group;

q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl or substituted C₂-C₆ alkynyl; and each of R₁ and R₂ is selected from hydrogen, hydroxyl, halogen, substituted or unsubstituted alkoxy, NJ₁J₂, SJ₁, N₃, OC(=X)J₁, OC(=X)NJ₁J₂, NJ₃C(=X)NJ₁J₂ and CN, wherein X is O, S or NJ₁ and each J₁, J₂ and J₃ is,
 10 independently, H or C₁-C₆ alkyl.

In certain embodiments, the modified THP nucleosides of Formula VII are provided wherein q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each H. In certain embodiments, at least one of q₁, q₂, q₃, q₄, q₅, q₆ and q₇ is other than H. In certain embodiments, at least one of q₁, q₂, q₃, q₄, q₅, q₆ and q₇ is methyl. In certain embodiments, THP nucleosides of Formula VII are provided wherein one of R₁ and R₂ is fluoro. In
 15 certain embodiments, R₁ is fluoro and R₂ is H; R₁ is methoxy and R₂ is H, and R₁ is methoxyethoxy and R₂ is H.

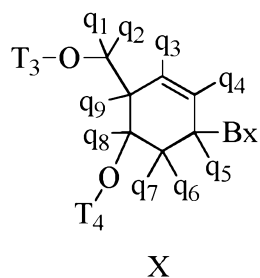
In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example nucleosides comprising morpholino sugar moieties and their use in oligomeric compounds has been reported (see for example: Braasch *et al.*, *Biochemistry*, 2002, 41, 4503-
 20 4510; and U.S. Patents 5,698,685; 5,166,315; 5,185,444; and 5,034,506). As used here, the term “morpholino” means a sugar surrogate having the following formula:



In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are referred to herein as “modified morpholinos.”

Combinations of modifications are also provided without limitation, such as 2'-F-5'-methyl substituted nucleosides (see PCT International Application WO 2008/101157 published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a bicyclic nucleic acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4'-CH₂-O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g., Srivastava et al., J. Am. Chem. Soc.* 2007, 129(26), 8362-8379).

In certain embodiments, antisense compounds comprise one or more modified cyclohexenyl nucleosides, which is a nucleoside having a six-membered cyclohexenyl in place of the pentofuranosyl residue in naturally occurring nucleosides. Modified cyclohexenyl nucleosides include, but are not limited to those described in the art (see for example commonly owned, published PCT Application WO 2010/036696, published on April 10, 2010, Robeyns *et al., J. Am. Chem. Soc.*, 2008, 130(6), 1979-1984; Horváth *et al., Tetrahedron Letters*, 2007, 48, 3621-3623; Nauwelaerts *et al., J. Am. Chem. Soc.*, 2007, 129(30), 9340-9348; Gu *et al., Nucleosides, Nucleotides & Nucleic Acids*, 2005, 24(5-7), 993-998; Nauwelaerts *et al., Nucleic Acids Research*, 2005, 33(8), 2452-2463; Robeyns *et al., Acta Crystallographica, Section F: Structural Biology and Crystallization Communications*, 2005, F61(6), 585-586; Gu *et al., Tetrahedron*, 2004, 60(9), 2111-2123; Gu *et al., Oligonucleotides*, 2003, 13(6), 479-489; Wang *et al., J. Org. Chem.*, 2003, 68, 4499-4505; Verbeure *et al., Nucleic Acids Research*, 2001, 29(24), 4941-4947; Wang *et al., J. Org. Chem.*, 2001, 66, 8478-82; Wang *et al., Nucleosides, Nucleotides & Nucleic Acids*, 2001, 20(4-7), 785-788; Wang *et al., J. Am. Chem.*, 2000, 122, 8595-8602; Published PCT application, WO 06/047842; and Published PCT Application WO 01/049687; the text of each is incorporated by reference herein, in their entirety). Certain modified cyclohexenyl nucleosides have Formula X.



wherein independently for each of said at least one cyclohexenyl nucleoside analog of Formula X:

Bx is a heterocyclic base moiety;

T₃ and T₄ are each, independently, an internucleoside linking group linking the cyclohexenyl nucleoside analog to an antisense compound or one of T₃ and T₄ is an internucleoside linking group linking the tetrahydropyran nucleoside analog to an antisense compound and the other of T₃ and T₄ is H, a hydroxyl protecting group, a linked conjugate group, or a 5'-or 3'-terminal group; and

q₁, q₂, q₃, q₄, q₅, q₆, q₇, q₈ and q₉ are each, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, substituted C₂-C₆ alkynyl or other sugar substituent group.

As used herein, "2'-modified" or "2'-substituted" refers to a nucleoside comprising a sugar comprising a substituent at the 2' position other than H or OH. 2'-modified nucleosides, include, but are not limited to, bicyclic nucleosides wherein the bridge connecting two carbon atoms of the sugar ring connects the 2' carbon and another carbon of the sugar ring; and nucleosides with non-bridging 2'substituents, such as allyl, amino, azido, thio, O-allyl, O-C₁-C₁₀ alkyl, -OCF₃, O-(CH₂)₂-O-CH₃, 2'-O(CH₂)₂SCH₃, O-(CH₂)₂-O-N(R_m)(R_n), or O-CH₂-C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H or substituted or unsubstituted C₁-C₁₀ alkyl. 2'-modified nucleosides may further comprise other modifications, for example at other positions of the sugar and/or at the nucleobase.

As used herein, "2'-F" refers to a nucleoside comprising a sugar comprising a fluoro group at the 2' position of the sugar ring.

As used herein, "2'-OMe" or "2'-OCH₃" or "2'-O-methyl" each refers to a nucleoside comprising a sugar comprising an -OCH₃ group at the 2' position of the sugar ring.

As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides. In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or

deoxyribonucleosides (DNA).

Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (see for example review article: Leumann, *Bioorg. Med. Chem.*, 2002, 10, 841-854). Such ring systems can undergo various additional
5 substitutions to enhance activity.

Methods for the preparations of modified sugars are well known to those skilled in the art. Some representative U.S. patents that teach the preparation of such modified sugars include without limitation, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265;
10 5,670,633; 5,700,920; 5,792,847 and 6,600,032 and International Application PCT/US2005/019219, filed June 2, 2005 and published as WO 2005/121371 on December 22, 2005, and each of which is herein incorporated by reference in its entirety.

In nucleotides having modified sugar moieties, the nucleobase moieties (natural, modified or a combination thereof) are maintained for hybridization with an appropriate nucleic acid target.

15 In certain embodiments, antisense compounds comprise one or more nucleosides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleosides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a bicyclic nucleoside having a (4'-CH(CH₃)-O-2') bridging group. In certain embodiments, the (4'-CH(CH₃)-O-2') modified nucleosides are arranged throughout the
20 wings of a gapmer motif.

Modified Nucleobases

Nucleobase (or base) modifications or substitutions are structurally distinguishable from, yet functionally interchangeable with, naturally occurring or synthetic unmodified nucleobases. Both natural and modified nucleobases are capable of participating in hydrogen bonding. Such nucleobase
25 modifications can impart nuclease stability, binding affinity or some other beneficial biological property to antisense compounds. Modified nucleobases include synthetic and natural nucleobases such as, for example, 5-methylcytosine (5-me-C). Certain nucleobase substitutions, including 5-methylcytosine substitutions, are particularly useful for increasing the binding affinity of an antisense compound for a target nucleic acid. For example, 5-methylcytosine substitutions have been shown to increase nucleic
30 acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278).

Additional modified nucleobases include 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 ($-C\equiv C-CH_3$) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 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, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Heterocyclic base moieties can also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Nucleobases that are particularly useful for increasing the binding affinity of antisense compounds include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2 aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

In certain embodiments, antisense compounds targeted to an androgen receptor nucleic acid comprise one or more modified nucleobases. In certain embodiments, shortened or gap-widened antisense oligonucleotides targeted to an androgen receptor nucleic acid comprise one or more modified nucleobases. In certain embodiments, the modified nucleobase is 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

Conjugated Antisense compounds

Antisense compounds may be covalently linked to one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the resulting antisense oligonucleotides. Typical conjugate groups include cholesterol moieties and lipid moieties. Additional conjugate groups include carbohydrates, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes.

Antisense compounds can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of antisense compounds to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect the antisense compound having terminal nucleic acid from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap), or at the 3'-terminus (3'-cap), or can be present on both termini. Cap structures are well known in the art and include, for example, inverted deoxy abasic caps. Further 3' and 5'-stabilizing groups that can

be used to cap one or both ends of an antisense compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

In certain embodiments, antisense compounds, including, but not limited to those particularly suited for use as ssRNA, are modified by attachment of one or more conjugate groups. In general, conjugate groups modify one or more properties of the attached oligonucleotide, including but not limited to pharmacodynamics, pharmacokinetics, stability, binding, absorption, cellular distribution, cellular uptake, charge and clearance. Conjugate groups are routinely used in the chemical arts and are linked directly or via an optional conjugate linking moiety or conjugate linking group to a parent compound such as an oligonucleotide. Conjugate groups includes without limitation, intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, thioethers, polyethers, cholesterol, thiocholesterol, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins and dyes. Certain conjugate groups have been described previously, for example: cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Lett., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., do-decan-diol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937).

For additional conjugates including those useful for ssRNA and their placement within antisense compounds, see e.g., PCT Publication No.; WO2013/033230.

Compositions and Methods for Formulating Pharmaceutical Compositions

Antisense oligonucleotides may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

An antisense compound targeted to an androgen receptor nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutically acceptable diluent is water, such as sterile water suitable for injection. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to an androgen receptor nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is water. In certain embodiments, the antisense compound is an antisense oligonucleotide provided herein.

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense compound.

In vitro testing of antisense oligonucleotides

Described herein are methods for treatment of cells with antisense oligonucleotides, which can be modified appropriately for treatment with other antisense compounds.

Cells may be treated with antisense oligonucleotides when the cells reach approximately 60-80% confluency in culture.

One reagent commonly used to introduce antisense oligonucleotides into cultured cells includes the cationic lipid transfection reagent LIPOFECTIN (Invitrogen, Carlsbad, CA). Antisense oligonucleotides may be mixed with LIPOFECTIN in OPTI-MEM 1 (Invitrogen, Carlsbad, CA) to achieve the desired final concentration of antisense oligonucleotide and a LIPOFECTIN concentration that may range from 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another reagent used to introduce antisense oligonucleotides into cultured cells includes LIPOFECTAMINE (Invitrogen, Carlsbad, CA). Antisense oligonucleotide is mixed with LIPOFECTAMINE in OPTI-MEM 1 reduced serum medium (Invitrogen, Carlsbad, CA) to achieve the

desired concentration of antisense oligonucleotide and a LIPOFECTAMINE concentration that may range from 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation.

5 Yet another technique used to introduce antisense oligonucleotides into cultured cells includes free uptake of the oligonucleotides by the cells.

Cells are treated with antisense oligonucleotides by routine methods. Cells may be harvested 16-24 hours after antisense oligonucleotide treatment, at which time RNA or protein levels of target nucleic acids are measured by methods known in the art and described herein. In general, when treatments are
10 performed in multiple replicates, the data are presented as the average of the replicate treatments.

The concentration of antisense oligonucleotide used varies from cell line to cell line. Methods to determine the optimal antisense oligonucleotide concentration for a particular cell line are well known in the art. Antisense oligonucleotides are typically used at concentrations ranging from 1 nM to 300 nM when transfected with LIPOFECTAMINE. Antisense oligonucleotides are used at higher concentrations
15 ranging from 625 to 20,000 nM when transfected using electroporation.

RNA Isolation

RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. RNA is prepared using methods well known in the art, for example, using the TRIZOL Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommended
20 protocols.

Embodiments:

E1. A compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 12-179.

25 E2. A compound comprising a modified oligonucleotide consisting of 16 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 12-179.

E3. A compound comprising a modified oligonucleotide consisting of the nucleobase sequence of any one of SEQ ID NOs: 12-179.

E 4. A compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175.

5 E 5. A compound comprising a modified oligonucleotide consisting of 16 to 30 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175.

E6. A compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence consisting of the nucleobase sequence of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175.

10 E7. A compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides complementary within nucleotides 2957-2972, 3079-3094, 3099-3114, 3109-3124, 3113-3128, 3120-3135, 3133-3148, 3224-3239, 3226-3241, 3351-3366, 3353-3368, 3361-3376, 3388-3403, 3513-3528, 3517-3532, 3519-3534, 3641-3656, 3735-3750, 3764-3779, 3768-3783, 3798-3813, 3799-3814, 3851-3866, 3870-3885, 3874-3889, 3876-3891, 3878-3893, 3884-3899, 3886-3901, 3888-3903, 15 3901-3916, 3956-3971, 3962-3977, 3964-3979, 3967-3982, 4019-4034, 4038-4053, 4049-4064, 4056-4071, 4059-4074, 4062-4077, 4066-4081, 4070-4085, 4101-4116, 4103-4118, 4105-4120, 4109-4124, 4305-4320, 4405-4420, 4532-4547, 4534-4549, 4537-4552, 4539-4554, 4555-4570, 4571-4586, 4573-4588, 4578-4593, 4597-4612, 4632-4647, 4655-4670, 4656-4671, 4662-4677, 4699-4714, 4747-4762, 4750-4765, 4752-4767, 4754-4769, 4755-4770, 4769-4784, 4798-4813, 4804-4819, 4807-4822, 4833-20 4848, 4837-4852, 4839-4854, 4865-4880, 4868-4883, 4872-4887, 4874-4889, 4876-4891, 4887-4902, 4889-4904, 4916-4931, 4918-4933, 4938-4953, 4942-4957, 4944-4959, 4951-4966, 5050-5065, 5052-5067, 5054-5069, 5056-5071, 5060-5075, 5061-5076, 5062-5077, 5133-5148, 5141-5156, 5155-5170, 5265-5280, 5293-5308, 5308-5323, 5392-5407, 5448-5463, 5469-5484, 5481-5496, 5483-5498, 5486-5501, 5488-5503, 5494-5509, 5521-5536, 5666-5681, 6222-6237, 6701-6716, 7543-7558, 8471-8486, 25 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58739, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58725-58740, 58750-58769, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-30 60917, 67454-67469, 114874-114889, 115272-115287, 115365-115380, 134971-134986, 102156-102171, 139682-139697, 139762-139777, 139782-139797, 144856-144871, 144938-144953, 148406-

148421, 148443-148458, 148520-148535, 181695-181710, 182958-182973, or 183049-183064 of SEQ ID NO: 1, wherein said modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

E8. A compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases

5 100% complementary to an equal length portion of nucleobases 2957-2972, 3079-3094, 3099-3114, 3109-3124, 3113-3128, 3120-3135, 3133-3148, 3224-3239, 3226-3241, 3351-3366, 3353-3368, 3361-3376, 3388-3403, 3513-3528, 3517-3532, 3519-3534, 3641-3656, 3735-3750, 3764-3779, 3768-3783, 3798-3813, 3799-3814, 3851-3866, 3870-3885, 3874-3889, 3876-3891, 3878-3893, 3884-3899, 3886-3901, 3888-3903, 3901-3916, 3956-3971, 3962-3977, 3964-3979, 3967-3982, 4019-4034, 4038-4053, 10 4049-4064, 4056-4071, 4059-4074, 4062-4077, 4066-4081, 4070-4085, 4101-4116, 4103-4118, 4105-4120, 4109-4124, 4305-4320, 4405-4420, 4532-4547, 4534-4549, 4537-4552, 4539-4554, 4555-4570, 4571-4586, 4573-4588, 4578-4593, 4597-4612, 4632-4647, 4655-4670, 4656-4671, 4662-4677, 4699-4714, 4747-4762, 4750-4765, 4752-4767, 4754-4769, 4755-4770, 4769-4784, 4798-4813, 4804-4819, 4807-4822, 4833-4848, 4837-4852, 4839-4854, 4865-4880, 4868-4883, 4872-4887, 4874-4889, 4876-15 4891, 4887-4902, 4889-4904, 4916-4931, 4918-4933, 4938-4953, 4942-4957, 4944-4959, 4951-4966, 5050-5065, 5052-5067, 5054-5069, 5056-5071, 5060-5075, 5061-5076, 5062-5077, 5133-5148, 5141-5156, 5155-5170, 5265-5280, 5293-5308, 5308-5323, 5392-5407, 5448-5463, 5469-5484, 5481-5496, 5483-5498, 5486-5501, 5488-5503, 5494-5509, 5521-5536, 5666-5681, 6222-6237, 6701-6716, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 20 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58739, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58725-58740, 58750-58769, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-60917, 67454-67469, 114874-114889, 115272-115287, 115365-115380, 134971-25 134986, 102156-102171, 139682-139697, 139762-139777, 139782-139797, 144856-144871, 144938-144953, 148406-148421, 148443-148458, 148520-148535, 181695-181710, 182958-182973, or 183049-183064 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is complementary to SEQ ID NO: 1.

E9. The compound of any one of E1, E7, or E8, wherein the compound comprises a modified 30 oligonucleotide consisting of 10 to 30 linked nucleosides complementary within exon 1 nucleotides 2957-2972, 3079-3094, 3099-3114, 3109-3124, 3113-3128, 3120-3135, 3133-3148, 3224-3239, 3226-3241, 3351-3366, 3353-3368, 3361-3376, 3388-3403, 3513-3528, 3517-3532, 3519-3534, 3641-3656, 3735-3750, 3764-3779, 3768-3783, 3798-3813, 3799-3814, 3851-3866, 3870-3885, 3874-3889, 3876-3891,

3878-3893, 3884-3899, 3886-3901, 3888-3903, 3901-3916, 3956-3971, 3962-3977, 3964-3979, 3967-3982, 4019-4034, 4038-4053, 4049-4064, 4056-4071, 4059-4074, 4062-4077, 4066-4081, 4070-4085, 4101-4116, 4103-4118, 4105-4120, 4109-4124, 4305-4320, 4405-4420, 4532-4547, 4534-4549, 4537-4552, 4539-4554, 4555-4570, 4571-4586, 4573-4588, 4578-4593, 4597-4612, 4632-4647, 4655-4670, 4656-4671, 4662-4677, 4699-4714, 4747-4762, 4750-4765, 4752-4767, 4754-4769, 4755-4770, 4769-4784, 4798-4813, 4804-4819, 4807-4822, 4833-4848, 4837-4852, 4839-4854, 4865-4880, 4868-4883, 4872-4887, 4874-4889, 4876-4891, 4887-4902, 4889-4904, 4916-4931, 4918-4933, 4938-4953, 4942-4957, 4944-4959, 4951-4966, 5050-5065, 5052-5067, 5054-5069, 5056-5071, 5060-5075, 5061-5076, 5062-5077, 5133-5148, 5141-5156, 5155-5170, 5265-5280, 5293-5308, 5308-5323, 5392-5407, 5448-5463, 5469-5484, 5481-5496, 5483-5498, 5486-5501, 5488-5503, 5494-5509, or 5521-5536 of SEQ ID NO:1.

E10. The compound of E9, wherein the compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides complementary within exon 1 nucleotides 5052-5067 of SEQ ID NO:1.

E11. The compound of any one of E1, E7, or E8, wherein the compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides complementary within intron 1 nucleotides 5666-5681, 6222-6237, 6701-6716, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58739, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58725-58740, 58750-58769, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-60917, 67454-67469, 114874-114889, 115272-115287, 115365-115380, or 134971-134986 of SEQ ID NO:1.

E12. The compound of E11, wherein the compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides complementary within intron 1 nucleotides 8638-8653, 11197-11212, 40615-40630, 58719-58734, 58720-58735, or 58721-58736 of SEQ ID NO:1.

E13. The compound of any one of E1-12, wherein the modified oligonucleotide comprises at least one modified sugar.

E14. The compound of E13, wherein at least one modified sugar comprises a 2'-O-methoxyethyl group.

E15. The compound of E13, wherein the at least one modified sugar is a bicyclic sugar.

E16. The compound of E15, wherein the bicyclic sugar comprises a 4'-CH(CH₃)-O-2' group.

E17. The compound of E15, wherein the bicyclic sugar comprises a 4'-CH₂-O-2' or 4'-(CH₂)₂-O-2' group.

E18. The compound of any one of E1-17, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

5 E19. The compound of E18, wherein each internucleoside linkage of the antisense oligonucleotide is a phosphorothioate internucleoside linkage.

E20. The compound of any one of E1-19, wherein the modified oligonucleotide comprises at least one modified nucleobase.

E21. The compound of E20, wherein the modified nucleobase is a 5-methylcytosine.

10 E22. The compound of any one of E1-21, wherein the modified oligonucleotide comprises:
a gap segment consisting of linked deoxynucleosides;
a 5' wing segment consisting of linked nucleosides; and
a 3' wing segment consisting of linked nucleosides;

15 wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

E23. The compound of E22, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;
a 5' wing segment consisting of 3 linked nucleosides; and
a 3' wing segment consisting of 3 linked nucleosides;

20 wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl sugar or a constrained ethyl sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

25 E24. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 35, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 9 linked deoxynucleosides;
a 5' wing segment consisting of three linked nucleosides; and
a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the three linked nucleosides of the 5' wing segment are each a constrained ethyl (cEt) sugar; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

E25. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 39, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 9 linked deoxynucleosides;

10 a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the three linked nucleosides of the 5' wing segment are each a constrained ethyl (cEt) sugar; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

E26. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 39, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

20 a gap segment consisting of 8 linked deoxynucleosides;

a 5' wing segment consisting of four linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

E27. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 39, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 8 linked deoxynucleosides;

5 a 5' wing segment consisting of five linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the five linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the three linked nucleosides of the 3' wing segment are each a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

E28. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 39, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 7 linked deoxynucleosides;

a 5' wing segment consisting of four linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the five linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

E29. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 35, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 7 linked deoxynucleosides;

30 a 5' wing segment consisting of six linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the six linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the three linked nucleosides of the 3' wing segment are each a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

E30. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 43, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

E31. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 124, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

E32. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 150, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

E33. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 155, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

10 a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

15 E34. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 169, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

20 a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

25 E35. A compound comprising a single-stranded modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 175, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; each nucleoside of each wing segment comprises a constrained ethyl (cEt) sugar; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

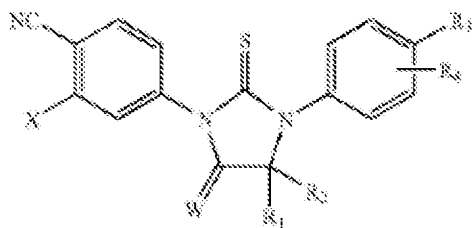
E36. The compound of any one of E1-35, wherein the modified oligonucleotide is at least 90% complementary to a nucleic acid encoding androgen receptor.

