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(54) Title: ANTISENSE OLIGONUCLEOTIDE CHEMOTHERAPY FOR BENIGN HYPERPLASIA OR CANCER OF THE PROSTATE

(57) Abstract
Methods of selectively inhibiting the growth of or killing prostatic cells, using antisense oligonucleotides to prostate specific genes, are disclosed. The oligonucleotides may have natural nucleic acid structures or may be modified oligonucleotides with enhanced stability or tissue specific targeting. The prostate specific genes to which the antisense may be directed include the PSA and the probasin gene. Pharmaceutical compositions including such antisense oligonucleotides are also described for use in the methods. The methods and products are of particular utility in the treatment of benign prostatic hyperplasia or prostate cancer.
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ANTISENSE OLGONUCLEOTIDE CHEMOTHERAPY FOR
BENIGN HYPERPLASIA OR CANCER OF THE PROSTATE

Field of the Invention

The present invention relates to the field of chemotherapy for hyperplasias and cancers and, in particular, to chemotherapy for benign hyperplasia or cancer of the prostate. In addition, the invention relates to the field of antisense oligonucleotides and their use in human hyperplasia and cancer therapy.

Background of the Invention

Treatment of carcinoma of the prostate was one of the first successes of cancer chemotherapy, using the therapeutic program of castration and/or anti-androgen hormonal treatments introduced by Charles Huggins in the 1940s. A remarkable relief of symptoms and objective regression of bony metastases occurs under this endocrine therapeutic program. Unfortunately, after a "golden period" which lasts roughly 18 months, regrowth of the prostate cancer cells occurs and, in the later stages of the disease, sensitivity to and repression by anti-androgen hormonal therapy ceases. The conventional regimen of combined chemotherapeutic agents also is typically ineffective after the golden period, and a downhill clinical course follows, terminating in death.

A key problem had been the silent onset of cancer of the prostate, with growth beyond its capsule and metastasis to bone too frequently occurring before the first visit to a physician. During the last half dozen years, there has been increasing recognition of the importance of early diagnosis and significant improvements in the available tests. As a consequence of early diagnosis, detection of prostatic cancer still contained within its capsule has become more frequent. For this situation, radical prostatectomy has largely supplanted the traditional castration/estrogen therapy. Radiation targeted to the prostate itself and to any proximal capsular infiltration has also become a prominent modality of therapy. When these two therapeutic approaches fail to halt progression of the disease, which is all too often (see, e.g., Gittes (1991); and Catalona (1994)), the prospect of benefit from available chemotherapy is gloomy.

Less severe but more common than prostatic cancer is benign prostatic hyperplasia (BPH). This condition may be a precursor to full blown prostatic cancer or may continue for decades without evolving into the deadly carcinoma. Depending upon the degree of hypertrophy and the age of the patient, treatment may range from "watchful waiting" to more aggressive
approaches employing anti-androgen hormonal therapy, transurethral resection, or radical prostatectomy (see, e.g., Catalona (1994)).

Prostatic specific antigen (PSA) was first described by Wang et al. (1979) as a specific marker for prostate tissue and was subsequently identified by Papsidero et al. (1980) as being present in the sera of prostate cancer patients. Since then, PSA in the sera has become the most prevalent diagnostic marker for cancer of the prostate (see, e.g., Gittes (1991); Catalona (1994); Oesterling (1995); and Pienta (1995)). Levels of serum PSA are also elevated in BPH but less so than in progressed prostatic cancer. The complete amino acid sequence of PSA was disclosed by Watt et al. (1986) and the complete gene encoding PSA was disclosed by Lundwall (1989) and Klobeck et al. (1989). PSA is a glycoprotein having a single polypeptide chain and a molecular mass of approximately 34kDa. PSA is produced exclusively by epithelial cells of the prostate and is localized to the rough endoplasmic reticulum and associated vesicles until it is secreted into the acini and ducts of the prostate (see, e.g., Sinha, et al. (1987)). There PSA functions as a neutral serine protease which serves to liquefy the seminal coagulum by degrading seminal vesicle proteins including fibronectin and semenogelin (see, e.g., Lilja (1985); Warhol and Logtine (1985)). Higher serum PSA levels are correlated with the presence and progression of prostate cancer. Despite variance between patients, PSA levels are useful both in monitoring the progress of individual patients and as an indicator for diagnosing or staging prostate cancer (see, e.g., El-Shirbiny (1994)).