E37. The compound of any one of E1-36, wherein the antisense oligonucleotide is 100% complementary to a nucleic acid encoding androgen receptor.

E38. The compound of E37, wherein the nucleic acid encoding androgen receptor comprises the nucleotide sequence of any one of SEQ ID NOs: 1-8.

E39. A composition comprising the compound of any one of E1-38, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent or carrier.

E40. A composition comprising the compound of any one of E1-38 and a diarylhydantoin Androgen Receptor (AR) inhibitor of Formula XVI:



(XVI)

wherein X is selected from the group consisting of trifluoromethyl and iodo, wherein W is selected from the group consisting of O and NR5, wherein R5 is selected from the group consisting of H, methyl, and



wherein D is S or O and E is N or O and G is alkyl, aryl, substituted alkyl or substituted aryl; or D is S or
 5 O and E-G together are C1-C4 lower alkyl,

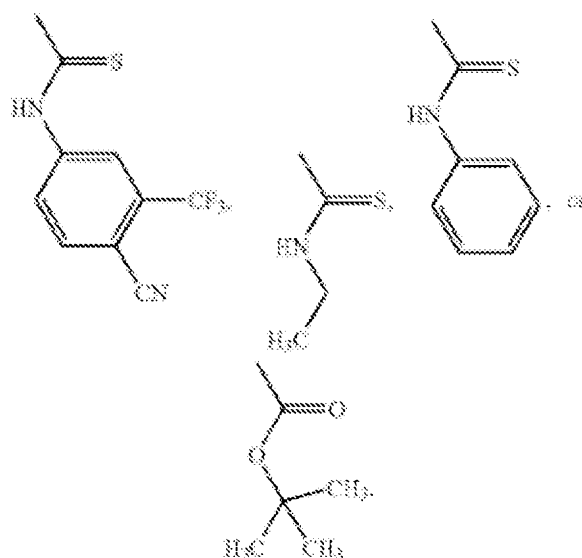
wherein R1 and R2 together comprise eight or fewer carbon atoms and are selected from the group
 consisting of alkyl, substituted alkyl including haloalkyl, and, together with the carbon to which they are
 linked, a cycloalkyl or substituted cycloalkyl group,

wherein R3 is selected from the group consisting of hydrogen, halogen, methyl, C1 -C4 alkoxy, formyl,
 10 haloacetoxymethyl, trifluoromethyl, cyano, nitro, hydroxyl, phenyl, amino, methylcarbamoyl, methoxycarbonyl,
 acetamido, methanesulfonamino, methanesulfonyl, 4-methanesulfonyl-1 -piperazinyl, piperazinyl, and
 C1-C6 alkyl or alkenyl optionally substituted with hydroxyl, methoxycarbonyl, cyano, amino, amido,
 nitro, carbamoyl, or substituted carbamoyl including methylcarbamoyl, dimethylcarbamoyl, and
 hydroxyethylcarbamoyl,

15 wherein R4 is selected from the group consisting of hydrogen, halogen, alkyl, and haloalkyl, and
 wherein R3 is not methylaminomethyl or dimethylaminomethyl.

R5 may be

20



E41. The composition of E40, wherein the diarylhydantoin Androgen Receptor (AR) inhibitor is MDV3100.

5 E42. A composition comprising the compound of any one of E1-38 and an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464.

E43. A method of treating cancer comprising administering to a subject having cancer the compound of any one of E1-38 or composition of any one of E39-42, thereby treating cancer in the subject.

10 E44. An antisense compound of any one of E1-38 or composition of any one of E39-42 for use in treating cancer

E45. The compound or composition of E44, wherein the cancer is prostate cancer, breast cancer, ovarian cancer, gastric cancer or bladder cancer.

15 E46. The compound or composition of E45, wherein the cancer is castrate-resistant prostate cancer.

E47. The compound or composition of E46, wherein the castrate-resistant prostate cancer is resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464.

20 E48. The method of E43, wherein the cancer is prostate cancer, breast cancer, ovarian cancer, gastric cancer or bladder cancer.

E49. The method of E48, wherein the cancer is castrate-resistant prostate cancer.

E50. The method of E49, wherein the castrate-resistant prostate cancer is resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464.

25 E51. The compound of E44-47 or the method of E49 or E50, wherein the antisense compound targets an AR splicing variant.

E52. The compound or method of E51, wherein the AR splicing variant lacks a functional ligand binding domain.

E53. The compound of E44-47 or the method of any one of E49-52, wherein the antisense compound is capable of reducing expression of full-length AR and an AR splicing variant lacking any one of exons 4-8.

E54. The compound or method of E51, wherein the AR splicing variant consists of exons 1-3.

E55. The compound of E44-47 or the method of any one of E49-52, wherein the antisense compound is targeted to AR upstream of the 3' end of exon 3 and is capable of inhibiting growth or proliferation of the prostate cancer cell to a greater extent than an antisense compound targeted to a region of AR downstream of the 3' end of exon 3.

E56. The compound or method of E55, wherein the antisense compound targeted to a region of AR downstream of the 3' end of exon 3 is capable of reducing levels of full-length AR but not an AR splicing variant consisting of exons 1-3.

E57. The compound or method of E56, wherein the region downstream of the 3' end of exon 3 comprises exon 4.

E58. The compound of E44-47 or the method of any one of E49-52, wherein the prostate cancer cell preferentially expresses an AR splicing variant over full-length AR.

E59. The compound or method of E58, wherein the AR splicing variant lacks a functional ligand binding domain.

E60. A method of treating prostate cancer resistant to a anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464 in a subject comprising administering to the subject an antisense compound targeted to human androgen receptor (AR) upstream of the 3' end of exon 3, thereby treating the prostate cancer.

E61. The method of E60, wherein the subject is diagnosed as having prostate cancer resistant to the anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464.

E62. The method of E60 or E61, wherein the antisense compound targets an AR splicing variant.

E63. The method of E62, wherein the AR splicing variant lacks a functional ligand binding domain.

E64. The method of any one of E60-63, wherein the antisense compound is capable of reducing expression of full-length AR and an AR splicing variant lacking any one of exons 4-8.

5 E65. The method of E64, wherein the AR splicing variant consists of exons 1-3.

E66. The method of any one of E60-65, wherein the antisense compound is targeted to AR upstream of the 3' end of exon 3 and is capable of inhibiting growth or proliferation of a prostate cancer cell resistant to the diarylhydantoin Androgen Receptor (AR) inhibitor to a greater extent than an antisense compound targeted to a region of AR downstream of the 3' end exon 3.

10 E67. The method of E66, wherein the antisense compound targeted to a region of AR downstream of the 3' end of exon 3 is capable of reducing levels of full-length AR but not an AR splicing variant lacking any one of exons 4-8.

E68. The method of E67, wherein the AR splicing variant consists of exons 1-3.

15 E69. The method of E68, wherein the region downstream of the 3' end of exon 3 comprises exon 4.

E70. The method of any one of E60-69, wherein the prostate cancer is castration-resistant.

E71. The method of any one of E60-70, wherein the prostate cancer comprises cells that preferentially express an AR splicing variant over full-length AR.

E72. The method of E71, wherein the AR splicing variant lacks any one of exons 4-8.

20 E73. The method of E72, wherein the AR splicing variant consists of exons 1-3.

E74. The method of E72, wherein the AR splicing variant lacks a functional ligand binding domain.

25 E75. A method of inhibiting prostate cancer cell growth or proliferation comprising contacting the prostate cancer cell with an antisense compound targeted to human androgen receptor (AR) and anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, wherein the antisense compound and the anti-androgenic agent synergize in combination to inhibit the growth or proliferation of the prostate cancer cell.

E76. The method of E75, wherein the antisense compound is targeted to AR upstream of the 3' end of exon 3.

E77. The method of E75 or E76, wherein the prostate cancer cell is contacted with an amount of the antisense compound and an amount of anti-androgenic agent that are each or both less in combination than the amount of either the antisense compound or anti-androgenic agent alone effective in inhibiting the growth or proliferation of said prostate cancer cell.

E78. The method of any one of E75-77, wherein the antisense compound and anti-androgenic agent provide a greater-than-additive effect compared to the antisense compound alone or anti-androgenic agent alone in inhibiting the growth or proliferation of said prostate cancer cell.

E79. The method of any one of E75-78, wherein the antisense compound targets an AR splicing variant.

E80. The method of E79, wherein the AR splicing variant lacks a functional ligand binding domain.

E81. The method of any one of E75-80, wherein the antisense compound is capable of reducing expression of full-length AR and an AR splicing variant consisting of exons 1-3.

E82. A method of inhibiting growth or proliferation of an androgen receptor (AR)-positive breast cancer cell comprising contacting the breast cancer cell with an antisense compound targeted to human androgen receptor (AR) wherein the growth or proliferation of the breast cancer cell is inhibited.

E83. A method of inhibiting AR expression in a subject having or at risk of having an androgen receptor (AR)-positive breast cancer comprising:

identifying a subject having or at risk of having AR-positive breast cancer, and

administering to the subject an antisense compound targeted to human AR,

wherein the antisense compound inhibits AR expression in the subject.

E84. A method of treating AR-positive breast cancer in a subject comprising administering to the subject an antisense compound targeted to human androgen receptor (AR), thereby treating the breast cancer in the subject.

E85. The method of any one of E82-84, wherein the AR-positive breast cancer or breast cancer cell is dependent on androgen expression for growth.

E86. The method of any one of E82-85, wherein the breast cancer or breast cancer cell is estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, or Her2/neu-negative.

E87. The method of any one of E82-85, wherein the breast cancer or breast cancer cell is ER-positive and AR-positive.

5 E88. The method of any one of E82-85, wherein the breast cancer or breast cancer cell is ER-negative and AR-positive.

E89. The method of any one of E82-88, wherein the breast cancer or breast cancer cell is an apocrine breast cancer or breast cancer cell.

10 E90. The method of any one of E60-88, wherein the antisense compound is the compound of any one of E1-38, or pharmaceutically acceptable salt thereof.

E91. The method of any one of E60-88, wherein the antisense compound is the compound of any one of E24-35, or pharmaceutically acceptable salt thereof.

EXAMPLES

Non-limiting disclosure and incorporation by reference

15 While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references recited in the present application is incorporated herein by reference in its entirety.

20 **Example 1: Antisense inhibition of human AR in HuVEC cells**

Antisense oligonucleotides were designed targeting an AR nucleic acid and were tested for their effects on AR mRNA *in vitro*. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation
25 with 500 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3559 (forward sequence TCCTTCACCAATGTCAACTCC, designated herein as SEQ ID NO: 9; reverse sequence GAGCCATCCAACTCTTGAGA, designated herein as SEQ ID NO: 10; probe sequence AGTACCGCATGCACAAGTCCCG, designated herein as SEQ ID NO: 11) was
30 used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as

measured by RIBOGREEN®. Results are presented as percent inhibition of AR, relative to untreated control cells. A total of 155 oligonucleotides were tested. Only those oligonucleotides which were selected for further study are shown in Tables 1 and 2.

The newly designed chimeric antisense oligonucleotides in Tables 1 and 2 were designed as 3-10-3 (S)-cET gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on both the 5' direction and on the 3' direction comprising three nucleosides. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an (S)-cEt modification. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in Tables 1 and 2 is targeted to either the human AR genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_011669.17 truncated from nucleotides 5079000 to 5270000) or the human AR mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_000044.3), or both. 'n/a' indicates that the oligonucleotide does not target that particular gene sequence.

Table 1

Target Start Site for SEQ ID NO: 1	Target Start Site for SEQ ID NO: 2	ISIS No	Sequence	% inhibition	SEQ ID NO
3799	937	549332	GCGCTCTGACAGCCTC	84	12
3851	989	549334	CACCTGCGGGAAGCTC	83	13
3888	1026	549338	GGCTGTGATGATGCGG	83	14
4047	1185	549345	TCTGGAACAGATTCTG	82	191
4059	1197	549347	CTTCGCGCACGCTCTG	84	15
4534	1672	549358	ATGGTGCTGGCCTCGC	91	16
4655	1793	549360	GGTCGAAGTGCCCCCT	89	17
4699	1837	549361	GACACCGACACTGCCT	84	18
4755	1893	549362	CCCGAAGCTGTTCCCC	85	19
4865	2003	549366	CTTGCCTGCGCTGTCG	84	20
5060	2198	549371	GTTGTAGTAGTCGCGA	93	21
5062	2200	549372	AAGTTGTAGTAGTCGC	92	22
5155	2293	549374	GCGCTGCCGTAGTCCA	93	23
5265	2403	549377	AGGATGAGGAAGCGGC	90	24
5392	2530	549379	GCTCCCGCCTCGCCGC	86	25
5448	2586	549380	CGCTTTCCTGGCCCGC	94	26
5483	2621	549381	GCCGCCAGGGTACCAC	89	27
n/a	2721	549383	CCAAACGCATGTCCCC	88	28

102155	2800	549386	GCTTCATCTCCACAGA	77	192
102156	2801	549387	AGCTTCATCTCCACAG	84	29
n/a	2871	549388	TCCCTTCAGCGGCTCT	88	30
144856	2801	549390	TTTCTGCTGGCGCACA	89	31

Table 2

Target Start Site for SEQ ID NO: 1	Target Start Site for SEQ ID NO: 2	ISIS No	Sequence	% inhibition	SEQ ID NO
181695	3602	549414	GTTCATTCGAAGTTCA	81	32
182958	4164	549432	GAGGATCATCACAGAT	90	33
183049	4255	549434	CTAAACTTCCCGTGGC	96	34
58721	n/a	549457	TTGATTTAATGGTTGC	98	35
58751					
58722	n/a	549458	GTTGATTTAATGGTTG	95	36
58752					
58725	n/a	549459	ATGGTTGATTTAATGG	96	37
58755					

Example 2: Dose-dependent antisense inhibition of human AR in HuVEC cells

5 Gapmers from the study described above exhibiting significant *in vitro* inhibition of AR mRNA were selected and tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 18.5 nM, 55.6 nM, 166.7 nM, 500.0 nM and 1500.0 nM concentrations of antisense oligonucleotide, as specified in Tables 3 and 4. After a treatment period of approximately 16 hours, RNA was isolated from the cells and AR mRNA levels were measured by

10 quantitative real-time PCR. Human AR primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of AR, relative to untreated control cells. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below.

15 The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Tables 3 and 4. As illustrated, AR mRNA levels were reduced in a dose-dependent manner in the antisense oligonucleotide treated cells.

Table 3

ISIS No	18.5 nM	55.6 nM	166.7 nM	500.0 nM	1500.0 nM	IC ₅₀ (nM)
549358	0	29	63	85	95	141
549360	2	44	58	79	83	116
549361	0	12	30	52	66	525
549362	0	10	23	57	74	447
549371	0	30	52	83	88	148
549372	0	22	51	85	89	150
549374	15	40	59	83	92	108
549377	0	13	52	72	93	216
549379	9	11	51	68	88	237
549380	14	50	87	94	98	62
549381	4	14	33	71	91	261
549383	2	10	34	75	88	270
549388	0	15	42	36	86	428
549390	12	0	35	55	91	369

Table 4

ISIS No	18.5 nM	55.6 nM	166.7 nM	500.0 nM	1500.0 nM	IC ₅₀ (nM)
549332	24	35	57	79	79	104
549334	9	29	46	63	72	253
549338	30	32	47	67	78	154
549347	5	15	37	62	71	357
549366	8	44	58	72	91	129
549387	2	9	41	68	92	261
549414	0	21	35	53	76	366
549432	10	15	46	80	92	179
549434	27	38	60	86	96	85
549457	50	70	95	99	99	18
549458	22	48	84	97	98	57
549459	51	61	90	94	97	18

Example 3: Antisense inhibition of human AR in HuVEC cells

5 Additional antisense oligonucleotides were designed targeting an AR nucleic acid and were tested for their effects on AR mRNA *in vitro*. Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation with 500 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3559 was used to measure mRNA levels. AR

mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of AR, relative to untreated control cells. A total of 82 oligonucleotides were tested. Only those oligonucleotides which were selected for further study are shown in Table 5.

The newly designed chimeric antisense oligonucleotides in Table 5 were designed as 3-10-3 (S)-cET gapmers or 5-10-5 MOE gapmers. The 3-10-3 (S)-cET gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on both the 5' direction and on the 3' direction comprising three nucleosides. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an (S)-cEt modification. The 5-10-5 MOE gapmer is 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in Table 5 is targeted to the human AR genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_011669.17 truncated from nucleotides 5079000 to 5270000)

Table 5

Target Start Site	Target Stop Site	ISIS No	ISIS No	Motif	% inhibition	SEQ ID NO
58721	58736	549457	TTGATTTAATGGTTGC	3-10-3	98	35
58751	58766					
58722	58737	549458	GTTGATTTAATGGTTG	3-10-3	94	36
58752	58767					
58725	58740	549459	ATGGTTGATTTAATGG	3-10-3	92	37
58755	58770					
58720	58739	560071	TGGTTGATTTAATGGTTGCA	5-10-5	73	38
58750	58769					
58720	58735	560098	TGATTTAATGGTTGCA	3-10-3	99	39
58750	58765					
58723	58738	560099	GGTTGATTTAATGGTT	3-10-3	95	40
58753	58768					
58724	58739	560100	TGGTTGATTTAATGGT	3-10-3	91	41
58754	58769					
58721	58736	560137	TTGATTTAATGGTTGC	3-10-3	95	35
58751	58766					

Example 4: Dose-dependent antisense inhibition of human AR in HuVEC cells

Gapmers from the studies described above exhibiting significant *in vitro* inhibition of AR mRNA were selected and tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 31.3 nM, 62.5 nM, 125.0 nM, 250.0 nM, 500.0 nM, and 1000.0 nM concentrations of antisense oligonucleotide, as specified in Table 6. After a treatment period of approximately 16 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human AR primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of AR, relative to untreated control cells. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 6. As illustrated, AR mRNA levels were reduced in a dose-dependent manner in the antisense oligonucleotide treated cells.

Table 6

ISIS No	31.25 nM	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	IC ₅₀ (μM)
549457	40	57	78	89	96	96	0.03
549458	15	25	47	70	88	93	0.1
549459	16	23	50	71	85	92	0.1
560071	7	0	19	40	57	76	0.4
560098	20	41	64	83	94	94	0.1
560099	13	29	58	72	89	94	0.1
560100	16	24	53	69	81	93	0.1
560137	27	49	61	82	91	96	0.1

Example 5: Antisense inhibition of human AR in HuVEC cells

Additional antisense oligonucleotides were designed targeting an AR nucleic acid and were tested for their effects on AR mRNA *in vitro*. Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation with 250 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results

are presented as percent inhibition of AR, relative to untreated control cells. A total of 40 oligonucleotides were tested. Only those oligonucleotides which were selected for further study are shown in Table 7.

The newly designed chimeric antisense oligonucleotides in Table 7 were designed as 3-10-3 (S)-cET gapmers or deoxy, MOE and (S)-cEt oligonucleotides. The 3-10-3 (S)-cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on both the 5' direction and on the 3' direction comprising three nucleosides. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an (S)-cEt modification. The deoxy, MOE and (S)-cEt oligonucleotides are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; the number indicates the number of deoxynucleosides; and 'e' indicates a MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. The SEQ ID NO listed in the table refers to the oligonucleotide sequence. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in Table 7 is targeted to the human AR genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_011669.17 truncated from nucleotides 5079000 to 5270000).

Table 7

Target Start Site	Target Stop Site	Sequence	ISIS No	Chemistry	% inhibition	SEQ ID NO
58721	58736	TTGATTTAATGGTTGC	549457	kkk-10-kkk	67	35
58751	58766					
58722	58737	GTTGATTTAATGGTTG	549458	kkk-10-kkk	71	36
58752	58767					
58720	58735	TGATTTAATGGTTGCA	560098	kkk-10-kkk	69	39
58750	58765					
58721	58736	TTGATTTAATGGTTGC	560131	kkk-9-kkke	74	35
58751	58766					
58721	58736	TTGATTTAATGGTTGC	560137	ekkk-8-kkke	66	35
58751	58766					
58720	58735	TGATTTAATGGTTGCA	569213	kkk-9-kkke	69	39
58750	58765					
58720	58735	TGATTTAATGGTTGCA	569216	ekkk-8-kkke	68	39
58750	58765					
58721	58736	TTGATTTAATGGTTGC	569222	eekkk-8-kkk	74	35
58751	58766					

58721	58736	TTGATTTAATGGTTGC	569228	eekkk-7-kkke	67	35
58751	58766					
58720	58735	TGATTTAATGGTTGCA	569236	ekkk-7-kkkee	66	39
58750	58765					

Example 6: Dose-dependent antisense inhibition of human AR in HuVEC cells

Gapmers from the studies described above exhibiting significant *in vitro* inhibition of AR mRNA were selected and tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 31.3 nM, 62.5 nM, 125.0 nM, 250.0 nM, 500.0 nM, and 1000.0 nM concentrations of antisense oligonucleotide, as specified in Table 8. After a treatment period of approximately 16 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human AR primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of AR, relative to untreated control cells. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 8. As illustrated, AR mRNA levels were reduced in a dose-dependent manner in the antisense oligonucleotide treated cells.

Table 8

ISIS No	31.25 nM	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	IC ₅₀ (μM)
549457	34	44	75	82	93	96	0.06
549458	30	36	54	70	85	90	0.10
560098	30	54	65	78	89	97	0.07
560131	16	48	65	82	89	97	0.09
560137	35	39	64	73	89	94	0.08
569213	35	53	65	83	94	96	0.06
569216	38	51	68	83	91	96	0.05
569222	36	48	67	83	91	98	0.06
569228	26	43	62	78	88	92	0.09
569236	17	39	54	79	84	92	0.11

Example 7: Dose-dependent antisense inhibition of human AR in HuVEC cells

Additional antisense oligonucleotides were designed as deoxy, MOE and (S)-cEt oligonucleotides targeting AR gene sequences and were tested at various doses in HuVEC cells. The oligonucleotides are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; the number indicates the number of deoxynucleosides; otherwise 'd' indicates deoxyribose; and 'e' indicates a MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. The SEQ ID NO listed in the table refers to the oligonucleotide sequence. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in Table 9 is targeted to the human AR genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_011669.17 truncated from nucleotides 5079000 to 5270000)

Table 9

Target Start Site	Target Stop Site	Sequence	ISIS No	Chemistry	SEQ ID NO
58720	58735	TGATTTAATGGTTGCA	569221	eekkk-8-kkk	39
58750	58765				
58720	58735	TGATTTAATGGTTGCA	569227	eekkk-7-kkke	39
58750	58765				
58720	58735	TGATTTAATGGTTGCA	569236	ekkk-7-kkkee	39
58750	58765				
58720	58735	TGATTTAATGGTTGCA	579666	ekkeekk-7-kk	39
58750	58765				
58721	58736	TTGATTTAATGGTTGC	579667	ekkeekk-7-kk	35
58751	58766				
58720	58735	TGATTTAATGGTTGCA	579670	ekkekk-7-kkk	39
58750	58765				
58721	58736	TTGATTTAATGGTTGC	579671	ekkekk-7-kkk	35
58751	58766				
58721	58736	TTGATTTAATGGTTGC	569228	eekkk-7-kkke	35
58751	58766				
58723	58738	GGTTGATTTAATGGTT	579669	ekkeekk-7-kk	40
58753	58768				
58722	58737	GTTGATTTAATGGTTG	579672	ekkekk-7-kkk	36
58752	58767				
58722	58737	GTTGATTTAATGGTTG	569217	ekkk-8-kkke	36

58752	58767				
58723	58738	GGTTGATTTAATGGTT	569214	kkk-9-kkke	40
58753	58768				
58723	58738	GGTTGATTTAATGGTT	560099	kkk-10-kkk	40
58753	58768				

Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 62.5 nM, 125.0 nM, 250.0 nM, 500.0 nM, and 1000.0 nM concentrations of antisense oligonucleotide, as specified in Tables 10-12. After a treatment period of approximately 16 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human AR primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of AR, relative to untreated control cells. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Tables 10-12. As illustrated, AR mRNA levels were reduced in a dose-dependent manner in some of the antisense oligonucleotide treated cells.

Table 10

ISIS No	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	IC ₅₀ (nM)
549458	25	46	55	64	78	203
569227	8	40	33	51	73	388
569228	29	44	63	77	87	158
569236	4	35	54	68	88	252
579666	33	34	47	64	80	229
579667	30	29	44	36	76	411

Table 11

ISIS No	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	IC ₅₀ (nM)
549458	16	22	44	64	74	324
579669	24	39	45	74	91	207
579670	27	28	55	75	70	236
579671	6	40	54	57	77	288
579672	9	30	50	72	86	258

Table 12

ISIS No	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	IC ₅₀ (nM)
549458	19	22	45	38	71	470
569214	20	26	61	62	76	265
569217	34	39	49	64	64	247
569221	12	32	59	57	73	294

Example 8: Antisense inhibition of human AR in HuVEC cells

5 Additional antisense oligonucleotides were designed targeting an AR nucleic acid and were tested for their effects on AR mRNA *in vitro*. Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation with 1,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of AR, relative to untreated control cells. A total of 75 oligonucleotides were tested. Only those oligonucleotides which were selected for further study are shown in Table 13.

The newly designed chimeric antisense oligonucleotides in Table 13 were designed as 3-10-3 (S)-cET gapmers, 3-9-4 (S)-cET gapmers, 4-8-4 (S)-cET gapmers, 4-9-3 (S)-cET gapmers, 5-7-4 (S)-cET gapmers, 5-8-3 (S)-cET gapmers, 6-7-3 (S)-cET gapmers, or deoxy, MOE and (S)-cET oligonucleotides. The 3-10-3 (S)-cET gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on both the 5' direction and on the 3' direction comprising three nucleosides. The 3-9-4 (S)-cET gapmers are 16 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked by a wing segment on the 5' direction comprising three nucleotides and on the 3' direction comprising four nucleosides. The 4-8-4 (S)-cET gapmers are 16 nucleosides in length, wherein the central gap segment comprises of eight 2'-deoxynucleosides and is flanked by wing segments on both the 5' direction and on the 3' direction comprising four nucleosides. The 4-9-3 (S)-cET gapmers are 16 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked by a wing segment on the 5' direction comprising four nucleotides and on the 3' direction comprising three nucleosides. The 5-7-4 (S)-cET gapmers are 16 nucleosides in length, wherein the central gap segment comprises of seven 2'-deoxynucleosides and is flanked by a wing segment on the 5' direction comprising five nucleotides and on the 3' direction comprising four nucleotides. The 5-8-3 (S)-cET gapmers are 16 nucleosides in length,

wherein the central gap segment comprises of eight 2'-deoxynucleosides and is flanked by a wing segment on the 5' direction comprising five nucleotides and on the 3' direction comprising three nucleosides. The 6-7-3 (S)-cEt gapmers are 16 nucleosides in length, wherein the central gap segment comprises of seven 2'-deoxynucleosides and is flanked by a wing segment on the 5' direction comprising six nucleotides and on the 3' direction comprising three nucleosides. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an (S)-cEt modification. The deoxy, MOE and (S)-cEt oligonucleotides are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; the number indicates the number of deoxynucleosides; otherwise 'd' indicates deoxyribose; and 'e' indicates a MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

The SEQ ID NO listed in the table refers to the oligonucleotide sequence. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in Table 13 is targeted to the human AR genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_011669.17 truncated from nucleotides 5079000 to 5270000).

Table 13

Target Start Site	Target Stop Site	Sequence	ISIS No	Chemistry	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	kkk-10-kkk	64	22
5061	5076	AGTTGTAGTAGTCGCG	585233	kkk-8-keeee	69	42
5062	5077	AAGTTGTAGTAGTCGC	585259	ekkk-9-kkk	71	22
5062	5077	AAGTTGTAGTAGTCGC	585262	kkk-9-kkke	77	22
5062	5077	AAGTTGTAGTAGTCGC	585263	kkk-8-kkkee	69	22
5062	5077	AAGTTGTAGTAGTCGC	585264	kkk-7-kkkeee	62	22
5062	5077	AAGTTGTAGTAGTCGC	585265	eekk-8-kkee	69	22
5062	5077	AAGTTGTAGTAGTCGC	585268	keke-8-ekkek	72	22
5062	5077	AAGTTGTAGTAGTCGC	585269	ekkek-8-ekkek	73	22
5062	5077	AAGTTGTAGTAGTCGC	585271	ekk-10-kke	57	22

5062	5077	AAGTTGTAGTAGTCGC	585274	kkk-10-kke	65	22
58719	58734	GATTTAATGGTTGCAA	586124	kkk-10-kkk	82	43
58720	58735	TGATTTAATGGTTGCA	569227	eekkk-7-kkke	51	39
58750	58765					
58722	58737	GTTGATTTAATGGTTG	560132	kkk-9-kkke	58	36
58752	58767					
58722	58737	GTTGATTTAATGGTTG	569229	eekkk-7-kkke	57	36
58752	58767					
58722	58737	GTTGATTTAATGGTTG	569238	ekkk-7-kkkee	51	36
58752	58767					
58722	58737	GTTGATTTAATGGTTG	549458	kkk-10-kkk	87	36
58752	58767					
58722	58737	GTTGATTTAATGGTTG	569223	eekkk-8-kkk	59	36
58752	58767					
58724	58739	TGGTTGATTTAATGGT	569215	kkk-9-kkke	59	41
58754	58769					
58725	58740	ATGGTTGATTTAATGG	560133	kkk-9-kkke	53	37
58755	58770					
58725	58740	ATGGTTGATTTAATGG	569220	ekkk-8-kkke	58	37
58755	58770					
58721	58736	TTGATTTAATGGTTGC	586224	kkkkk-8-kkk	90	35
58751	58766					
58722	58737	GTTGATTTAATGGTTG	586225	kkkkk-8-kkk	88	36
58752	58767					
58720	58735	TGATTTAATGGTTGCA	586227	kkkkk-8-kkk	87	39
58750	58765					

Example 9: Dose-dependent antisense inhibition of human AR in HuVEC cells

Antisense oligonucleotides from the studies described above exhibiting significant *in vitro* inhibition of AR mRNA were selected and tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 31.25 nM, 62.5 nM, 125.0 nM, 250.0 nM, 500.0 nM, and 1000.0 nM concentrations of antisense oligonucleotide, as specified in Table 14. After a treatment period of approximately 16 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human AR primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of AR, relative to untreated control cells. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 14. As illustrated, AR mRNA levels were reduced in a dose-dependent manner in the antisense oligonucleotide treated cells.

Table 14

ISIS No	31.25 nM	62.5 nM	125.0 nM	250.0 nM	500.0 nM	1000.0 nM	IC ₅₀ nM
549372	2	17	31	51	61	80	271
549458	0	19	40	63	74	90	196
560132	8	19	21	53	65	85	252
560133	17	15	24	35	58	79	336
569215	12	2	26	55	71	90	234
569220	11	29	34	43	59	78	275
569223	21	20	30	59	73	87	191
569227	13	22	45	46	61	74	255
569229	16	14	36	47	74	84	220
569238	4	32	33	54	71	88	202

5

Example 10: Dose-dependent antisense inhibition of human AR in HuVEC cells

Gapmers from Example 8 exhibiting significant *in vitro* inhibition of AR mRNA were selected and tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 46.9 nM, 187.5 nM, 750.0 nM, and 3000.0 nM concentrations of antisense oligonucleotide, as specified in Table 15. After a treatment period of approximately 16 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human AR primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of AR, relative to untreated control cells.

10

15

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Table 15. As illustrated, AR mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 15

ISIS No	46.9 nM	187.5 nM	750.0 nM	3000.0 nM	IC ₅₀ (μ M)
549372	9	41	66	87	0.29
549458	15	50	85	96	0.19

586124	28	47	84	94	0.13
586224	39	75	93	98	0.05
586225	17	61	89	97	0.13
586227	20	60	88	96	0.13

Example 11: Antisense inhibition of human AR in HuVEC cells

Additional antisense oligonucleotides were designed targeting an AR nucleic acid and were tested for their effects on AR mRNA *in vitro*. Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation with 500 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of AR, relative to untreated control cells. A total of 616 oligonucleotides were tested. Only those oligonucleotides which were selected for further study are shown in Tables 16-23.

The newly designed chimeric antisense oligonucleotides in Tables 16-23 were designed as 3-10-3 (S)-cET gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on both the 5' direction and on the 3' direction comprising three nucleosides. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an (S)-cEt modification. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

The SEQ ID NO listed in the table refers to the oligonucleotide sequence. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in Tables 16-23 is targeted to either the human AR genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_011669.17 truncated from nucleotides 5079000 to 5270000) or the human AR mRNA sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NM_000044.3), or both. 'n/a' indicates that the oligonucleotide does not target that particular gene sequence.

Table 16

Target	Target	Sequence	ISIS No	%	SEQ ID

Start Site	Stop Site			inhibition	NO
5062	5077	AAGTTGTAGTAGTCGC	549372	47	22
58722	58737	GTTGATTTAATGGTTG	549458	60	36
58752	58767				
2957	2972	ACAGCACTGGAGCGGC	583542	45	44
3079	3094	AACTTCACCGAAGAGG	583556	43	45
3099	3114	AGTCTTTAGCAGCTTT	583559	52	46
3109	3124	GCTTCCTCCGAGTCTT	583564	45	47
3113	3128	CCTTGCTTCCTCCGAG	583566	47	48
3120	3135	GCACTTTCCTTGCTTC	583567	52	49
3133	3148	TCAGTCCTACCAGGCA	583571	43	50
3224	3239	GACTGAGGCAGCTGCG	583583	45	51
3226	3241	CCGACTGAGGCAGCTG	583584	44	52

Table 17

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	40	22
58722	58737	GTTGATTTAATGGTTG	549458	46	36
58752	58767				
3351	3366	GCTAGCTCGCCCGCTC	583608	51	53
3353	3368	CAGTAGCTCGCCCGC	583609	51	54
3361	3376	GCAATGTGCAGCTAGC	583613	51	55
3388	3403	GTCGCCTGGCTCCTAA	583620	41	56
3513	3528	CTGGCTCCGCACTCGG	583635	50	57
3517	3532	ATCTCTGGCTCCGCAC	583637	43	58
3519	3534	TGATCTCTGGCTCCGC	583638	51	59
3641	3656	AGTGTCCACTGAAGTA	583642	42	60
3735	3750	AGGCTCACAGTCTGTC	583649	46	61
3764	3779	GACACACGGTGGACAA	583660	44	62
3768	3783	AGAAGACACACGGTGG	583662	51	63
3798	3813	CGCTCTGACAGCCTCA	583667	42	64

Table 18

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	26	22
58722	58737	GTTGATTTAATGGTTG	549458	48	36
58752	58767				
3870	3885	GTCGCTGCAGCTAGCT	583685	47	65

3874	3889	GGTAGTCGCTGCAGCT	583687	41	66
3876	3891	GCGGTAGTCGCTGCAG	583688	38	67
3878	3893	ATGCGGTAGTCGCTGC	583689	39	68
3884	3899	GTGATGATGCGGTAGT	583692	41	69
3886	3901	CTGTGATGATGCGGTA	583693	36	70
3901	3916	GAAGAGTTCAACAGGC	583700	36	71
3956	3971	GCTTGGCTGAATCTTC	583709	39	72
3962	3977	CCTTGAGCTTGGCTGA	583712	37	73
3964	3979	ATCCTTGAGCTTGGCT	583713	36	74
3967	3982	TCCATCCTTGAGCTTG	583714	36	75
4019	4034	GTAGGTCTTGGACGGC	583719	36	76
4038	4053	GATTCTGGAAAGCTCC	583727	40	77
4049	4064	GCTCTGGAACAGATTC	583728	45	78
4056	4071	CGCGCACGCTCTGGAA	583731	34	79
4062	4077	TCACTTCGCGCACGCT	583734	46	80
4066	4081	TGGATCACTTCGCGCA	583736	47	81
4070	4085	GTTCTGGATCACTTCG	583738	36	82
4101	4116	CGCTCGCGGCCTCTGG	583745	40	83
4103	4118	TGCGCTCGCGGCCTCT	583746	32	84
4105	4120	GCTGCGCTCGCGGCCT	583747	35	85

Table 19

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	39	22
58722	58737	GTTGATTTAATGGTTG	549458	50	36
58752	58767				
4109	4124	AGGTGCTGCGCTCGCG	583749	36	86
4305	4320	GCTGTTCCATCCAG	583759	38	87
4405	4420	TGCTGCGGCAGCCCCT	583771	40	88
4532	4547	GGTGCTGGCCTCGCTC	583787	37	89
4537	4552	TGCATGGTGCTGGCCT	583789	39	90
4539	4554	GTTGCATGGTGCTGGC	583790	39	91
4555	4570	TGCTGTTGCTGAAGGA	583795	63	92
4571	4586	GGATACTGCTTCCTGC	583796	65	93
4573	4588	TCGGATACTGCTTCCT	583797	35	94
4578	4593	TGCCTTCGGATACTGC	583799	65	95
4597	4612	CTCGCTCTCCCGCTGC	583802	37	96
4632	4647	TGTCCTTGGAGGAAGT	583809	45	97
4656	4671	TGGTCGAAGTGCCCCC	583818	42	98
4662	4677	CAGAAATGGTCGAAGT	583821	42	99

Table 20

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	23	22
58722	58737	GTTGATTTAATGGTTG	549458	54	36
58752	58767				
4747	4762	TGTTCCCCTGGACTCA	583833	37	100
4750	4765	AGCTGTTCCCCTGGAC	583834	52	101
4752	4767	GAAGCTGTTCCCCTGG	583835	44	102
4754	4769	CCGAAGCTGTTCCCCT	583836	37	103
4769	4784	GTACATGCAATCCCCC	583843	35	104
4798	4813	ACAGCGGGTGGAACTC	583847	34	105
4804	4819	GGACGCACAGCGGGTG	583850	38	106
4807	4822	GTGGGACGCACAGCGG	583851	33	107
4833	4848	TGCATTGCGCCAATGG	583853	33	108
4837	4852	CCTTTGCATTGCGCCA	583855	44	109
4839	4854	AACCTTTGCATTGCGC	583856	45	110
4868	4883	GCTCTTGCTGCGCTG	583862	32	111
4872	4887	CAGTGCTCTTGCCTGC	583864	46	112
4874	4889	TTCAGTGCTCTTGCCT	583865	45	113
4876	4891	TCTTCAGTGCTCTTGC	583866	32	114
4887	4902	ACTCAGCAGTATCTTC	583868	34	115
4889	4904	ATACTCAGCAGTATCT	583871	47	116
4916	4931	TTTGGTGTAACCTCCC	583880	39	117
4918	4933	CCTTTGGTGTAACCTC	583881	47	118
4938	4953	CTAGGCTCTCGCCTTC	583890	32	119
4942	4957	CAGCCTAGGCTCTCGC	583892	35	120
4944	4959	AGCAGCCTAGGCTCTC	583893	34	121
4951	4966	CTGCCAGAGCAGCCTA	583896	37	122

Table 21

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	37	22
58722	58737	GTTGATTTAATGGTTG	549458	47	36
58752	58767				
5050	5065	TCGCGACTCTGGTACG	583917	37	123

5052	5067	AGTCGCGACTCTGGTA	583918	47	124
5054	5069	GTAGTCGCGACTCTGG	583919	55	125
5056	5071	TAGTAGTCGCGACTCT	583920	42	126
5061	5076	AGTTGTAGTAGTCGCG	583922	37	42
5133	5148	TCTCCAGCTTGATGCG	583932	39	127
5141	5156	CAGCGGGTTCTCCAGC	583933	38	128
5293	5308	CCTTCTTCGGCTGTGA	583969	44	129
5308	5323	GGTCCATACAACTGGC	583975	42	130

Table 22

Target Start Site on SEQ ID NO: 1	Target Start Site on SEQ ID NO: 2	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	2200	AAGTTGTAGTAGTCGC	549372	46	22
58722	n/a	GTTGATTTAATGGTTG	549458	39	36
58752	n/a				
5469	2607	ACACATCAGGTGCGGT	583990	30	131
5481	2619	CGCCAGGGTACCACAC	583996	33	132
5486	2624	CATGCCGCCAGGGTAC	583998	45	133
5488	2626	ACCATGCCGCCAGGGT	583999	29	134
5494	2632	CTGCTCACCATGCCGC	584002	30	135
5521	2659	ACACAAGTGGGACTGG	584006	33	136
n/a	2870	CCCTTCAGCGGCTCTT	584044	29	137

Table 23

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	25	22
58722	58737	GTTGATTTAATGGTTG	549458	51	36
58752	58767				
144938	144953	CAGAGTCATCCCTGCT	584069	36	138
148406	148421	CACCCTCAAGATTCTT	584100	36	139
148443	148458	AAGGTAGTCTTTAAGG	584106	30	140
148520	148535	GTTTTCAAATGCAGCC	584111	33	141
139682	139697	GCCATGAGACAGCTTT	584125	35	142
139762	139777	ATTCTTGACTGTCTGA	584128	38	143
139782	139797	GCATGCCAGCTGGCTC	584130	29	144

5666	5681	CGCGCAGGTAGGAGCC	584138	35	145
6222	6237	TCTAAACATGACGGTT	584139	37	146
6701	6716	ATGCAATTGCCTGCCA	584141	39	147

Example 12: Antisense inhibition of human AR in HuVEC cells

Additional antisense oligonucleotides were designed targeting an AR nucleic acid and were tested for their effects on AR mRNA *in vitro*. Cultured HuVEC cells at a density of 20,000 cells per well were transfected using electroporation with 1,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of AR, relative to untreated control cells. A total of 385 oligonucleotides were tested. Only those oligonucleotides which were selected for further study are shown in Tables 24-28.