Probasin is a prostate specific basic protein first isolated by Matuo et al. (1982) from rat dorsolateral prostate. A cDNA to the rat probasin was disclosed by Spence et al. (1989) and revealed two in-frame translation initiation codons which are believed to account for the secreted and nuclear forms of the protein. The physiological role of probasin is unknown but it is a heparin binding protein that co-purifies with heparin binding growth factor-1 (HBGF-1) and is positively regulated by androgen. Probasin appears to have minor mitogenic activity (0.2-1% of HBGF-1) but this may be an artifact of its co-purification with HBGF-1 (Matuo et al. (1989)).

Summary of the Invention

The present invention provides methods for treating a patient diagnosed as having benign prostatic hyperplasia or a prostatic cancer. The methods include administering to the patient a therapeutically effective amount of a composition comprising an antisense oligonucleotide which
selectively hybridizes to a PSA or probasin gene or mRNA sequence of the patient, thereby inhibiting the expression of the PSA or probasin gene or mRNA sequence. This inhibition of the PSA or probasin genes or mRNAs by antisense oligonucleotides results in a significant inhibition of the growth or survival of prostatic cells. As a result, the methods provide a useful new means of treating benign prostatic hyperplasia and prostatic cancer.

The PSA antisense oligonucleotides may comprise at least 10 consecutive bases from SEQ ID NO.: 1, at least 10 consecutive bases from the joined exons of SEQ ID NO.: 1; or oligonucleotides that hybridize to the complements of these sequences under physiological conditions. More preferably, the antisense oligonucleotides comprise at least 15 consecutive bases, and most preferably, 20-30 consecutive bases from the above-described sequences.

The probasin antisense oligonucleotides may comprise at least 10 consecutive bases from SEQ ID NO.: 2, at least 10 consecutive bases from a genomic sequence corresponding to SEQ ID NO.: 2, or oligonucleotides that hybridize to the complements of these sequences under physiological conditions. More preferably, the antisense oligonucleotides comprise at least 15 consecutive bases, and most preferably, 20-30 consecutive bases from the above-described sequences.

Examples of sequences of the invention include, but are not limited to, those disclosed as SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, SEQ ID NO.: 6, SEQ ID NO.: 7, and SEQ ID NO.: 8.

In preferred embodiments, all of the above-described oligonucleotides are modified oligonucleotides. In one set of embodiments, the modified oligonucleotide includes at least one synthetic internucleoside linkage such as a phosphorothioate, alkylphosphonate, phosphorodithioate, phosphate ester, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, or carboxymethyl ester.

In other embodiments with modified oligonucleotides, the modified oligonucleotide has at least one low molecular weight organic group covalently bound to a phosphate group of said oligonucleotide. In another set of embodiments, the modified oligonucleotide has at least one low molecular weight organic group covalently bound to a 2' position of a ribose of said oligonucleotide. Such low molecular weight organic groups include lower alkyl chains or aliphatic groups (e.g., methyl, ethyl, propyl, butyl), substituted alkyl and aliphatic groups (e.g., aminomethyl, aminopropyl, aminohydroxyethyl, aminohydroxypropyl), small saccharides or
glycosyl groups.

In another set of embodiments the modified oligonucleotide has covalently attached thereto a prostate-targeting compound such as an androgen, androgen derivative, estrogen, estrogen derivative, estramustine, emcyt or estracyt.