The newly designed chimeric antisense oligonucleotides in Tables 24-28 were designed as 3-10-3 (S)-cET gapmers. The gapmers are 16 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on both the 5' direction and on the 3' direction comprising three nucleosides. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has an (S)-cEt modification. The internucleoside linkages throughout each gapmer are phosphorothioate linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

The SEQ ID NO listed in the table refers to the oligonucleotide sequence. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in Tables 24-28 is targeted to the human AR genomic sequence, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NT_011669.17 truncated from nucleotides 5079000 to 5270000)

Table 24

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	63	22
58722	58737	GTTGATTTAATGGTTG	549458	88	36
58752	58767				
7543	7558	ATGGGAGTAACTTTTG	584145	76	148
8471	8486	CATATTATTGTGCTGC	584148	85	149

125

8638	8653	GTCAATATCAAAGCAC	584149	85	150
9464	9479	GAGTTGTGATTCAGG	584152	88	151
10217	10232	TTGATGGAATGCTGAT	584157	69	152
10250	10265	GGTAACTTTCTCTGA	584158	69	153
10865	10880	TGGATTGTAAATTACG	584162	82	154
11197	11212	GAACATTATTAGGCTA	584163	81	155
11855	11870	TCAATCTAGATACCAT	584165	70	156
13189	13204	CACATCAGAAGGAGTA	584166	89	157
13321	13336	GAGTGTTAATGAAGAC	584167	78	158
13346	13361	CTGATTAGCTATGACC	584168	70	159
16555	16570	ATGAGTCCTCAGAATC	584179	74	160
16793	16808	GTAGATTCTAGCTTTG	584180	81	161
16968	16983	ACAGGCTCTGACTAGG	584183	76	162
17206	17221	TGTGTGACCCTTGAC	584184	78	163
18865	18880	AAGTATGAGCATGGTT	584192	73	164

Table 25

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	59	22
58722	58737	GTTGATTTAATGGTTG	549458	76	36
58752	58767				
29329	29344	GGATTCTCTACACACA	584233	62	165
32290	32305	CCATTTGTGCCAAACC	584242	62	166
33315	33330	AGGTTAGGGAGTAGGC	584245	70	167
39055	39070	TAGGGTTTGGTCAGAA	584263	56	168
40615	40630	CCTTATGGATGCTGCT	584269	57	169
42017	42032	GTTATCTTACTCTCCC	584274	70	170

Table 26

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	58	22
58722	58737	GTTGATTTAATGGTTG	549458	79	36
58752	58767				
56050	56065	GATTGTGTATAGCTGC	584312	65	171
60902	60917	GGTTATGGTTCTGTCT	584329	58	172
67454	67469	CTTCATTGCAGGTCTG	584361	61	173

Table 27

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	70	22
58722	58737	GTTGATTTAATGGTTG	549458	76	36
58752	58767				
114874	114889	TAGCCAACCTTTCTTTA	584465	58	174
115272	115287	CATTGTACTATGCCAG	584468	64	175
115365	115380	TTTGGTAACATTAGGC	584469	74	176
134971	134986	ATGGTTGTCCTGTACA	584495	58	177

Table 28

Target Start Site	Target Stop Site	Sequence	ISIS No	% inhibition	SEQ ID NO
5062	5077	AAGTTGTAGTAGTCGC	549372	54	22
58722	58737	GTTGATTTAATGGTTG	549458	65	36
58752	58767				
114874	114889	TAGCCAACCTTTCTTTA	584465	54	174
115365	115380	TTTGGTAACATTAGGC	584469	63	176
134971	134986	ATGGTTGTCCTGTACA	584495	53	177

5 Example 13: Dose-dependent antisense inhibition of human AR in HuVEC cells

Gapmers from the studies described above exhibiting significant *in vitro* inhibition of AR mRNA were selected and tested at various doses in HuVEC cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 46.9 nM, 187.5 nM, 750.0 nM, and 3000.0 nM concentrations of antisense oligonucleotide, as specified in Tables 29-37. After a treatment period of approximately 16 hours, RNA was isolated from the cells and AR mRNA levels were measured by quantitative real-time PCR. Human AR primer probe set RTS3559 was used to measure mRNA levels. AR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN[®]. Results are presented as percent inhibition of AR, relative to untreated control cells.

The half maximal inhibitory concentration (IC₅₀) of each oligonucleotide is also presented in Tables 29-37. As illustrated, AR mRNA levels were reduced in a dose-dependent manner in some of the antisense oligonucleotide treated cells.

Table 29

ISIS No	46.9 nM	187.5 nM	750.0 nM	3000.0 nM	IC ₅₀ (μM)
549372	7	41	70	91	0.32
549458	21	72	91	97	0.11
583542	9	28	47	66	0.90
583556	19	47	68	66	0.34
583559	30	49	63	80	0.22
583564	16	33	55	74	0.52
583566	0	28	50	74	0.73
583567	17	34	60	79	0.43
583571	18	36	53	59	0.80
583583	21	31	49	64	0.79
583584	24	44	52	73	0.41
583608	12	46	67	76	0.35
583609	16	48	63	73	0.36
583613	24	60	70	75	0.19
583635	35	56	69	78	0.13
583638	33	64	79	85	0.11
583649	28	50	68	84	0.20
583660	21	39	61	72	0.42
583662	27	59	75	75	0.15

Table 30

ISIS No	46.9 nM	187.5 nM	750.0 nM	3000.0 nM	IC ₅₀ (μM)
549372	13	29	69	90	0.37
549458	22	62	92	97	0.13
583620	0	17	44	54	1.85
583637	22	32	59	75	0.45
583642	18	35	67	74	0.46
583667	35	55	73	82	0.14
583685	32	53	73	81	0.16
583687	34	67	83	81	0.08
583688	3	26	50	60	1.05
583689	20	34	62	74	0.44
583692	8	47	61	71	0.44
583709	8	50	70	84	0.29
583712	15	47	72	78	0.29
583727	18	49	70	76	0.29
583728	9	48	67	70	0.40
583734	29	60	74	75	0.12
583736	21	38	60	63	0.51
583738	16	40	56	71	0.51

583745	19	51	68	77	0.27

Table 31

ISIS No	46.9 nM	187.5 nM	750.0 nM	3000.0 nM	IC ₅₀ (μ M)
549372	5	36	69	88	0.36
549458	24	59	92	98	0.13
583693	12	39	64	80	0.38
583700	14	34	57	71	0.55
583713	29	51	67	74	0.22
583714	22	34	59	79	0.40
583719	22	46	65	72	0.32
583731	18	24	47	58	1.31
583746	24	44	65	67	0.35
583747	13	38	50	69	0.64
583771	17	27	47	69	0.77
583789	30	49	71	85	0.19
583790	17	42	65	81	0.32
583795	37	61	83	90	0.09
583796	38	69	83	90	0.07
583799	29	60	76	85	0.14
583809	13	37	68	81	0.36
583818	9	46	71	84	0.31
583821	11	35	61	77	0.46

Table 32

ISIS No	46.9 nM	187.5 nM	750.0 nM	3000.0 nM	IC ₅₀ (μ M)
549372	15	39	70	86	0.30
549458	19	58	89	96	0.15
583749	34	40	75	87	0.17
583759	5	28	61	67	0.63
583787	15	31	66	74	0.43
583797	21	50	74	82	0.22
583802	17	25	47	60	1.07
583834	34	54	73	84	0.13
583835	20	55	74	88	0.19
583836	11	27	67	86	0.39
583850	9	21	54	78	0.60
583855	22	50	81	91	0.18
583856	31	55	74	89	0.14
583864	30	49	72	85	0.17

583864	0	47	62	85	0.37
583865	33	42	68	85	0.19
583871	28	30	68	87	0.28
583880	13	52	78	92	0.22
583881	28	50	85	91	0.15

Table 33

ISIS No	46.9 nM	187.5 nM	750.0 nM	3000.0 nM	IC ₅₀ (μ M)
549372	14	33	64	90	0.34
549458	21	61	90	96	0.13
583833	26	43	70	74	0.26
583843	22	40	67	85	0.30
583847	8	30	60	84	0.46
583851	8	24	54	76	0.61
583853	24	51	70	80	0.21
583862	15	37	64	79	0.41
583866	17	48	71	91	0.24
583868	19	31	59	81	0.41
583890	0	0	17	33	>30
583892	22	38	68	83	0.27
583893	15	35	62	79	0.42
583896	13	17	49	69	0.86
583918	16	47	68	86	0.30
583919	27	60	85	91	0.14
583920	11	16	50	72	0.76
583969	12	26	66	86	0.44
583975	19	49	55	88	0.36

Table 34

ISIS No	46.9 nM	187.5 nM	750.0 nM	3000.0 nM	IC ₅₀ (μ M)
549372	14	36	64	88	0.32
549458	14	53	84	95	0.18
583917	6	30	50	70	0.64
583922	16	43	76	92	0.23
583932	9	35	64	81	0.38
583933	22	25	56	81	0.41
583990	0	9	33	56	1.92
583996	26	12	50	70	0.71
583998	4	25	38	70	0.89
583999	13	12	30	64	1.53

130

584002	12	46	70	92	0.25
584006	21	26	59	88	0.35
584044	23	30	51	78	0.44
584069	18	40	63	82	0.30
584100	6	5	20	44	7.79
584125	12	12	47	76	0.72
584128	20	22	41	72	0.74
584139	13	33	56	85	0.4
584141	22	37	61	85	0.29

Table 35

ISIS No	46.9 nM	187.5 nM	750.0 nM	3000.0 nM	IC ₅₀ (μ M)
549372	0	28	64	88	0.42
549458	13	49	84	91	0.19
584106	3	13	12	32	>30
584111	22	30	59	84	0.33
584130	0	10	11	37	>30
584138	2	40	62	89	0.37
584145	6	32	63	88	0.36
584148	16	48	79	95	0.20
584149	11	37	68	89	0.31
584152	28	59	87	95	0.11
584162	24	45	80	92	0.18
584163	19	37	74	90	0.25
584166	34	52	84	92	0.10
584167	13	45	76	93	0.21
584179	1	25	62	87	0.44
584180	26	56	84	96	0.12
584183	3	41	64	87	0.31
584184	9	42	76	93	0.23
584192	1	34	73	95	0.30

Table 36

ISIS No	46.9 nM	187.5 nM	750.0 nM	3000.0 nM	IC ₅₀ (μ M)
549372	2	26	61	85	0.42
549458	1	51	83	96	0.23
584157	6	6	52	82	0.59
584158	14	37	70	89	0.26

584165	12	34	66	89	0.30
584168	5	32	70	91	0.32
584233	0	30	66	86	0.39
584242	12	38	66	93	0.27
584245	4	33	69	90	0.32
584263	9	24	67	90	0.34
584269	6	26	69	92	0.34
584274	17	36	74	93	0.23
584312	17	37	65	93	0.26
584329	0	17	67	86	0.46
584361	0	18	71	87	0.41
584465	0	0	32	51	2.5
584468	9	26	60	90	0.37
584469	13	46	73	89	0.22
584495	0	14	55	74	0.65

Table 37

ISIS No	46.9 nM	187.5 nM	750.0 nM	3000.0 nM	IC ₅₀ (μ M)
549372	9	41	66	87	0.29
549458	15	50	85	96	0.19
586124	28	47	84	94	0.13
586195	41	62	90	95	0.07
586197	27	47	77	94	0.14
586198	39	62	89	96	0.07
586199	25	56	89	97	0.13
586200	23	44	85	95	0.15
586205	34	67	89	95	0.07
586207	0	39	79	93	0.3
586208	32	70	88	93	0.08
586212	20	60	86	94	0.13
586221	39	72	94	98	0.04
586224	39	75	93	98	0.05
586225	17	61	89	97	0.13
586227	20	60	88	96	0.13
586232	24	45	82	91	0.17
586240	14	49	83	93	0.18
586570	16	44	81	91	0.21

Example 14: Selection of antisense oligonucleotides targeting human androgen receptor (AR) mRNA for assays with prostate cancer cell lines

Antisense oligonucleotides from those presented in the studies above, targeting different regions of the human AR genomic sequence, were selected for further studies in prostate cancer cell lines. AR-V7 and AR-V567es are major AR splice variants detected in cancer patients as described in Hornberg, E. et al., PLoS One 2011. Vol. 6.

The following ISIS oligonucleotides were selected for further studies: ISIS 549372, which targets the human AR genomic sequence at exon 1; ISIS 549434, which targets the human AR genomic sequence at the 3'-end of exon 8 beyond the stop codon of AR; ISIS 560131, which targets the human AR genomic sequence at intron 1; and ISIS 569236, which targets the human AR genomic sequence at intron 1. Another antisense oligonucleotide, ISIS 554221 (ACCAAGTTTCTTCAGC, designated herein as SEQ ID NO: 178), was designed as a 3-10-3 LNA gapmer with phosphorothioate backbone targeted to exon 4, (*i.e.* the ligand binding domain) of AR identical to an antisense oligonucleotide designated as SEQ ID NO: 58 of US 7,737,125 for use as a benchmark.

Example 15: Effect of antisense inhibition of human androgen receptor (AR) mRNA on androgen receptor protein levels in MDV3100-resistant C4-2B cells

C4-2B cells are androgen-independent human prostate adenocarcinoma cells commonly used in the field of oncology and have been established as clinically relevant cultured cells (Thalmann, G.N. *et al.*, Cancer Res. 1994. 54: 2577). MDV3100 or Enzalutamide is an experimental androgen receptor antagonist drug developed by Medivation for the treatment of castration-resistant prostate cancer. ISIS 549372, ISIS 554221, and ISIS 549434 were tested in MDV3100-resistant (MR) C4-2B cells.

The cells were cultured in the presence of 5 μ M concentration of MDV3100 over the course of 2 months to induce MDV3100 resistance. ISIS 549372, ISIS 549434, and ISIS 554221 at 1 μ M concentration of antisense oligonucleotide were each added to the culture media at 1 μ M concentration for free uptake by the cells. After a treatment period of 2 days, cells were harvested in RIPA buffer containing protease inhibitors. The presence of bands for full-length AR, as well as the variant form, AR-V7, was detected by western blot using AR antibody (N-20, Santa Cruz). Treatment of the cells with ISIS 549372 reduced full-length AR and AR-V7 more extensively than treatment with either ISIS 554221 or ISIS 549434.

Example 16: Effect of antisense inhibition of human androgen receptor (AR) mRNA on AR-target genes in MDV3100-resistant C4-2B cells

The effect of antisense inhibition of AR on AR target genes was analyzed. ISIS 549372, ISIS 549458, ISIS 554221, and ISIS 549434 were tested in C4-2B MR cells.

Cells were plated at a density of 40,000 cells per well in 96-well plates and cultured in RPMI1640 medium with 10% fetal bovine serum. The cells were cultured in the presence of 5 μ M concentration of MDV3100 over the course of 2 months to induce MDV3100 resistance. ISIS 549372, ISIS 549458, ISIS 549434, and ISIS 554221 were each added at 0.04 μ M, 0.20 μ M, 1.00 μ M, and 5.00 μ M concentrations of antisense oligonucleotide to culture media for free uptake by the cells. A control oligonucleotide, ISIS 347526 (sequence TCTTATGTTTCCGAACCGTT (SEQ ID NO: 179) 5-10-5 MOE gapmer) with no known target region in human gene sequences, was included as a negative control. After a treatment period of 24 hrs, total AR mRNA levels were measured by quantitative real-time PCR using primer probe set RTS3559. Human AR primer probe set hAR_LTS00943 (forward sequence GCCCCTGGATGGATAGCTACT, designated herein as SEQ ID NO: 180; reverse sequence CCACAGATCAGGCAGGTCTTC, designated herein as SEQ ID NO: 181; probe sequence ACTGCCAGGGACCATGTTTTGCCC, designated herein as SEQ ID NO: 182) was used to measure AR-V7 mRNA levels. AR mRNA levels were adjusted to human actin mRNA levels. Results are presented in Table 38 as percent inhibition of total AR, relative to untreated control cells. Treatment of the cells with ISIS 549372, ISIS 549458, and ISIS 549434 reduced total AR transcript levels in a dose dependent manner more extensively than treatment with ISIS 554221.

Western analysis of full-length AR, as well as the AR-V7 variant, was also conducted in a manner similar to the assay described above. The assay demonstrated that treatment with ISIS 549372 and ISIS 549458 reduced levels of full-length AR and AR-V7. Treatment with ISIS 549434 reduced levels of full-length AR but not that of AR-V7. Treatment with ISIS 554221 reduced levels of full-length AR less extensively compared to ISIS 549372, and did not reduce levels of AR-V7. The control oligonucleotide ISIS 347526 did not reduce protein levels, as expected.

The mRNA level of the AR target gene, KLK2 was measured using the primer probe set hKLK2_LTS00963 (forward sequence CTTGCGCCCCAGGAGTCT, designated herein as SEQ ID NO: 183; reverse sequence CTCAGAGTAAGCTCTAGCACACATGTC, designated herein as SEQ ID NO: 184; probe sequence AGTGTGTGAGCCTCCATCTCCTGTCCAA, designated herein as SEQ ID NO: 185). The mRNA level of the AR target gene, KLK3 was measured using the primer probe set RTS1072 (forward sequence GCCAAGGAGGGAGGGTCTT, designated herein as SEQ ID NO: 186; reverse sequence CCCCCATAGTGAATCAGCTT, designated herein as SEQ ID NO: 187; probe sequence ATGAAGTAAGGAGAGGGACTGGACCCCC, designated herein as SEQ ID NO: 188). As presented in

Tables 39 and 40, treatment with I ISIS 549372, ISIS 549458, and ISIS 549434 reduced target gene levels in a dose dependent manner more extensively than treatment with ISIS 554221.

Table 38

Percent inhibition of full-length AR mRNA in C4-2B MR cells

ISIS No	0.04 μ M	0.20 μ M	1.00 μ M	5.00 μ M
549372	35	47	88	91
549434	9	36	66	88
549458	41	78	94	97
554221	0	0	0	23
347526	28	35	31	17

5

Table 39

Percent inhibition of KLK3 mRNA in C4-2B MR cells

ISIS No	0.04 μ M	0.20 μ M	1.00 μ M	5.00 μ M
549372	17	35	68	80
549434	10	47	42	64
549458	0	42	81	92
554221	0	0	47	56
347526	5	38	42	16

Table 40

Percent inhibition of KLK2 mRNA in C4-2B MR cells

ISIS No	0.04 μ M	0.20 μ M	1.00 μ M	5.00 μ M
549372	14	16	57	87
549434	5	27	49	68
549458	35	47	87	93
554221	24	25	56	66
347526	28	29	23	22

10

Example 17: Effect of antisense inhibition of human androgen receptor (AR) mRNA on the proliferative ability of MDV3100-resistant C4-2B cells

The effect of antisense inhibition of AR on the proliferative ability of cancer cells was analyzed. ISIS 549372, ISIS 549458, ISIS 554221, and ISIS 549434 were tested in C4-2B MR cells.

15 ISIS 549372, ISIS 549434, ISIS 549458, and ISIS 554221 were each added to the culture media at 0.04 μ M, 0.20 μ M, 1.00 μ M, and 5.00 μ M concentration of antisense oligonucleotide. ISIS 347526 was included as a negative control. After a treatment period of 6 days, the proliferative capacity of the cancer cells was measured with using CellTiter 96® AQueous One Solution Cell Proliferation kit (Promega),

following the manufacturer's instructions. Results are presented in Table 41 as percent inhibition of proliferation, relative to non-treated cells. Treatment of the cells with ISIS 549372, ISIS 549434, and ISIS 549458 reduced proliferation of the cells in a dose dependent manner more extensively than treatment with ISIS 554221.

5

Table 41

Percent inhibition of C4-2B MR cell proliferation

ISIS No	0.04 μ M	0.20 μ M	1.00 μ M	5.00 μ M
549372	0	4	25	43
549434	0	0	21	22
549458	8	16	41	56
554221	11	12	0	24
347526	11	22	7	16

Example 18: Effect of antisense inhibition of human androgen receptor (AR) mRNA on MDV3100-resistant LMR20 cells

10 An MDV3100-resistant cell line, designated as LMR20, was created. The effect of antisense inhibition of AR on the proliferative ability and AR mRNA levels of LMR20 cells was analyzed. ISIS 560131, ISIS 549458, and ISIS 569236 were tested along with the LNA gapmer, ISIS 554221.

15 LNCaP cells were incubated with increasing concentrations of MDV3100 for approximately 6 months. A single clone was selected after extensive culturing in the presence of 20 μ M MDV3100. The clone, LMR20, maintained the ability to allow free uptake of antisense oligonucleotides without lipid-mediated transfection, while demonstrating an approximately ten-fold increase in IC₅₀ when treated with MDV3100, compared to parental LNCaP cells.

Study 1

20 LMR20 cells were plated at 1,500 cells per well in phenol red-free medium with charcoal-stripped fetal bovine serum (CSS), to remove any androgens from the medium (Life Technologies). ISIS 560131, ISIS 549458, ISIS 569236, and ISIS 554221 were individually added to the culture media at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M concentration. ISIS 549148, which has no known human target sequence, was included as a control. The synthetic androgen agonist, R1881, (Takeda, A.N. et al., Mol. Pharmacol. 2007. 71: 473-82) was added on day 1 at 1 nM dose to a set of cells also treated with each of

25 the antisense oligonucleotides. DHT was added on day 1 at a dose of 10 nM to another set of cells also treated with each of the antisense oligonucleotides. MDV3100 was added on day 1 at a dose of 10 nM to another set of cells untreated with antisense oligonucleotide, which served as a control. After a treatment

period of 5 days, the proliferative ability of the cancer cells was measured by the standard MTT assay. Results are presented in Table 42 as percent inhibition of proliferation, relative to non-treated cells.

As presented in Table 42, in the presence of androgen agonists R1881 or DHT, ISIS 560131, ISIS 549458, and ISIS 569236 significantly inhibited MDV3100-resistant prostate cancer cell proliferation in a dose dependent manner more extensively than ISIS 554221. Inhibition of proliferation by the antisense oligonucleotides was also either comparable or more potent than with treatment with MDV3100.

Table 42

Percent inhibition of LMR20 cell proliferation

Treatment	ASO (μ M)	ISIS 560131	ISIS 569236	ISIS 549458	ISIS 554221	MDV3100
CSS	0.04	0	0	0	0	0
	0.20	0	10	0	1	5
	1.0	9	0	0	2	0
	5.0	16	12	5	16	11
CSS+ R1881	0.04	0	0	0	1	0
	0.20	13	2	22	10	5
	1.0	55	34	59	19	31
	5.0	70	61	74	54	67
CSS+ DHT	0.04	0	0	0	0	0
	0.20	13	10	25	0	1
	1.0	57	32	60	10	13
	5.0	71	57	70	36	41

Study 2

LMR20 cells were plated at 1,500 cells per well in phenol red-free medium with CSS. ISIS 560131, ISIS 549458, ISIS 569236, and the LNA gapmer ISIS 554221 were individually added to the culture media at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M concentration. ISIS 549148, which has no known human target sequence, was included as a control. MDV3100 was added on day 1 at a dose of 10 nM to a set of cells, and served as a control. DHT was added on day 1 at a dose of 10 nM for 72 hrs to one set of cells also treated with each of the antisense oligonucleotides or MDV3100. R1881 was added on day 1 at a dose of 10 nM for 72 hrs to another set of cells also treated with each of the antisense oligonucleotides or MDV3100. mRNA levels of AR, prostate-specific antigen (PSA) and TMPRSS2, an androgen-regulated gene (Lin, B., et al., Cancer Res. 1999. 59: 4180), were measured. Results are presented in

Tables 43-45 as mRNA levels expressed as a percentage of the baseline values. mRNA levels may be lowered or increased after treatment.

As presented in Tables 43-45, ISIS 560131, ISIS 549458, and ISIS 569236 reduced AR mRNA levels in LMR20 cells, treated with or without either AR agonist, in a dose dependent manner relative to the baseline. Treatment with the LNA gapmer ISIS 554221 did not alter AR mRNA levels. ISIS 560131, ISIS 549458, and ISIS 569236 reduced PSA levels and TMPRSS2 more extensively than the LNA gapmer ISIS 554221 or MDV3100. Treatment with MDV3100 increased the levels of AR mRNA in cells treated with AR agonist, and did not reduce either PSA or TMPRSS2 mRNA levels.

Table 43

mRNA levels (% baseline value) of cells without AR agonist treatment

Gene	ASO (μ M)	560131	569236	549458	554221	MDV3100
AR	0.04	107	104	101	124	106
	0.20	74	87	75	140	101
	1.0	29	42	30	132	99
	5.0	17	27	25	98	92
PSA	0.04	113	122	135	106	98
	0.20	83	90	85	118	93
	1.0	75	78	50	58	90
	5.0	71	73	72	87	113
TMPRSS2	0.04	92	96	110	95	101
	0.20	67	81	85	117	119
	1.0	52	59	54	77	119
	5.0	45	48	62	73	141

Table 44

mRNA levels (% baseline value) after treatment with DHT

Gene	ASO (μ M)	560131	569236	549458	554221	MDV3100
AR	0.04	89	94	91	137	105
	0.20	55	77	66	135	124
	1.0	25	44	34	136	110
	5.0	20	34	31	100	143
PSA	0.04	74	108	93	97	124

138

	0.20	61	79	71	86	108
	1.0	35	46	47	64	95
	5.0	35	46	47	64	95
TMPRSS2	0.04	112	113	127	121	134
	0.20	108	123	119	118	144
	1.0	93	111	106	122	132
	5.0	71	110	91	114	124

Table 45

mRNA levels (% baseline value) after treatment with R1881

Gene	ASO (μ M)	560131	569236	549458	554221	MDV3100
AR	0.04	87	89	88	131	94
	0.20	65	80	56	133	107
	1.0	30	44	25	124	115
	5.0	26	37	32	99	136
PSA	0.04	92	90	93	100	84
	0.20	77	90	67	93	101
	1.0	44	57	50	80	92
	5.0	35	41	44	57	87
TMPRSS2	0.04	132	126	137	136	114
	0.20	117	131	119	134	125
	1.0	88	98	96	125	133
	5.0	76	95	96	122	139

Example 19: Effect of antisense inhibition of human androgen receptor (AR) mRNA in combination with MDV3100 on the proliferative ability of C4-2B cells

The effect of antisense inhibition of AR in combination with different doses of MDV3100 on the proliferative ability of cancer cells was analyzed. ISIS 549372, ISIS 549434, ISIS 549458, and ISIS 554221 were tested in C4-2B cells.

C4-2B cells were plated at 1,500 cells per well. ISIS 549372, ISIS 549434, ISIS 549458, or ISIS 554221 were individually added to the culture media at 0.1 μ M concentration. ISIS 347526 was included as a negative control. MDV3100 was also added on day 1 at doses of 0.25 μ M or 1.00 μ M. After a treatment period of 6 days, the proliferative capacity of the cancer cells was measured with CellTiter 96®

Aqueous One Solution Cell Proliferation kit (Promega), following the manufacturer's instructions. Results are presented in Table 46 as percent inhibition of proliferation, relative to non-treated cells. Treatment of the cells with ISIS 549372 or ISIS 549458 reduced proliferation of the cells more extensively than treatment with ISIS 554221. For instance, as presented in Table 46, treatment with ISIS 549372 alone reduced cell proliferation by 59% and treatment with ISIS 549458 reduced cell proliferation by 74% compared to ISIS 554221 alone, which reduced cell proliferation by 23%.

As presented in Tables 46 and 47, ISIS 549372 or ISIS 549458 in combination with MDV3100 inhibited prostate cancer cell proliferation to a greater extent than an equal molar concentration of ISIS 554221 in combination of MDV3100.

To find out whether treatment of the cells with ISIS 549372 or ISIS 549458 was synergistic with MDV3100, the assay was repeated at 0.1 μ M ASO. As presented in Table 46, treatment with ISIS 549372 or ISIS 549458 was synergistic with MDV3100. For instance, MDV3100 alone at 0.25 μ M inhibited proliferation by 4%; ISIS 549372 alone at 0.1 μ M inhibited cell proliferation by 23%; in combination, cell proliferation was inhibited by 66%. Similarly, ISIS 549458 alone at 0.1 μ M inhibited cell proliferation by 39%; in combination, cell proliferation was inhibited by 75%. Hence, the combination of ISIS 549372 or ISIS 549458 and MDV3100 was synergistic (*i.e.* greater than additive) in terms of inhibition of prostate cancer cell proliferation.

Table 46

Percent inhibition of C4-2B cell proliferation with 0.1 μ M ASO

	MDV3100		
	0 μ M	0.25 μ M	1 μ M
PBS	0	9	38
ISIS 549372	23	44	66
ISIS 549458	39	59	75
ISIS 554221	9	29	59
ISIS 141923	0	4	38

Table 47

Percent inhibition of C4-2B cell proliferation with 0.2 μ M ASO

	MDV3100		
	0 μ M	0.25 μ M	1 μ M
PBS	0	20	46

140

ISIS 549372	59	69	77
ISIS 549458	74	75	79
ISIS 554221	23	45	67
ISIS 141923	0	5	50

Example 20: Effect of antisense inhibition of human androgen receptor (AR) mRNA in combination with MDV3100 on the proliferative ability of LNCaP cells

The effect of antisense inhibition of AR in combination with different doses of MDV3100 on the proliferative ability of cancer cells was analyzed. ISIS 560131 and ISIS 569236 were tested in LNCaP cells.

LNCaP cells were plated at 1,000 cells per well. ISIS 560131 or ISIS 569236 was individually added to the culture media at 0.08 μ M, 0.04 μ M, 0.2 μ M, or 1.0 μ M concentration. ISIS 549148 was included as a negative control. MDV3100 was added to the ISIS oligonucleotide-treated cells on day 2 at doses of 0.016 μ M, 0.08 μ M, 0.4 μ M, or 2.0 μ M. After a treatment period of 5 days, the proliferative capacity of the cancer cells was measured with CellTiter 96® AQueous One or CellTiter-Glo® Solution Cell Proliferation kit (Promega), following the manufacturer's instructions. Results are presented in Tables 48-52 as percent inhibition of proliferation, relative to non-treated cells.

As presented in the Tables, treatment with ISIS 560131 or ISIS 569236 was synergistic with MDV3100. For instance, MDV3100 with control oligonucleotide, ISIS 549148, at 0.08 μ M inhibited proliferation by an average of 7%; ISIS 560131 alone at 0.04 μ M inhibited cell proliferation by 24%; in combination, cell proliferation was inhibited by 41%. Similarly, ISIS 569236 alone at 0.04 μ M inhibited cell proliferation by 9%; in combination, cell proliferation was inhibited by 26%. Hence, the combination of ISIS 560131 or ISIS 569236 and MDV3100 was synergistic (*i.e.* greater than additive) in terms of inhibition of prostate cancer cell proliferation.

Table 48

Proliferation (% untreated control) in LNCaP without MDV-3100

	ASO Dose			
	0.08 μ M	0.04 μ M	0.2 μ M	1.0 μ M
ISIS 560131	106	76	50	26
ISIS 569236	106	91	60	35
ISIS 549148	104	101	91	82

Table 49

141

Proliferation (% untreated control) in LNCaP with 0.016 μ M MDV-3100

	ASO Dose			
	0.08 μ M	0.04 μ M	0.2 μ M	1.0 μ M
ISIS 560131	103	71	49	25
ISIS 569236	104	92	58	29
ISIS 549148	106	86	83	59

Table 50Proliferation (% untreated control) in LNCaP with 0.08 μ M MDV-3100

	ASO Dose			
	0.08 μ M	0.04 μ M	0.2 μ M	1.0 μ M
ISIS 560131	99	59	48	27
ISIS 569236	98	74	51	31
ISIS 549148	93	101	89	90

Table 51

5

Proliferation (% untreated control) in LNCaP with 0.4 μ M MDV-3100

	ASO Dose			
	0.08 μ M	0.04 μ M	0.2 μ M	1.0 μ M
ISIS 560131	68	50	40	26
ISIS 569236	61	48	41	27
ISIS 549148	65	57	50	48

Table 52Proliferation (% untreated control) in LNCaP with 2.0 μ M MDV-3100

	ASO Dose			
	0.08 μ M	0.04 μ M	0.2 μ M	1.0 μ M
ISIS 560131	45	42	38	23
ISIS 569236	44	41	35	23
ISIS 549148	39	42	41	32

Example 21: Effect of antisense inhibition of human androgen receptor (AR) mRNA in combination with MDV3100 on the proliferative ability of C4-2B cells

10 The effect of antisense inhibition of AR in combination with different doses of MDV3100 on the proliferative ability of cancer cells was analyzed. ISIS 560131 and ISIS 569236 were tested in C4-2B cells.

C4-2B cells were plated at 1,000 cells per well. ISIS 560131 or ISIS 569236 was individually added to the culture media at 0.08 μ M, 0.04 μ M, 0.2 μ M, or 1.0 μ M concentration. ISIS 549148 was included as a negative control. MDV3100 was added to the ISIS oligonucleotide-treated cells on day 2 at doses of 0.016 μ M, 0.08 μ M, 0.4 μ M, or 2.0 μ M. After a treatment period of 5 days, the proliferative capacity of the cancer cells was measured with CellTiter 96® AQueous One Solution Cell Proliferation kit (Promega), following the manufacturer's instructions. Results are presented in Tables 53-57 as percent inhibition of proliferation, relative to non-treated cells.

As presented in the Tables, treatment with ISIS 560131 or ISIS 569236 was synergistic with MDV3100. For instance, MDV3100 with control oligonucleotide, ISIS 549148, at 0.4 μ M inhibited proliferation by an average of 6%; ISIS 560131 alone at 0.08 μ M inhibited cell proliferation by 16%; in combination, cell proliferation was inhibited by 31%. Similarly, MDV3100 with control oligonucleotide, ISIS 549148, at 0.08 μ M did not inhibit proliferation (0%); ISIS 569236 alone at 0.2 μ M inhibited cell proliferation by 37%; in combination, cell proliferation was inhibited by 52%. Hence, the combination of ISIS 560131 or ISIS 569236 and MDV3100 was synergistic (*i.e.* greater than additive) in terms of inhibition of prostate cancer cell proliferation.

Table 53

Proliferation (% untreated control) in C4-2B without MDV-3100

	ASO Dose			
	0.08 μ M	0.04 μ M	0.2 μ M	1.0 μ M
ISIS 560131	84	59	47	41
ISIS 569236	100	72	63	51
ISIS 549148	111	117	118	126

Table 54Proliferation (% untreated control) in C4-2B with 0.016 μ M MDV-3100

	ASO Dose			
	0.08 μ M	0.04 μ M	0.2 μ M	1.0 μ M
ISIS 560131	104	71	53	39
ISIS 569236	107	74	65	55
ISIS 549148	110	107	124	103

Table 55Proliferation (% untreated control) in C4-2B with 0.08 μ M MDV-3100

	ASO Dose	

143

	0.08 μ M	0.04 μ M	0.2 μ M	1.0 μ M
ISIS 560131	66	73	56	42
ISIS 569236	89	79	51	43
ISIS 549148	84	125	123	114

Table 56Proliferation (% untreated control) in C4-2B with 0.4 μ M MDV-3100

	ASO Dose			
	0.08 μ M	0.04 μ M	0.2 μ M	1.0 μ M
ISIS 560131	69	69	48	48
ISIS 569236	90	63	48	39
ISIS 549148	89	110	88	88

Table 57Proliferation (% untreated control) in C4-2B with 2.0 μ M MDV-3100

	ASO Dose			
	0.08 μ M	0.04 μ M	0.2 μ M	1.0 μ M
ISIS 560131	37	42	49	43
ISIS 569236	44	45	48	46
ISIS 549148	47	40	52	59

5

Example 22: Effect of antisense inhibition of human androgen receptor (AR) mRNA in combination with MDV3100 on the proliferative ability of 22RV1 cells

The effect of antisense inhibition of AR in combination with different doses of MDV3100 on the proliferative ability of cancer cells was analyzed. ISIS 560131 and ISIS 569236 were tested in 22RV1 cells.

10

22RV1 cells were plated at 2,000 cells per well in 5% CSS medium for 48 hours. Cells were transfected using RNAiMAX reagent with ISIS 560131 or ISIS 569236 at 0.4 nM, 1.34 nM, 4 nM, or 13.4 nM concentrations. ISIS 549148 was included as a negative control. DHT at 1 nM and/or MDV3100 at doses of 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M were added after 4 hours. After a treatment period of 3 days, the proliferative capacity of the cancer cells was measured with CellTiter 96® AQueous One Solution Cell Proliferation kit (Promega), following the manufacturer's instructions. Results are presented in Tables 58-62 as percent inhibition of proliferation, relative to non-treated cells.

15

As presented in the Tables, treatment with ISIS 560131 or ISIS 569236 was synergistic with MDV3100. For instance, MDV3100 with control oligonucleotide, ISIS 549148, at 1.0 μ M inhibited proliferation by an average of 5%; ISIS 560131 alone at 1.34 nM inhibited cell proliferation by 3%; in combination, cell proliferation was inhibited by 23%. Similarly, MDV3100 with control oligonucleotide, ISIS 549148, at 1.0 μ M inhibited proliferation by 5%; ISIS 569236 alone at 1.0 μ M inhibited cell proliferation by 17%; in combination, cell proliferation was inhibited by 30%. Hence, the combination of ISIS 560131 or ISIS 569236 and MDV3100 was synergistic (*i.e.* greater than additive) in terms of inhibition of prostate cancer cell proliferation.

Table 58

Proliferation (% untreated control) in 22RV1 without MDV-3100

	ASO Dose			
	0.4 nM	1.34 nM	4.0 nM	13.4 nM
ISIS 560131	103	97	77	57
ISIS 569236	97	83	69	37
ISIS 549148	109	109	109	99

Table 59Proliferation (% untreated control) in 22RV1 cells with 0.04 μ M MDV-3100

	ASO Dose			
	0.4 nM	1.34 nM	4.0 nM	13.4 nM
ISIS 560131	96	80	65	39
ISIS 569236	83	70	61	24
ISIS 549148	106	106	100	85

Table 60Proliferation (% untreated control) in 22RV1 cells with 0.2 μ M MDV-3100

	ASO Dose			
	0.4 nM	1.34 nM	4.0 nM	13.4 nM
ISIS 560131	95	90	76	51
ISIS 569236	93	77	60	20
ISIS 549148	101	115	110	96

Table 61Proliferation (% untreated control) in 22RV1 cells with 1.0 μ M MDV-3100

	ASO Dose			
	0.4 nM	1.34 nM	4.0 nM	13.4 nM

145

ISIS 560131	96	77	63	40
ISIS 569236	79	70	52	18
ISIS 549148	106	95	98	82

Table 62Proliferation (% untreated control) in 22RV1 cells with 5.0 μ M MDV-3100

	ASO Dose			
	0.4 nM	1.34 nM	4.0 nM	13.4 nM
ISIS 560131	91	76	63	41
ISIS 569236	82	72	52	24
ISIS 549148	96	102	98	85

Example 23: Effect of antisense inhibition of human androgen receptor (AR) mRNA on CWR22-RV1 cells

The effect of antisense inhibition of AR on the proliferative ability of cancer cells was analyzed. ISIS 549372, ISIS 549434, ISIS 549458, and ISIS 554221 were tested in CWR22-RV1 cells.

CWR22-RV1 cells were plated and transfected using RNAiMax reagent (Life Technologies) with ISIS oligonucleotides at 1.7 nM, 5.0 nM, 16.7 nM, or 50 nM concentrations. ISIS 347526 was included as a negative control. After a treatment period of 6 days, the target reduction and proliferative capacity of the cancer cells was measured.

Antisense inhibition of AR full-length mRNA was measured with the RTS3559 primer probe set. The results are presented in Table 63 as percent inhibition relative to non-treated cells. The reduction in V7 splice variant of the AR mRNA was also measured by RT-PCR using SYBR Green staining (Hu, R. et al., Cancer Res. 2009. 69: 16-22). The results are presented in Table 64, as percent reduction, relative to non-treated cells. Cell proliferation was measured with CellTiter 96® AQueous One Solution Cell Proliferation kit (Promega), following the manufacturer's instructions. Results are presented in Table 65 as percent inhibition of proliferation, relative to non-treated cells.

Table 63

Percent inhibition of AR full-length mRNA

Dose (nM)	ISIS 549372	ISIS 549434	ISIS 549458	ISIS 554221	ISIS 347526
1.7	24	27	28	24	0
5.0	53	46	41	41	3
16.7	64	69	61	67	4
50.0	78	86	78	72	0

Table 64

Percent inhibition of AR splice variant, V7

Dose (nM)	ISIS 549372	ISIS 549434	ISIS 549458	ISIS 554221	ISIS 347526
1.7	23	0	18	25	17
5.0	35	20	34	1	0
16.7	56	4	58	7	0
50.0	82	23	82	35	10

5

Table 65

Percent inhibition of cell proliferation

Dose (nM)	ISIS 549372	ISIS 549434	ISIS 549458	ISIS 554221	ISIS 347526
1.7	0	8	0	17	0
5.0	0	15	0	11	0
16.7	25	13	17	27	0
50.0	53	38	40	47	0

Example 24: Effect of antisense inhibition of human androgen receptor (AR) mRNA by free uptake of antisense oligonucleotide by C4-2B cells

10 The effect of free uptake of antisense oligonucleotides on AR mRNA levels was investigated. ISIS 549372, ISIS 549434, ISIS 549458, and ISIS 554221 were tested.

Cells were plated at a concentration of 1,000 cells/well in 96-well plates to measure cell proliferation, and at 4,000 cells/well to measure target reduction. ISIS 549458, ISIS 549372, ISIS 549434, and ISIS 554221 were added individually at 0.04 μ M, 0.20 μ M, 1.00 μ M, or 5.00 μ M. After an
 15 incubation period of 24 hrs, mRNA levels were measured using hAR_LTS00943. The data is presented in Table 66. The results indicate that ISIS 549458, ISIS 549372, and ISIS 549434 inhibited AR mRNA expression more potently than ISIS 554221.

On day 6, cells plated for measuring proliferation were incubated with MTT reagent until the development of color. Color intensity was measured using a spectrophotometer at 490 nm. The data is
 20 presented in Table 67.

Table 66

Percent inhibition of AR full-length mRNA

Dose (μ M)	ISIS 549372	ISIS 549434	ISIS 549458	ISIS 554221
0.04	10	10	16	0
0.20	36	35	48	0
1.00	73	52	80	0
5.00	80	55	86	0

5

Table 67

Percent inhibition of cell proliferation

Dose (μ M)	ISIS 549372	ISIS 549434	ISIS 549458	ISIS 554221
0.04	8	0	7	0
0.20	34	14	31	10
1.00	44	35	45	21
5.00	45	37	41	30

Example 25: Effect of antisense inhibition of human androgen receptor (AR) mRNA by free uptake of antisense oligonucleotide by LnCaP cells

10 The effect of free uptake of antisense oligonucleotides on AR mRNA levels was investigated.

Cells were plated at a concentration of 4,000 cells/well in 96-well plates. ISIS oligonucleotides, specified in Table 68, were added individually at 0.02 μ M, 0.10 μ M, 0.50 μ M, 2.50 μ M, or 10.00 μ M. After an incubation period of 24 hrs, mRNA levels were measured using primer probe set hAR_LTS00943. The data is presented in Table 68. The results indicate that most of the ISIS
 15 oligonucleotides inhibited AR mRNA expression more potently than ISIS 554221 at each concentration.

Table 68

Percent inhibition of AR mRNA

ISIS No	0.02 μ M	0.1 μ M	0.5 μ M	2.5 μ M	10 μ M
554221	0	0	0	0	17
549372	0	0	21	63	78
549458	4	14	67	86	89
560131	0	0	13	31	57
569213	3	0	31	59	78

569216	15	17	49	66	82
569221	18	31	49	78	91
569227	0	0	4	33	55
569236	3	2	21	43	70
579666	0	8	30	49	68
579667	0	0	8	12	40
579671	15	0	19	54	71
583918	8	0	0	0	13
584149	0	0	0	14	39
584163	0	0	19	41	70
584269	0	0	0	12	23
584468	0	0	10	44	73
586124	0	0	19	64	82
586227	0	0	14	44	59

Example 26: Effect of antisense inhibition of human androgen receptor (AR) mRNA in the presence of DHT on the proliferative ability of 22RV1 cells

Dihydrotestosterone (DHT) is an androgen hormone and AR activator. The effect of antisense inhibition of AR on the proliferative ability of cancer cells treated with DHT was analyzed. ISIS 560131 and ISIS 569236 were tested in the human prostate carcinoma cell line, 22RV1.

22RV1 cells were plated at 1,500 cells per well. ISIS 560131 and ISIS 569236 were individually transfected into the cells using RNAiMAX™ reagent (Life Technologies) at 1.34 nM, 4.00 nM, 13.4 nM, or 40.0 nM concentration. ISIS 549148, which has no known human target sequence, was included as a control. Separate sets of cells, also treated with each of the antisense oligonucleotides, were treated with DHT added on day 1 at a final concentration of 1 nM. After a treatment period of 5 days, the proliferative ability of the cancer cells was measured using the standard MTT assay. Results are presented in Table 69 as percent inhibition of proliferation, relative to non-treated cells.

As presented in Table 69, both ISIS 560131 and ISIS 569236 significantly inhibited prostate cancer cell proliferation even in the presence of AR activator, DHT, compared to the control. The control oligonucleotide did not show any effect on proliferation, as expected.

Table 69

Percent inhibition of 22RV1 cell proliferation

	ASO (nM)	ISIS 560131	ISIS 569236	ISIS 549148
- DHT	1.34	0	0	0

149

	4.0	2	18	0
	13.4	29	47	4
	40.0	54	64	0
+ DHT	1.34	0	0	0
	4.0	1	6	0
	13.4	13	32	3
	40.0	34	56	0

Example 27: Time-course study of treatment C4-2B cells with ISIS oligonucleotides targeting AR

The effect of antisense inhibition of on C4-2B cancer cells on gene expression was analyzed. ISIS 560131 and ISIS 569236 were tested.

5 *AR mRNA analysis*

C4-2B cells were plated at 1,000 cells per well in complete medium. ISIS 560131 or ISIS 569236 was individually added to the culture media to the final concentrations of 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M concentrations without using transfection reagent. ISIS 549148 was included as a negative control. MDV3100 was added at dose of 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M in a separate set of cells. After a treatment period of 8 hours, 24 hours, and 48 hours, AR expression was measured with primer probe set hAR-LTS00943. Results are presented in Tables 70-72 as percent expression of AR, relative to non-treated cells. Treatment of the cells with ISIS 560131 or ISIS 569236 reduced AR expression in the cells relative to the control set. Treatment with MDV-3100 increased AR expression at the 48 hour time-point.

15

Table 70

Percent expression of AR compared to the control group in 8 hours

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	110	85	68	45
ISIS 569236	100	87	84	58
ISIS 549148	116	105	111	110
MDV-3100	99	100	92	103

Table 71

Percent expression of AR compared to the control group in 24 hours

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M

150

ISIS 560131	47	18	5	4
ISIS 569236	103	35	15	5
ISIS 549148	87	85	87	107
MDV-3100	88	99	96	84

Table 72

Percent expression of AR compared to the control group in 48 hours

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	33	5	6	4
ISIS 569236	80	19	7	2
ISIS 549148	98	90	87	99
MDV-3100	94	94	113	126

5 *AR Protein analysis*

Protein levels in the cells were also analyzed. The cells were harvested in RIPA buffer containing protease inhibitors. The presence of bands for full-length AR was detected by western blot using AR antibody (N-20, SC-816, Santa Cruz Biotechnology). Full-length AR was significantly reduced in cells treated with ISIS 560131 or ISIS 569236 for 24 hours and 48 hours, normalized to the levels of the house-keeping gene, GAPDH.

mRNA expression analysis of downstream genes

Expression analysis of prostate-specific antigen (PSA) and TMPRSS2 were also analyzed. Results are presented in Tables 73-75 as percent inhibition of PSA expression and Tables 76-78 as percent inhibition of TMPRSS2 expression, relative to non-treated cells. Treatment of the cells with ISIS 560131 or ISIS 569236 reduced PSA and TMPRSS2 expression in the cells relative to the control set at the 24 hr and 48 hr time points. Treatment with MDV-3100 also reduced downstream gene expressions but not as potently as that with the ISIS oligonucleotides.

Table 73

Percent inhibition of PSA expression compared to the control group in 8 hours

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	12	0	3	1
ISIS 569236	18	3	0	0

151

ISIS 549148	1	8	8	0
MDV-3100	0	3	23	33

Table 74

Percent inhibition of PSA expression compared to the control group in 24 hours

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	27	46	56	60
ISIS 569236	10	34	44	54
ISIS 549148	22	13	16	6
MDV-3100	24	24	53	65

Table 75

5

Percent inhibition of PSA expression compared to the control group in 48 hours

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	20	61	71	80
ISIS 569236	4	45	68	76
ISIS 549148	2	0	18	10
MDV-3100	5	5	32	63

Table 76

Percent inhibition of TMPRSS2 expression compared to the control group in 8 hours

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	0	0	6	0
ISIS 569236	0	0	0	0
ISIS 549148	5	0	0	0
MDV-3100	0	6	45	52

Table 77

Percent inhibition of TMPRSS2 expression compared to the control group in 24 hours

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	35	57	66	67
ISIS 569236	10	32	57	66
ISIS 549148	29	10	29	10
MDV-3100	23	31	63	72

Table 78

Percent inhibition of TMPRSS2 expression compared to the control group in 48 hours

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	46	71	77	76
ISIS 569236	22	57	70	75
ISIS 549148	0	4	0	0
MDV-3100	5	16	46	59

Example 28: Antisense inhibition of AR mRNA in LNCaP cells cultured in complete media and CSS media

5 The effect of antisense inhibition of AR in LNCaP cells cultured in complete medium, as well as CSS medium with DHT, was investigated.

Gene expression in complete medium

Cells were plated at 1,000 cells per well. ISIS 560131 or ISIS 569236 was added individually at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M. ISIS 549148 was included as a negative control. MDV3100 was
 10 added a dose of 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M. μ M in a separate set of cells. After an incubation period of 48 hours, RNA levels of AR, PSA and TMPRSS2 were measured. The data is presented in Tables 79-81.

Protein analysis of full-length AR also demonstrated a dose-dependent decrease of expression, normalized to levels of the house-keeping gene, GAPDH.

15

Table 79

Percent expression of AR in LNCaP cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	101	53	17	7
ISIS 569236	98	90	47	20
ISIS 549148	102	111	109	109
MDV-3100	111	133	121	139

Table 80

Percent inhibition of PSA expression in LNCaP cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	0	60	87	90

153

ISIS 569236	0	19	63	81
ISIS 549148	0	0	0	0
MDV-3100	0	35	84	87

Table 81

Percent inhibition of TMPRSS2 expression in LNCaP cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	0	25	50	51
ISIS 569236	0	5	40	48
ISIS 549148	0	0	0	0
MDV-3100	0	0	34	39

Gene expression in CSS medium and CSS+DHT media

5 Cells were plated at 2,000 cells per well and cultured in phenol red-free RPMI supplemented with 5% charcoal stripped serum (Gibco) media for 16 hours. ISIS 560131 or ISIS 569236 was added individually at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M to each cell set. ISIS 549148 was included as a negative control. MDV3100 was added at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M in a separate set of cells. After an incubation period of 4 hrs, DHT was added to the medium to a final concentration of 1 nM as indicated. RNAs were collected 48 hrs later and levels of AR, PSA and TMPRSS2 were measured. The data is presented in Table 82-85. In the absence of DHT, AR expression in LNCaP cells was 95%, PSA expression was 7% and TMPRSS2 expression was 24% compared to the untreated control.

Table 82

Percent expression of AR in LNCaP cells cultured in CSS medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	81	46	16	5
ISIS 569236	94	66	35	13
ISIS 549148	106	97	96	104
MDV-3100	91	67	64	77

Table 83

Percent expression of AR in LNCaP cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	101	71	27	10

154

ISIS 569236	104	86	55	21
ISIS 549148	98	102	96	111
MDV-3100	107	121	110	113

Table 84

Percent inhibition of PSA expression in LNCaP cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	10	21	21	72
ISIS 569236	4	11	45	59
ISIS 549148	0	8	0	9
MDV-3100	15	38	81	82

Table 85

Percent inhibition of TMPRSS2 expression in LNCaP cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	6	11	26	64
ISIS 569236	6	8	40	50
ISIS 549148	0	0	1	10
MDV-3100	8	24	60	69

5

Effect on proliferation in CSS medium and CSS+DHT media

After a treatment period of 5 days in complete medium or CSS+ 1 nM DHT medium, the proliferative capacity of the cancer cells was measured with using CellTiter 96® AQueous One Solution or CellTiter-Glo® solution Cell Proliferation kit (Promega), following the manufacturer's instructions.

10 Results are presented in Tables 86 and 87 as percent inhibition of proliferation, relative to non-treated cells. Treatment of the cells with ISIS 560131, ISIS 569236, and MDV-3100 reduced proliferation of the cells in a dose dependent compared to the control. Treatment with ISIS oligonucleotides in CSS+DHT medium reduced the proliferative capacity to a greater extent than treatment with MVD-3100. The proliferative capacity of cells cultured in CSS medium without DHT is 17% of untreated control levels.

15

Table 86

Proliferation (% untreated control) in LNCaP cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	96	70	48	45
ISIS 569236	100	85	68	54

155

ISIS 549148	101	95	94	110
MDV-3100	107	88	65	45

Table 87

Proliferation (% untreated control) in LNCaP cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	97	81	46	8
ISIS 569236	95	99	54	17
ISIS 549148	112	96	95	89
MDV-3100	112	95	74	33

Example 29: Antisense inhibition of AR mRNA in C4-2 cells cultured in complete media and CSS media

The effect of antisense inhibition of AR mRNA levels in C4-2 cells cultured in complete medium, as well as CSS medium with DHT, was investigated.

Gene expression in complete medium

Cells were plated at 1,000 cells per well. ISIS 560131 or ISIS 569236 was added individually at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M. ISIS 549148 was included as a negative control. MDV3100 was added at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M in a separate set of cells. After an incubation period of 48 hrs, RNA levels of AR, PSA and TMPRSS2 were measured. The data is presented in Tables 88-90. Treatment with ISIS oligonucleotide inhibited AR expression, whereas treatment with MDV-3100 increased AR expression in the cells.

Protein analysis of full-length AR and PSA also demonstrated a dose-dependent decrease of expression, normalized to levels of the house-keeping gene, GAPDH.

Table 88

Percent expression of AR in C4-2 cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	48	13	8	8
ISIS 569236	72	27	11	9
ISIS 549148	89	90	84	86
MDV-3100	95	99	132	137

Table 89

Percent inhibition of PSA expression in C4-2 cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	48	78	88	89
ISIS 569236	35	62	83	88
ISIS 549148	15	24	24	23
MDV-3100	28	40	72	89

Table 90

Percent inhibition of TMPRSS2 expression in C4-2 cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	29	62	76	71
ISIS 569236	17	54	67	67
ISIS 549148	2	7	10	0
MDV-3100	10	20	44	67

5

Gene expression in CSS+DHT media

Cells were plated at 2,000 cells per well and cultured in CSS media with 1 nM DHT. ISIS 560131 or ISIS 569236 was added individually at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M to each cell set. ISIS 549148 was included as a negative control. MDV3100 was added at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M in a separate set of cells. After an incubation period of 48 hrs, RNA levels of AR, PSA and TMPRSS2 were measured. The data is presented in Table 91-93. In the absence of DHT, AR expression in C4-2 cells was 153%, PSA expression was 42% and TMPRSS2 expression was 23% compared to the untreated control. Treatment with ISIS oligonucleotide inhibited AR expression, whereas treatment with MDV-3100 increased AR expression in the cells.

15

Table 91

Percent expression of AR in C4-2 cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	88	57	20	15
ISIS 569236	89	82	52	23
ISIS 549148	101	101	118	111
MDV-3100	101	109	156	148

Table 92

Percent inhibition of PSA expression in C4-2 cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	10	24	49	74
ISIS 569236	0	4	57	64
ISIS 549148	0	8	21	22
MDV-3100	9	8	51	73

Table 93

Percent inhibition of TMPRSS2 expression in C4-2 cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	10	17	51	78
ISIS 569236	0	11	61	67
ISIS 549148	3	0	22	28
MDV-3100	9	0	44	78

5

Effect on proliferation in CSS medium and CSS+DHT media

After a treatment period of 5 days in complete medium or CSS+ 1 nM DHT medium, the proliferative capacity of the cancer cells was measured with using CellTiter 96® AQueous One Solution or CellTiter-Glo® Cell Proliferation kit (Promega), following the manufacturer's instructions. Results are presented in Tables 94 and 95 as percent inhibition of proliferation, relative to non-treated cells.

10

Treatment of the cells with ISIS 560131, ISIS 569236, and MDV-3100 reduced proliferation of the cells in a dose dependent manner compared to the control. The proliferative capacity of cells cultured in CSS medium without DHT is 17% of untreated control levels.

Table 94

15

Proliferation (% untreated control) in C4-2 cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	104	82	70	51
ISIS 569236	103	81	57	58
ISIS 549148	106	112	91	94
MDV-3100	105	108	71	67

Table 95

Proliferation (% untreated control) in C4-2 cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	106	94	47	31
ISIS 569236	99	99	88	51
ISIS 549148	102	82	82	91
MDV-3100	122	124	87	22

Example 30: Antisense inhibition of AR mRNA in C4-2B cells cultured in complete media and CSS media

The effect of antisense inhibition of AR mRNA levels in C4-2B cells cultured in complete medium, as well as CSS medium with DHT, was investigated.

Gene expression in complete medium

Cells were plated at 1,000 cells per well. ISIS 560131 or ISIS 569236 was added individually at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M. ISIS 549148 was included as a negative control. MDV3100 was added at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M in a separate set of cells. After an incubation period of 48 hrs, RNA levels of AR, PSA and TMPRSS2 were measured. The data is presented in Tables 96-98. Treatment with ISIS oligonucleotide inhibited AR expression, whereas treatment with MDV-3100 increased AR expression in the cells.

Protein analysis of full-length AR also demonstrated a dose-dependent decrease of expression, normalized to levels of the house-keeping gene, GAPDH.

Table 96

Percent expression of AR in C4-2B cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	34	15	14	14
ISIS 569236	61	23	20	16
ISIS 549148	101	91	88	87
MDV-3100	108	121	157	182

Table 97

Percent inhibition of PSA expression in C4-2B cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	56	84	89	92

159

ISIS 569236	30	72	81	89
ISIS 549148	3	11	18	14
MDV-3100	8	27	73	88

Table 98

Percent inhibition of TMPRSS2 expression in C4-2B cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	46	71	72	75
ISIS 569236	33	59	69	73
ISIS 549148	0	2	4	0
MDV-3100	3	24	55	71

Gene expression in CSS+DHT media

5 Cells were plated at 2,000 cells per well and cultured in CSS media with 1 nM DHT. ISIS 560131 or ISIS 569236 was added individually at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M to each cell set. ISIS 549148 was included as a negative control. MDV3100 was added at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M in a separate set of cells. After an incubation period of 48 hrs, RNA levels of AR, PSA and TMPRSS2 were measured. The data is presented in Tables 99-101. In the absence of DHT, AR expression in C4-2

10 cells was 188%, PSA expression was 43% and TMPRSS2 expression was 27% compared to the untreated control. Treatment with ISIS oligonucleotide inhibited AR expression, whereas treatment with MDV-3100 increased AR expression in the cells.

Table 99

Percent expression of AR in C4-2B cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	55	31	15	13
ISIS 569236	67	49	24	19
ISIS 549148	91	104	101	95
MDV-3100	112	144	165	173

Table 100

Percent inhibition of PSA expression in C4-2B cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	0	17	50	61

160

ISIS 569236	0	5	33	46
ISIS 549148	0	0	0	0
MDV-3100	0	0	37	45

Table 101

Percent inhibition of TMPRSS2 expression in C4-2B cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	0	34	60	76
ISIS 569236	0	6	43	59
ISIS 549148	0	0	0	3
MDV-3100	0	11	48	66

Effect on proliferation in CSS medium and CSS+DHT media

5 After a treatment period of 5 days in complete medium or CSS+ 1 nM DHT medium, the proliferative capacity of the cancer cells was measured with using CellTiter 96® AQueous One or CellTiter-Glo® Solution Cell Proliferation kit (Promega), following the manufacturer's instructions. Results are presented in Tables 102 and 103 as percent inhibition of proliferation, relative to non-treated cells. Treatment of the cells with ISIS 560131, ISIS 569236, and MDV-3100 reduced proliferation of the

10 cells in a dose dependent compared to the control. Treatment with ISIS oligonucleotides in CSS+DHT medium reduced the proliferative capacity to a greater extent than treatment with MVD-3100. The proliferative capacity of cells cultured in CSS medium without DHT is 12% of untreated control levels.

Table 102

Proliferation (% untreated control) in C4-2B cells cultured in complete medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	93	50	50	41
ISIS 569236	98	64	55	48
ISIS 549148	119	97	103	98
MDV-3100	131	105	72	60

Table 103

Proliferation (% untreated control) in C4-2B cells cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1 μ M	5.0 μ M
ISIS 560131	111	75	49	40
ISIS 569236	109	109	67	39

ISIS 549148	109	131	119	114
MDV-3100	125	100	83	17

Example 31: Antisense inhibition of AR mRNA in VCaP cells cultured in complete media and CSS media

The effect of antisense inhibition of AR in VCaP prostate cancer cells (Korenchuk, S. et al., In Vivo. 2001. 15: 163-168) cultured in complete medium, as well as CSS medium with DHT, was investigated. VCaP cells express both full length AR, as well as the V7 variant.

Gene expression in complete medium

Cells were plated at 10,000 cells per well. ISIS 560131 or ISIS 569236 was added individually at 1.34 nM, 4 nM, 13.4 nM, or 40 nM using RNAiMax transfection reagent. ISIS 549148 was included as a negative control. After an incubation period of 48 hrs, RNA levels of full length AR, the V7 variant, PSA and TMPRSS2 were measured. The data is presented in Tables 104-107.

Protein analysis of full-length AR and the V7 variant also demonstrated a dose-dependent decrease of expression of both compared to levels of the house-keeping gene, GAPDH.

Table 104

Percent inhibition of full-length AR in VCaP cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	0	59	77	84
ISIS 569236	0	41	49	74
ISIS 549148	0	8	5	17

Table 105

Percent inhibition of AR V7 variant in VCaP cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	0	57	78	84
ISIS 569236	0	40	53	80
ISIS 549148	0	8	0	14

Table 106

Percent inhibition of PSA expression in VCaP cells cultured in complete medium

162

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	2	24	35	46
ISIS 569236	7	19	40	52
ISIS 549148	2	0	0	20

Table 107

Percent inhibition of TMPRSS2 expression in VCaP cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	0	0	0	4
ISIS 569236	0	0	0	36
ISIS 549148	0	0	0	0

5 A separate set of cells was treated with MDV-3100 at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M. After an incubation period of 48 hrs, RNA levels of full length AR, the V7 variant, PSA and TMPRSS2 were measured. The data is presented in Tables 108 expressed as percent expression of gene levels compared to the untreated control.

Table 108

Percent of gene expression in VCaP cells treated with MDV-3100 and cultured in complete medium

	0.04 μ M	0.2 μ M	1.0 μ M	5.0 μ M
Full length AR	136	135	160	178
AR V7 variant	172	179	244	237
PSA	105	76	75	61
TMPRSS2	131	121	135	141

10

Gene expression in CSS+DHT media

15 Cells were plated at 15,000 cells per well and cultured in CSS media for 16 hours. Cells were then transfected using RNAiMax reagent with ISIS 560131 or ISIS 569236 at 1.34 nM, 4 nM, 13.4 nM, or 40 nM to each cell set. ISIS 549148 was included as a negative control. After 4 hrs, 1 nM DHT was added. MDV3100 was added in a separate set of cells at doses of 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M. After an incubation period of 48 hrs, RNA levels of AR, PSA and TMPRSS2 were measured. The data is presented in Tables 109-113. In the absence of DHT, AR expression in VCaP cells was 555%, V7 variant

expression was 656%, PSA expression was 11%, and TMPRSS2 expression was 22% compared to the untreated control.

Table 109

Percent inhibition of full-length AR in VCaP cells cultured in CSS+DHT medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	12	16	37	38
ISIS 569236	23	21	38	35
ISIS 549148	0	0	0	0

Table 110

Percent inhibition of AR V7 variant in VCaP cells cultured in CSS+DHT medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	27	31	39	41
ISIS 569236	37	33	48	39
ISIS 549148	12	0	0	5

Table 111

Percent inhibition of PSA expression in VCaP cells cultured in CSS+DHT medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	0	35	69	73
ISIS 569236	8	25	62	74
ISIS 549148	0	3	9	0

Table 112

Percent inhibition of TMPRSS2 expression in VCaP cells cultured in CSS+DHT medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	0	21	49	57
ISIS 569236	6	19	40	54
ISIS 549148	0	0	0	0

Table 113

Percent of gene expression in VCaP cells treated with MDV-3100 and cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1.0 μ M	5.0 μ M
Full length AR	114	94	142	233
AR V7 variant	82	65	101	181

164

PSA	90	72	57	30
TMPRSS2	115	96	70	42

Effect on proliferation

After a treatment period of 5 days in complete medium or CSS+ 1 nM DHT medium, the proliferative capacity of the cancer cells was measured with using CellTiter 96® AQueous One or CellTiter-Glo® Solution Cell Proliferation kit (Promega), following the manufacturer's instructions. Results are presented in Tables 114-116 as percent inhibition of proliferation, relative to non-treated cells. Treatment of the cells with ISIS 560131, ISIS 569236, and MDV-3100 reduced proliferation of the cells in a dose dependent compared to the control. Treatment with ISIS oligonucleotides in CSS+DHT medium reduced the proliferative capacity to a greater extent than treatment with MVD-3100. The proliferative capacity of cells cultured in CSS medium without DHT is 12% of untreated control levels.

Table 114

Proliferation (% untreated control) in VCaP cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	98	66	53	48
ISIS 569236	98	76	68	59
ISIS 549148	98	98	113	106

Table 115

Proliferation (% untreated control) in VCaP cells cultured in CSS+DHT medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	95	65	42	37
ISIS 569236	83	68	61	45
ISIS 549148	114	123	104	92

Table 116

Proliferation (% untreated control) in VCaP cells treated with MDV-3100

	Complete medium	CSS+DHT medium
0.04 μ M	49	117
0.2 μ M	44	119
1.0 μ M	27	71
5.0 μ M	17	65

Effect on apoptosis

After a treatment period of 72 hours in complete medium, apoptosis of the cancer cells was measured with Caspase-Glo 3/7 assay (Promega). Results are presented in Tables 117 and 118 as percent apoptosis of the cells, relative to non-treated cells. Treatment of the cells with ISIS 560131, ISIS 569236, and MDV-3100 increased apoptosis of the cells in a dose dependent compared to the control.

Apoptosis was also measured by protein western blot analysis of cleaved PARP levels, which were shown to be increased in a dose-dependent manner in cells treated with ISIS 560131, ISIS 569236, and MDV-3100.

Table 117

Apoptosis (% untreated control) in VCaP cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	189	253	356	262
ISIS 569236	176	293	402	581
ISIS 549148	131	108	103	146

Table 118

Apoptosis (% untreated control) in VCaP cells treated with MDV-3100

	%
0.04 μ M	186
0.2 μ M	210
1.0 μ M	612
5.0 μ M	528

Example 32: Antisense inhibition of AR mRNA in 22RV1 cells cultured in complete media and CSS media

The effect of antisense inhibition of AR in 22RV1 cells cultured in complete medium, as well as CSS medium with DHT, was investigated.

Gene expression in complete medium

Cells were plated at 1,000 cells per well. ISIS 560131 or ISIS 569236 was added individually at 1.34 nM, 4 nM, 13.4 nM, or 40 nM using RNAiMax transfection reagent. ISIS 549148 was included as a negative control. After an incubation period of 48 hrs, RNA levels of full length AR, the V7 variant, PSA and TMPRSS2 were measured. The data is presented in Tables 119-122.

Protein analysis of full-length AR and the V7 variant also demonstrated a dose-dependent decrease of expression compared to levels of the house-keeping gene, GAPDH.

Table 119

Percent inhibition of full-length AR in 22RV1 cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	7	19	49	76
ISIS 569236	17	15	37	71
ISIS 549148	6	0	11	17

Table 120

Percent inhibition of AR V7 variant in 22RV1 cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	12	29	57	81
ISIS 569236	30	2	46	81
ISIS 549148	0	0	22	26

Table 121

Percent inhibition of PSA expression in 22RV1 cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	10	20	27	36
ISIS 569236	0	17	25	7
ISIS 549148	9	11	17	27

Table 122

Percent inhibition of TMPRSS2 expression in 22RV1 cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	7	3	19	32
ISIS 569236	0	13	21	36
ISIS 549148	15	9	14	4

A separate set of cells was treated with MDV-3100 at 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M. After an incubation period of 48 hrs, RNA levels of full length AR, the V7 variant, PSA and TMPRSS2 were measured. The data is presented in Tables 123 expressed as percent expression of gene levels compared to the untreated control.

Table 123

Percent of gene expression in 22RV1 cells treated with MDV-3100 and cultured in complete medium

	0.04 μ M	0.2 μ M	1.0 μ M	5.0 μ M
Full length AR	103	93	81	83
AR V7 variant	106	98	87	77
PSA	83	70	71	86
TMPRSS2	101	80	82	93

Gene expression in CSS+DHT media

5 Cells were plated at 2,000 cells per well and cultured in CSS media for 16 hours. Cells were then transfected using RNAiMax reagent with ISIS 560131 or ISIS 569236 at 1.34 nM, 4 nM, or 13.4 nM to each cell set. ISIS 549148 was included as a negative control. After 4 hrs, 1 nM DHT was added. MDV3100 was added in a separate set of cells at doses of 0.04 μ M, 0.2 μ M, 1.0 μ M, or 5.0 μ M. After an incubation period of 48 hrs, RNA levels of AR, AR V7 variant, PSA and TMPRSS2 were measured. The data is presented in Tables 124-128. In the absence of DHT, AR expression in VCaP cells was 555%, V7 variant expression was 656%, PSA expression was 11%, and TMPRSS2 expression was 22% compared to the untreated control.

15 Treatment with ISIS oligonucleotides resulted in significant inhibition of full length AR and the V7 variant, as well as downstream gene expression. Treatment with ISIS oligonucleotides resulted in inhibition of gene expression to a greater extent than treatment with MVD-3100.

Table 124

Percent inhibition of full-length AR in 22RV1 cells cultured in CSS+DHT medium

	1.34 nM	4.0 nM	13.4 nM
ISIS 560131	65	85	93
ISIS 569236	59	89	97
ISIS 549148	2	13	22

Table 125

Percent inhibition of AR V7 variant in 22RV1 cells cultured in CSS+DHT medium

	1.34 nM	4.0 nM	13.4 nM
ISIS 560131	63	83	93

168

ISIS 569236	54	88	97
ISIS 549148	19	19	32

Table 126

Percent inhibition of PSA expression in 22RV1 cells cultured in CSS+DHT medium

	1.34 nM	4.0 nM	13.4 nM
ISIS 560131	3	50	66
ISIS 569236	28	49	70
ISIS 549148	8	23	29

Table 127

Percent inhibition of TMPRSS2 expression in 22RV1 cells cultured in CSS+DHT medium

	1.34 nM	4.0 nM	13.4 nM
ISIS 560131	39	50	59
ISIS 569236	27	50	75
ISIS 549148	0	3	1

Table 128

Percent of gene expression in 22RV1 cells treated with MDV-3100 and cultured in CSS+DHT medium

	0.04 μ M	0.2 μ M	1.0 μ M	5.0 μ M
Full length AR	5	11	6	18
AR V7 variant	16	17	19	12
PSA	15	19	18	16
TMPRSS2	17	9	26	18

Effect on proliferation

After a treatment period of 5 days in complete medium, the proliferative capacity of the cancer cells was measured with using CellTiter 96® AQueous One or CellTiter-Glo® Solution Cell Proliferation kit (Promega), following the manufacturer's instructions. Results are presented in Tables 129 and 130 as percent inhibition of proliferation, relative to non-treated cells. Treatment of the cells with ISIS 560131, ISIS 569236, and MDV-3100 reduced proliferation of the cells in a dose dependent compared to the control. Treatment with ISIS oligonucleotides in CSS+DHT medium reduced the proliferative capacity to a greater extent than treatment with MVD-3100. The proliferative capacity of cells cultured in CSS medium without DHT is 12% of untreated control levels.

Table 129

Proliferation (% untreated control) in 22RV1 cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	94	72	50	17
ISIS 569236	92	53	20	7
ISIS 549148	97	97	101	83

Table 130

Proliferation (% untreated control) in 22RV1 cells treated with MDV-3100

	%
0.04 μ M	87
0.2 μ M	83
1.0 μ M	81
5.0 μ M	74

5 *Effect on apoptosis*

After a treatment period of 72 hours in complete medium or CSS+DHT medium, apoptosis of the cancer cells was measured with Caspase-glo 3/7 assay kit (Promega). Results are presented in Tables 131 and 132 as percent apoptosis of the cells, relative to non-treated cells. Treatment of the cells with ISIS 560131 and ISIS 569236 increased apoptosis of the cells in a dose dependent compared to the control.

Table 131

Apoptosis (% untreated control) in 22RV1 cells cultured in complete medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	99	127	131	566
ISIS 569236	91	141	333	1452
ISIS 549148	81	76	72	123

Table 132

Apoptosis (% untreated control) in 22RV1 cells cultured in CSS+DHT medium

	1.34 nM	4.0 nM	13.4 nM	40 nM
ISIS 560131	121	113	172	518
ISIS 569236	127	106	257	1136
ISIS 549148	113	94	102	108

Example 33: Effect of ISIS antisense oligonucleotides targeting human androgen receptor in cynomolgus monkeys

Cynomolgus monkeys were treated with ISIS antisense oligonucleotides selected from studies described above. Antisense oligonucleotide efficacy and tolerability were evaluated. The human antisense oligonucleotides tested are cross-reactive with the rhesus genomic sequence (GENBANK Accession No. NW_001218131.1 truncated from nucleotides 134001 to 308000 and designated herein as SEQ ID NO: 189). The target start site and target region of each oligonucleotide to SEQ ID NO: 189, as well as the details of their chemistry and sequence, is presented in Table 133.

Table 133

Antisense oligonucleotides complementary to SEQ ID NO: 189

ISIS No	Target Start Site	Target Region	Sequence	Chemistry	SEQ ID NO
560131	59450	Intron	TTGATTTAATGGTTGC	Deoxy, MOE, and (S)-cEt	35
569213	59449	Intron	TGATTTAATGGTTGCA	Deoxy, MOE, and (S)-cEt	39
	59479		TGATTTAATGGTTGCA		39
569216	59449	Intron	TGATTTAATGGTTGCA	Deoxy, MOE, and (S)-cEt	39
	59479		TGATTTAATGGTTGCA		39
569221	59449	Intron	TGATTTAATGGTTGCA	Deoxy, MOE, and (S)-cEt	39
	59479		TGATTTAATGGTTGCA		39
569236	59449	Intron	TGATTTAATGGTTGCA	Deoxy, MOE, and (S)-cEt	39
	59479		TGATTTAATGGTTGCA		39
579671	59450	Intron	TTGATTTAATGGTTGC	Deoxy, MOE, and (S)-cEt	35
586124	59448	Intron	GATTTAATGGTTGCAA	3-10-3 (S)-cEt	43
583918	3754	Exon	AGTCGCGACTCTGGTA	3-10-3 (S)-cEt	124
584149	7260	Intron	GTCAATATCAAAGCAC	3-10-3 (S)-cEt	150
584163	9811	Intron	GAACATTATTAGGCTA	3-10-3 (S)-cEt	155
584269	41322	Intron	CCTTATGGATGCTGCT	3-10-3 (S)-cEt	169
584468	109552	Intron	CATTGTACTATGCCAG	3-10-3 (S)-cEt	175

Treatment

Prior to the study, the monkeys were kept in quarantine for a 30-day period, during which the animals were observed daily for general health. The monkeys were 2-4 years old and weighed between 2 and 4 kg. Thirteen groups of four randomly assigned male cynomolgus monkeys each were injected subcutaneously with ISIS oligonucleotide or PBS. PBS solution or ISIS oligonucleotides, at a dose of 40 mg/kg, were administered with a loading regimen consisting of four doses on the first week of the study

(days 1, 3, 5, and 7), followed by a maintenance regimen consisting of once weekly administration starting on Day 14 (weeks 2 to 6). Subcutaneous injections were performed in clock-wise rotations at 4 sites on the back; one site per dose. The injection sites were delineated by tattoo, while sedated using ketamine, and were separated by a minimum of 3 cm.

- 5 During the study period, the monkeys were observed a minimum of once daily for signs of illness or distress. The protocols described in the Example were approved by the Institutional Animal Care and Use Committee (IACUC).

Target Reduction

RNA analysis

- 10 RNA was extracted from liver, heart, skeletal muscle, kidney, and prostate tissues for real-time PCR analysis of AR using primer probe set RTS3559. The results were normalized to RIBOGREEN®. Results are presented as percent inhibition of AR mRNA, relative to PBS control. As shown in Table 134, treatment with ISIS antisense oligonucleotides resulted in significant reduction of AR mRNA, relative to the PBS control. 'n/a' indicates that mRNA levels were not measured in that organ.

15 **Table 134**

Percent Inhibition of AR mRNA in the cynomolgus monkey relative to the PBS control

ISIS No	Heart	Skeletal Muscle	Kidney	Liver	Prostate
560131	32	30	19	65	27
569221	52	35	31	60	n/a
569236	42	47	42	33	32
579671	24	31	53	33	n/a
583918	76	74	73	88	58
584149	33	63	77	93	45
584163	53	73	90	98	58
584269	72	76	92	96	41
584468	33	53	88	97	50

Protein analysis

- 20 Serum testosterone protein levels were measured in the plasma with an ELISA kit (Enzo Life Sciences), following the manufacturer's instructions. The results are presented in Table 135, expressed in ng/mL. The results indicate that some of the ISIS oligonucleotides reduced testosterone protein levels.

Table 135

Testosterone protein levels in the cynomolgus monkey

	ng/mL
PBS	12.6
ISIS 560131	14.7
ISIS 569221	8.8
ISIS 569236	12.7
ISIS 579671	7.3
ISIS 584269	14.1
ISIS 584468	13.6

Tolerability studies*Body and organ weight measurements*

- 5 To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body and organ weights were measured. Body weights were measured on day 42 and are presented in Table 136. Organ weights were measured at the time of euthanasia and the data is also presented in Table 136. Specifically, treatment with ISIS 560131 was well tolerated in terms of the body and organ weights of the monkeys.

10

Table 136

Final body and organ weights in cynomolgus monkeys

Treatment	Body Wt (kg)	Spleen (g)	Heart (g)	Kidney (g)	Mesenteric lymph nodes (g)	Liver (g)
PBS	2.5	2.6	8.5	13	1.4	58
ISIS 560131	2.4	2.5	9.8	12	2.0	58
ISIS 569213	2.4	5.3	8.3	16	2.4	69
ISIS 569216	2.6	4.9	9.3	15	2.7	71
ISIS 569221	2.5	3.3	8.5	14	3.5	68
ISIS 569236	2.4	3.2	8.4	12	2.4	56
ISIS 579671	2.4	3.2	8.8	14	2.5	62
ISIS 586124	2.5	3.3	9.4	14	2.8	58
ISIS 583918	2.5	4.6	8.9	12	3.5	60
ISIS 584149	2.5	2.2	9.3	13	2.1	60
ISIS 584163	2.5	3.2	8.4	15	3.3	54
ISIS 584269	2.5	4.7	8.7	13	3.6	60
ISIS 584468	2.5	4.1	8.3	13	3.8	60

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, the monkeys were fasted overnight. Approximately, 1.5 mL of blood samples were collected on day 44 from all the study groups. Blood was collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 min and then centrifuged at 3,000 rpm for 10 min. Levels of various liver function markers were measured using a Toshiba 120FR NEO chemistry analyzer (Toshiba Co., Japan). The results are presented in Table 137. Specifically, treatment with ISIS 560131 was well tolerated in terms of the liver function markers.

Table 137

Liver function markers in cynomolgus monkey plasma

Treatment	Albumin (g/dL)	AST (IU/L)	ALT (IU/L)
PBS	4.2	37	39
ISIS 560131	4.0	87	68
ISIS 569213	3.7	80	47
ISIS 569216	3.7	93	75
ISIS 569221	4.0	73	48
ISIS 569236	4.1	45	35
ISIS 579671	4.0	53	56
ISIS 586124	3.9	94	56
ISIS 583918	4.1	73	75
ISIS 584149	4.5	58	57
ISIS 584163	4.2	68	50
ISIS 584269	4.0	81	75
ISIS 584468	4.0	52	46

Hematology

To evaluate any effect of ISIS oligonucleotides in cynomolgus monkeys on hematologic parameters, blood samples of approximately 0.5 mL of blood was collected day 44 from each of the available study animals in tubes containing K₂-EDTA. Samples were analyzed for red blood cell (RBC) count, white blood cells (WBC) count, platelet count, hemoglobin content and hematocrit, using an ADVIA2120i hematology analyzer (SIEMENS, USA). The data is presented in Table 138.

The data indicate treatment with most of the oligonucleotides did not cause any changes in hematologic parameters outside the expected range for antisense oligonucleotides at this dose. Specifically, treatment with ISIS 560131 was well tolerated in terms of the hematology of the monkeys.

Table 138

Hematological parameters in cynomolgus monkeys

Treatment	RBC ($\times 10^6/\mu\text{L}$)	Platelets ($\times 10^3/\mu\text{L}$)	WBC ($\times 10^3/\mu\text{L}$)	Hemoglobin (g/dL)	HCT (%)
PBS	5.3	426	13.6	13.2	43
ISIS 560131	5.8	392	11.3	13.1	44
ISIS 569213	5.6	426	12.9	12.5	42
ISIS 569216	5.6	504	12.2	12.8	43
ISIS 569221	5.6	406	11.1	12.9	45
ISIS 569236	5.7	358	14.4	13.1	44
ISIS 579671	5.4	438	10.0	12.5	42
ISIS 586124	5.8	391	10.4	13.6	45
ISIS 583918	5.8	435	12.7	13.3	46
ISIS 584149	5.7	478	11.3	13.7	45
ISIS 584163	5.5	461	9.1	12.8	44
ISIS 584269	5.2	522	9.8	12.4	41
ISIS 584468	5.9	408	11.1	13.5	45

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, the monkeys were fasted overnight. Approximately, 1.5 mL of blood samples were collected from all the study groups on day 44. Blood was collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 min and then centrifuged at 3,000 rpm for 10 min. Levels of BUN and creatinine were measured using a Toshiba 120FR NEO chemistry analyzer (Toshiba Co., Japan). Results are presented in Table 139, expressed in mg/dL. The plasma chemistry data indicate that most of the ISIS oligonucleotides did not have any effect on the kidney function outside the expected range for antisense oligonucleotides. Specifically, treatment with ISIS 560131 was well tolerated in terms of the kidney function of the monkeys.

Kidney function was also assessed by urinalysis. Fresh urine from all animals was collected on day 44 using a clean cage pan on wet ice. Food was removed overnight the day before fresh urine collection was done but water was supplied. The total protein and creatinine levels were measured using

a Toshiba 120FR NEO automated chemistry analyzer (Toshiba Co., Japan) and the protein to creatinine ratio was calculated. The results are presented in Table 140.

Table 139

5

Plasma BUN and creatinine levels (mg/dL) in cynomolgus monkeys

Treatment	BUN	Creatinine
PBS	30.5	0.78
ISIS 560131	23.7	0.84
ISIS 569213	29.4	0.91
ISIS 569216	28.4	0.81
ISIS 569221	20.2	0.86
ISIS 569236	24.9	0.87
ISIS 579671	22.7	0.74
ISIS 586124	23.8	0.87
ISIS 583918	24.5	0.87
ISIS 584149	26.4	0.85
ISIS 584163	22.4	0.82
ISIS 584269	21.8	0.89
ISIS 584468	22.2	0.78

Table 140

Urine protein/creatinine ratio in cynomolgus monkeys

Treatment	Ratio
PBS	0.00
ISIS 560131	0.02
ISIS 569213	0.02
ISIS 569216	0.08
ISIS 569221	0.00
ISIS 569236	0.02
ISIS 579671	0.00
ISIS 586124	0.01
ISIS 583918	0.01
ISIS 584149	0.01
ISIS 584163	0.01
ISIS 584269	0.00
ISIS 584468	0.00

C-reactive protein level analysis

To evaluate any inflammatory effect of ISIS oligonucleotides in cynomolgus monkeys, the monkeys were fasted overnight. Approximately, 1.5 mL of blood samples were collected from all the study groups on day 44. Blood was collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 min and then centrifuged at 3,000 rpm for 10 min. C-reactive protein (CRP), which is synthesized in the liver and which serves as a marker of inflammation, was measured on day 43 using a Toshiba 120FR NEO chemistry analyzer (Toshiba Co., Japan). Complement C3 was also measured similarly, and the data is presented as a percentage of baseline values. The results are presented in Table 141 and indicate that treatment with most of the ISIS oligonucleotides did not cause any inflammation in monkeys.

Table 141

C-reactive protein and C3 levels in cynomolgus monkey plasma

Treatment	CRP (mg/dL)	C3 (% of baseline)
PBS	2.5	118
ISIS 560131	1.7	100
ISIS 569213	2.8	60
ISIS 569216	3.6	94
ISIS 569221	4.9	91
ISIS 569236	2.6	103
ISIS 579671	4.5	101
ISIS 586124	4.0	93
ISIS 583918	3.5	89
ISIS 584149	1.7	110
ISIS 584163	1.0	102
ISIS 584269	4.9	102
ISIS 584468	1.3	111

Pharmacokinetics studies

The concentrations of the full-length oligonucleotide in the kidney and the liver of select treatment groups were measured. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer 2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTTT, designated

herein as SEQ ID NO: 190) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 µg/g.

The results are presented in Table 142, expressed as µg/g tissue. The kidney to liver ratio was also calculated and is presented in Table 142.

5

Table 142

Oligonucleotide concentration of in cynomolgous monkeys

Treatment	Liver	Kidney	K/L ratio
ISIS 560131	793	2029	2.6
ISIS 569221	966	1372	1.4
ISIS 569236	898	1282	1.4
ISIS 579671	871	2576	3.0
ISIS 584269	698	2823	4.0
ISIS 584468	474	2441	5.2

Example 34: Effect of antisense inhibition of androgen receptor (AR) on an androgen receptor-dependent breast cancer orthotopic model

10

MDA-MB-453 cells express AR in the absence of estrogen receptors and progesterone receptor (Hall, R.E. et al., Eur. J. Cancer 1994. 30: 484-490). The effect of inhibition of AR mRNA expression with antisense oligonucleotides was examined in MDA-MB-453 tumor-bearing mice.

Study 1

15

ISIS 569216 (TGATTTAATGGTTGCA; SEQ ID NO: 39), which is the antisense oligonucleotide tested in the assay, was designed as a deoxy, MOE and (S)cEt oligonucleotide, and is 16 nucleosides in length. The chemistry of the oligonucleotide is 5' - Te Gk Ak Tk Td Td Ad Ad Td Gd Gd Td Tk Gk Ck A, where 'e' denotes a 2'-O-methoxyethyl ribose; 'k' denotes an (S)-cEt; 'd' denotes a 2'-deoxyribose. The internucleoside linkages throughout the oligonucleotide are phosphorothioate (P=S) linkages. All cytosine residues throughout the oligonucleotide are 5-methylcytosines. ISIS 569216 has two target start sites, 58720 and 58750, on the human AR genomic sequence (GENBANK Accession No. NT_011669.17 truncated from nucleosides 5079000 to 5270000, SEQ ID NO: 1).

20

Treatment

MDA-MB-453 breast carcinoma cells (5×10^6), mixed with 50% Matrigel, were injected into the mammary fat pad of 10 female NSG mice. Dihydrotestosterone (DHT) pellets, the active form of the major circulating androgen, testosterone, were implanted subcutaneously at the same time. Once the tumor reached a size of 100 mm^3 , the mice were randomly divided into two treatment groups. The first treatment group was injected with ISIS 569216 administered by subcutaneous injection at a dose of 50 mg/kg five times a week for 4 weeks. The second treatment group was injected with vehicle only, administered by subcutaneous injection five times a week for 4 weeks, and served as the control group. Tumor growth was monitored once a week and mice were sacrificed on day 32 after treatment. Tumor tissue and TB-interface samples were collected and processed for further analysis.

10 *RNA analysis*

Tumors were excised and the tissue was processed for RNA extraction and qPCR analyses. AR mRNA expression was assessed at the TB-interface and normalized to actin mRNA expression. AR mRNA expression in mice treated with ISIS 569216 was inhibited by 48% compared to the control group.

Measurement of tumor volume

15 Tumor volumes were measured on a regular basis throughout the study period, using Vernier calipers. As shown in Table 143, tumor volumes were significantly decreased in mice treated with ISIS 569216 compared to the control group.

Table 143

Tumor volume on different days in the MDA_MB-453 cancer orthotopic model

	Day 16	Day 23	Day 30	Day 37	Day 44	Day 51
ISIS 569216	134	142	173	125	92	73
Control	111	141	155	195	287	347

20

Study 2.

Treatment

MDA-MB-453 cells obtained from ATCC were maintained in Leibovitz's L-15 media with 10% FBS. Female NSG mice (Jackson Laboratories) were implanted in the mammary fat pad with 5×10^6 tumor cells in growth-factor-reduced matrigel (1:1). DHT pellets were also implanted at the same time in the mice between the shoulder blades.

25 After 20 days, the mice were then randomly divided into treatment groups. Groups of mice were injected with 50 mg/kg of ISIS 569236 or ISIS 560131 administered subcutaneously 5 days per week for

2 weeks. A group of mice were similarly treated with control oligonucleotide, ISIS 549148 (a 3-10-3 (S)-cEt gapmer with sequence GGCTACTACGCCGTCA, designated herein as SEQ ID NO: 193, with no known human sequence). Another control group of mice was similarly treated with PBS.

Measurement of tumor growth

5 Tumor volumes were measured on a regular basis throughout the study period, using Vernier calipers. As shown in Table 144, tumor volumes were decreased in mice treated with antisense oligonucleotides targeting AR compared to the control group.

Table 144

Tumor volumes in the MDA-MB-453 model

	Day 0	Day 8	Day 13	Day 20	Day 23	Day 27	Day 29
PBS	136	336	331	358	338	417	481
ISIS 549148	148	303	312	365	413	490	550
ISIS 560131	144	261	243	204	232	233	258
ISIS 569236	134	283	260	230	264	329	323

RNA analysis

RNA extraction was performed using an RNA extraction kit from Qiagen. AR RNA expression was measured using primer probe set LTS00943 and normalized to human actin mRNA expression.

Human AR RNA expression was assessed in tumor tissue. AR RNA expression in mice treated with ISIS 560131 was inhibited by 35 % and AR expression in mice treated with ISIS 569236 was inhibited by 19% compared to the control group.

WHAT IS CLAIMED:

1. A compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 12-179.

2. A compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NO: 35, 39, 43, 124, 150, 155, 169, or 175.

3. A compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides complementary within nucleotides 2957-2972, 3079-3094, 3099-3114, 3109-3124, 3113-3128, 3120-3135, 3133-3148, 3224-3239, 3226-3241, 3351-3366, 3353-3368, 3361-3376, 3388-3403, 3513-3528, 3517-3532, 3519-3534, 3641-3656, 3735-3750, 3764-3779, 3768-3783, 3798-3813, 3799-3814, 3851-3866, 3870-3885, 3874-3889, 3876-3891, 3878-3893, 3884-3899, 3886-3901, 3888-3903, 3901-3916, 3956-3971, 3962-3977, 3964-3979, 3967-3982, 4019-4034, 4038-4053, 4049-4064, 4056-4071, 4059-4074, 4062-4077, 4066-4081, 4070-4085, 4101-4116, 4103-4118, 4105-4120, 4109-4124, 4305-4320, 4405-4420, 4532-4547, 4534-4549, 4537-4552, 4539-4554, 4555-4570, 4571-4586, 4573-4588, 4578-4593, 4597-4612, 4632-4647, 4655-4670, 4656-4671, 4662-4677, 4699-4714, 4747-4762, 4750-4765, 4752-4767, 4754-4769, 4755-4770, 4769-4784, 4798-4813, 4804-4819, 4807-4822, 4833-4848, 4837-4852, 4839-4854, 4865-4880, 4868-4883, 4872-4887, 4874-4889, 4876-4891, 4887-4902, 4889-4904, 4916-4931, 4918-4933, 4938-4953, 4942-4957, 4944-4959, 4951-4966, 5050-5065, 5052-5067, 5054-5069, 5056-5071, 5060-5075, 5061-5076, 5062-5077, 5133-5148, 5141-5156, 5155-5170, 5265-5280, 5293-5308, 5308-5323, 5392-5407, 5448-5463, 5469-5484, 5481-5496, 5483-5498, 5486-5501, 5488-5503, 5494-5509, 5521-5536, 5666-5681, 6222-6237, 6701-6716, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58739, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58725-58740, 58750-58769, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-60917, 67454-67469, 114874-114889, 115272-115287, 115365-115380, 134971-134986, 102156-102171, 139682-139697, 139762-139777, 139782-139797, 144856-144871, 144938-144953, 148406-148421, 148443-148458, 148520-148535, 181695-181710, 182958-182973, or 183049-183064 of SEQ ID NO: 1, wherein said modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

4. A compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides complementary within intron 1 nucleotides 5666-5681, 6222-6237, 6701-6716, 7543-7558, 8471-8486, 8638-8653, 9464-9479, 10217-10232, 10250-10265, 10865-10880, 11197-11212, 11855-11870, 13189-13204, 13321-13336, 13346-13361, 16555-16570, 16793-16808, 16968-16983, 17206-17221, 18865-18880, 29329-29344, 32290-32305, 33315-33330, 39055-39070, 40615-40630, 42017-42032, 56050-56065, 58719-58734, 58720-58739, 58721-58736, 58722-58737, 58723-58738, 58724-58739, 58725-58740, 58750-58769, 58751-58766, 58752-58767, 58753-58768, 58754-58769, 58755-58770, 60902-60917, 67454-67469, 114874-114889, 115272-115287, 115365-115380, or 134971-134986 of SEQ ID NO:1.

5. The compound of claim 4, wherein the compound comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides complementary within intron 1 nucleotides 8638-8653, 11197-11212, 40615-40630, 58719-58734, 58720-58735, or 58721-58736 of SEQ ID NO:1.

6. The compound of any one of claims 1-5, wherein the modified oligonucleotide comprises at least one modified sugar.

7. The compound of claim 6, wherein at least one modified sugar comprises a 2'-O-methoxyethyl group.

8. The compound of claim 6, wherein the at least one modified sugar is a bicyclic sugar.

9. The compound of claim 8, wherein the bicyclic sugar comprises a group selected from: 4'-CH(CH₃)-O-2', 4'-CH₂-O-2' and 4'-(CH₂)₂-O-2'.

10. The compound of any one of claims 1-9, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

11. The compound of claim 10, wherein each internucleoside linkage of the antisense oligonucleotide is a phosphorothioate internucleoside linkage.

12. The compound of any one of claims 1-11, wherein the modified oligonucleotide comprises at least one modified nucleobase.

13. The compound of claim 12, wherein the modified nucleobase is a 5-methylcytosine.

14. The compound of any one of claims 1-13, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

15. A compound comprising a single-stranded modified oligonucleotide consisting of 16
5 linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 35, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 9 linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of four linked nucleosides;

10 wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the three linked nucleosides of the 5' wing segment are each a constrained ethyl (cEt) sugar; the four linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

15

16. A compound comprising a single-stranded modified oligonucleotide consisting of 16
linked nucleosides having a nucleobase sequence consisting of the sequence of SEQ ID NO: 39, or a pharmaceutically acceptable salt thereof, wherein the modified oligonucleotide comprises:

a gap segment consisting of 7 linked deoxynucleosides;

20 a 5' wing segment consisting of four linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; the four linked nucleosides of the 5' wing segment are a 2'-O-methoxyethyl sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, and a constrained ethyl (cEt) sugar in the 5' to 3' direction; the five
25 linked nucleosides of the 3' wing segment are a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a constrained ethyl (cEt) sugar, a 2'-O-methoxyethyl sugar, and a 2'-O-methoxyethyl sugar in the 5' to 3' direction; each internucleoside linkage is a phosphorothioate linkage; and each cytosine is a 5-methylcytosine.

17. The compound of any one of claims 1-16, wherein the modified oligonucleotide is at
30 least 90% complementary to a nucleic acid encoding androgen receptor.

18. The compound of claim 17, wherein the nucleic acid encoding androgen receptor comprises the nucleotide sequence of any one of SEQ ID NOs: 1-8.

19. A composition comprising the compound of any one of claims 1-18, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent or carrier.

5 20. A composition comprising the compound of any one of claims 1-18 and an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464.

21. The composition of claim 20, wherein the anti-androgenic agent is MDV3100.

10 22. A method of treating cancer comprising administering to a subject having cancer the compound of any one of claims 1-18 or composition of any one of claims 19-21, thereby treating cancer in the subject.

23. The method of claim 22, wherein the cancer is prostate cancer, breast cancer, ovarian cancer, gastric cancer or bladder cancer.

24. The method of claim 22, wherein the cancer is castrate-resistant prostate cancer.

15 25. The method of claim 24, wherein the castrate-resistant prostate cancer is resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464.

26. An antisense compound of any one of claims 1-18 or composition of any one of claims 19-21 for use in treating cancer.

20 27. The compound or composition of claim 26, wherein the cancer is prostate cancer, breast cancer, ovarian cancer, gastric cancer or bladder cancer.

28. The compound or composition of claim 27, wherein the cancer is castrate-resistant prostate cancer.

25 29. The compound or composition of claim 28, wherein the castrate-resistant prostate cancer is resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464.

30. A method of inhibiting growth or proliferation of a prostate cancer cell resistant to an anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700

and VT464, comprising contacting the prostate cancer cell with an antisense compound targeted to human androgen receptor (AR) upstream of the 3' end of exon 3.

31. The method of claim 30, wherein the agent is MDV3100.

32. A method of treating prostate cancer resistant to an anti-androgenic agent selected from:
5 MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464, in a subject comprising administering to the subject an antisense compound targeted to human androgen receptor (AR) upstream of the 3' end of exon 3.

33. The method of claim 32, wherein the subject is diagnosed as having prostate cancer resistant to the anti-androgenic agent selected from: MDV3100, ARN-059, ODM-201, abiraterone,
10 TOK001, TAK700 and VT464.

34. A method of treating prostate cancer in a patient in need thereof, comprising administering to the patient an antisense compound of any one of claims 1-18 or composition of any one of claims 19-21 and an anti-androgenic agent.

35. The method as claimed in claim 34, wherein the anti-androgenic agent is selected from:
15 MDV3100, ARN-059, ODM-201, abiraterone, TOK001, TAK700 and VT464.

36. The method of claim 34 or 35, wherein the antisense compound and the anti-androgenic agent synergize in combination to inhibit the growth or proliferation of the prostate cancer cell.

37. The method of any one of claims 34-36, wherein the prostate cancer cell is contacted with an amount of the antisense compound and an amount of anti-androgenic agent that are each or both less in
20 combination than the amount of either the antisense compound or anti-androgenic agent alone effective in inhibiting the growth or proliferation of said prostate cancer cell.

38. A method of inhibiting growth or proliferation of an androgen receptor (AR)-positive breast cancer cell comprising contacting the breast cancer cell with an antisense compound targeted to human androgen receptor (AR) wherein the growth or proliferation of the breast cancer cell is inhibited.

25 39. The method of any one of claims 30-38, wherein the antisense compound is the compound of any one of claims 1-18, or pharmaceutically acceptable salt thereof.

40. The method of any one of claims 30-38, wherein the antisense compound is the compound of any one of claims 15 or 16, or pharmaceutically acceptable salt thereof.

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t t t t a t g t a a	g t a t g g c t t t	g a t t c t c t c t	c t c a c t c c a g	g t t t t t g t t a	g g g a a g a a a t	106200
g c a a g t g a a c	c c t c a t t g a a	c t c t t t c t g t	c c t t t a a a t c	c a t t c t t t c c	c a c c t c a a c t	106260
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cag	gaa	gca	gta	tcc	gaa	ggc	agc	agc	agc	ggg	aga	gcg	agg	gag	gcc	1757
Gln	Glu	Ala	Val	Ser	Glu	Gly	Ser	Ser	Ser	Gly	Arg	Ala	Arg	Glu	Ala	
	200					205					210					
tcg	ggg	gct	ccc	act	tcc	tcc	aag	gac	aat	tac	tta	ggg	ggc	act	tcg	1805
Ser	Gly	Ala	Pro	Thr	Ser	Ser	Lys	Asp	Asn	Tyr	Leu	Gly	Gly	Thr	Ser	
215					220					225					230	
acc	att	tct	gac	aac	gcc	aag	gag	ttg	tgt	aag	gca	gtg	tcg	gtg	tcc	1853
Thr	Ile	Ser	Asp	Asn	Ala	Lys	Glu	Leu	Cys	Lys	Ala	Val	Ser	Val	Ser	
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atg	ggc	ctg	ggt	gtg	gag	gcg	ttg	gag	cat	ctg	agt	cca	ggg	gaa	cag	1901
Met	Gly	Leu	Gly	Val	Glu	Ala	Leu	Glu	His	Leu	Ser	Pro	Gly	Glu	Gln	
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ctt	cgg	ggg	gat	tgc	atg	tac	gcc	cca	ctt	ttg	gga	gtt	cca	ccc	gct	1949
Leu	Arg	Gly	Asp	Cys	Met	Tyr	Ala	Pro	Leu	Leu	Gly	Val	Pro	Pro	Ala	
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gtg	cgt	ccc	act	cct	tgt	gcc	cca	ttg	gcc	gaa	tgc	aaa	ggt	tct	ctg	1997
Val	Arg	Pro	Thr	Pro	Cys	Ala	Pro	Leu	Ala	Glu	Cys	Lys	Gly	Ser	Leu	

200157W0SEQ
290

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cta Leu 295	gac Asp	gac Asp	agc Ser
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act Thr	gaa Glu	gat Asp 305	act Thr
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cct Pro	ttc Phe	aag Lys	gga Gly
ggt Gly 315	tac Tyr	acc Thr	aaa Lys
ggg Gly	cta Leu 320	gaa Glu	ggc Gly
gag Glu	agc Ser	cta Leu 325	ggc Gly
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tgc Cys	tct Ser	ggc Gly	agc Ser 330
gct Ala	gca Ala	gca Ala	ggg Gly
agc Ser 335	tcc Ser	ggg Gly	aca Thr
ctt Leu	gaa Glu 340	ctg Leu	ccg Pro
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tct Ser	acc Thr	ctg Leu 345	tct Ser
ctc Leu	tac Tyr	aag Lys	tcc Ser 350
gga Gly	gca Ala	ctg Leu	gac Asp 355
gag Glu 355	gca Ala	gct Ala	gcg Ala
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tac Tyr	cag Gln 360	agt Ser	cgc Arg
gac Asp	tac Tyr 365	tac Tyr	aac Asn
ttt Phe	cca Pro	ctg Leu	gct Ala 370
ctg Leu	gcc Ala	gga Gly	ccg Pro
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ccg Pro 375	ccc Pro	cct Pro	ccg Pro
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gct Ala 385	cgc Arg	atc Ile	aag Lys
ctg Leu	gag Glu 390		
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aac Asn	ccg Pro	ctg Leu	gac Asp
tac Tyr 395	ggc Gly	agc Ser	gcc Ala
tgg Trp	gcg Ala	gct Ala	gcg Ala
gca Ala	gca Ala	gca Ala	gca Ala
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cgc Arg	tat Tyr	ggg Gly	gac Asp 410
ctg Leu	gca Ala	agc Ser	ctg Leu
cat His 415	ggc Gly	gca Ala	gca Ala
gca Ala	gca Ala	gca Ala	gca Ala
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ggc Gly	tct Ser	ggg Gly	tca Ser
ccc Pro	tca Ser	gcc Ala	gcc Ala
gct Ala	tcc Ser	tca Ser	tcc Ser
tgg Trp 435	cac His	act Thr	ctc Leu
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ttc Phe	aca Thr 440	gcc Ala	gaa Glu
gaa Glu	ggc Gly	cag Gln 445	ttg Leu
tat Tyr	gga Gly	ccg Pro	tgt Cys 450
ggc Gly	ggc Gly	ggc Gly	ggc Gly
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agc Ser 495	gac Asp	ttc Phe	acc Thr
gca Ala	cct Pro 500	gat Asp	gtg Val
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tgg Trp	tac Tyr	cct Pro 505	ggc Gly
ggc Gly	atg Met	gtg Val	agc Ser 510
aga Arg	gtg Val	ccc Pro	tat Tyr
ccc Pro	ccc Pro	agg Arg	gac Asp 545
cat His	ggt Val	ttg Leu	ccc Pro
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tgt Cys	gtc Val 520	aaa Lys	agc Ser
gaa Glu	atg Met	ggc Gly 525	ccc Pro
tgg Trp	atg Met	gat Asp 530	agc Ser
tac Tyr	tcc Ser	gga Gly	cct Pro
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tac Tyr 535	ggg Gly	gac Asp	atg Met
cgt Arg	ttg Leu 540	gag Glu	act Thr
gcc Ala	agg Arg	gac Asp 545	cat His
ggt Val	ttg Leu	ccc Pro	att Ile 550
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gac Asp	tat Tyr	tac Tyr	ttt Phe
cca Pro	ccc Pro	cag Gln	aag Lys
acc Thr	tgc Cys	ctg Leu	atc Ile
tgt Cys	gga Gly	gat Asp	gaa Glu
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Phe	Phe	Lys	Arg	Ala	Ala	Glu	Gly	Lys	Gln	Lys	Tyr	Leu	Cys	Ala	Ser	
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aga	aat	gat	tgc	act	att	gat	aaa	ttc	cga	agg	aaa	aat	tgt	cca	tct	2957
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Cys	Arg	Leu	Arg	Lys	Cys	Tyr	Glu	Ala	Gly	Met	Thr	Leu	Gly	Ala	Arg	
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Lys	Leu	Lys	Lys	Leu	Gly	Asn	Leu	Lys	Leu	Gln	Glu	Glu	Gly	Glu	Ala	
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tcc	agc	acc	acc	agc	ccc	act	gag	gag	aca	acc	cag	aag	ctg	aca	gtg	3101
Ser	Ser	Thr	Thr	Ser	Pro	Thr	Glu	Glu	Thr	Thr	Gln	Lys	Leu	Thr	Val	
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Ser	His	Ile	Glu	Gly	Tyr	Glu	Cys	Gln	Pro	Ile	Phe	Leu	Asn	Val	Leu	
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gaa	gcc	att	gag	cca	ggg	gta	gtg	tgt	gct	gga	cac	gac	aac	aac	cag	3197
Glu	Ala	Ile	Glu	Pro	Gly	Val	Val	Cys	Ala	Gly	His	Asp	Asn	Asn	Gln	
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ccc	gac	tcc	ttt	gca	gcc	ttg	ctc	tct	agc	ctc	aat	gaa	ctg	gga	gag	3245
Pro	Asp	Ser	Phe	Ala	Ala	Leu	Leu	Ser	Ser	Leu	Asn	Glu	Leu	Gly	Glu	
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Cys	Met	Lys	Ala	Leu	Leu	Leu	Phe	Ser	Ile	Ile	Pro	Val	Asp	Gly	Leu	
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200157W0SEQ

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Arg	Arg	Phe	Tyr	Gln	Leu	Thr	Lys	Leu	Leu	Asp	Ser	Val	Gln	Pro	Ile	
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Gln	Val	Pro	Lys	Ile	Leu	Ser	Gly	Lys	Val	Lys	Pro	Ile	Tyr	Phe	His	
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Thr	Gln															
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200157WSEQ

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gcg Ala	tac Tyr	cag Gln	agt Ser 365	cgc Arg	gac Asp	tac Tyr	tac Tyr	aac Asn 370	ttt Phe	cca Pro	ctg Leu	gct Ala	ctg Leu 375	gcc Ala	gga Gly
ccg Pro	ccg Pro	ccc Pro 380	cct Pro	ccg Pro	ccg Pro	cct Pro	ccc Pro 385	cat His	ccc Pro	cac His	gct Ala	cgc Arg 390	atc Ile	aag Lys	ctg Leu
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cgg Arg	aaa Lys	tgt Cys 620	tat Tyr	gaa Glu	gca Ala	ggg Gly	att Ile 625	ctg Leu	gga Gly	gca Ala	gct Ala	gtt Val 630	gtt Val	gtt Val	tct Ser	2226
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		Met	Glu	Val	Gln	Leu	Gly	Leu	Gly	Arg						
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Val	Tyr	Pro	Arg	Pro	Pro	Ser	Lys	Thr	Tyr	Arg	Gly	Ala	Phe	Gln	Asn	25
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Pro	Glu	Ala	Ala	Ser	Ala	Ala	Pro	Pro	Gly	Ala	Ser	Leu	Leu	Leu	Gln	55
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Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	Gln	60
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4039

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Leu	Phe	Gln	Ser	Val	Arg	Glu	Val	Ile	Gln	Asn	Pro	Gly	Pro	Arg	His	
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Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
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cag cag cag cag cag cag cag cag cag cag cag caa gag act agc ccc 594
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cgt aga ggc ccc aca ggc tac ctg gtc ctg gat gag gaa cag caa cct 690
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Ser	Gln	Pro	Gln	Ser	Ala	Leu	Glu	Cys	His	Pro	Glu	Arg	Gly	Cys	Val	
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155 160 165

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ctt aaa gac atc ctg agc gag gcc agc acc atg caa ctc ctt cag caa 930
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200157W0SEQ

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