In preferred embodiments, the antisense oligonucleotides are administered intravenously at a dosage between 1.0 μg and 100 mg per kg body weight of the patient.

The present invention also provides for any or all of the above-described antisense oligonucleotides, including the various modified oligonucleotides, in a pharmaceutical composition. The antisense oligonucleotides are admixed with a sterile pharmaceutically acceptable carrier in a therapeutically effective amount such that the isolated antisense oligonucleotide selectively hybridizes to the PSA or probasin gene or mRNA sequence when administered to a patient. A pharmaceutical kit is also provided in which such a pharmaceutical composition is combined with a pharmaceutically acceptable carrier for intravenous administration.

Detailed Description of the Invention

The present invention provides new methods for the treatment of cancer of the prostate and pharmaceutical compositions useful therefor. It is now disclosed that antisense oligonucleotides complementary to genes which are expressed only or predominantly in prostatic cells are effective for inhibiting the growth of and/or killing hyperplastic or cancerous cells of prostatic origin. In particular, the present invention provides oligonucleotides, including modified oligonucleotides, which have antisense homology to a sufficient portion of either the PSA or probasin gene such that they inhibit the expression of that gene. The expression of both of these genes is believed to be tissue specific to the prostate. Surprisingly, inhibition of either of these genes, both of which encode secreted proteins with no known function within prostate cells, inhibits the growth of these cells. Because the antisense oligonucleotides of the invention can be administered systemically but selectively inhibit prostate cells, the present invention has particular utility in late stage prostate cancer which has metastasized.

Definitions

In order to describe more clearly and concisely the subject matter of the present
invention, the following definitions are provided for specific terms used in the claims appended hereto:

**PSA.** As used herein, the abbreviation “PSA” refers to the prostatic specific antigen well known in the art and described in the various references cited herein. Genomic DNA sequences of the human PSA gene were disclosed in Lundwall (1989) and Klobeck et al. (1989). The Klobeck et al. (1989) sequence is available on GenBank (Accession number X14810) and is reproduced here as SEQ ID NO.: 1. The translation initiation codon of this gene is found at base positions 401-403 and the stop codon is at positions 5566-5568 of SEQ ID NO.: 1. The gene consists of five exons which are indicated on SEQ. ID NO.: 1. A TATA signal is found at positions 332-338 and a transcriptional start region appears at positions 355-365. As will be obvious to one of ordinary skill in the art, other alleles of the PSA gene, including other human alleles and homologues from other mammalian species, encoding a PSA protein and hybridizing to SEQ ID NO.: 1 under stringent hybridization conditions, will exist in natural populations and are embraced by the term “PSA gene” as used herein. A slightly different sequence for PSA is also available on GenBank (Accession number M27274). The PSA gene of the invention is intended to encompass all such sequences.

**Probasin.** As used herein, the term “probasin” refers to the probasin protein known in the art and described in the various references cited herein. A cDNA to one allele of the rat probasin gene was disclosed in Spence, et al. (1989). The Spence et al. (1989) sequence is available on GenBank (Accession number M27156) and is reproduced here as SEQ. ID NO.: 2. This gene has two potential translation initiation start codons which are in frame with each other. The first is at positions 41-43 of SEQ ID NO. 2 and the second is at positions 92-94. The stop codon is at positions 572-574. The bases between the first and second initiation codons encode a hydrophobic sequence consistent with a secretory signal sequence. Thus, it is believed that the initiation of translation from the first start codon leads to production of the secreted form of probasin whereas translation from the second results in the nuclear form of the protein. As used herein, the term “probasin gene” is specifically intended to include a gene encoding either or both forms of the probasin protein. In addition, as will be obvious to one of ordinary skill in the art, other alleles of the probasin gene, including other human alleles and homologues from other mammalian species, encoding a probasin protein and hybridizing to SEQ ID NO.: 2 under stringent hybridization conditions, will exist in natural populations and are embraced by the term
"probasin gene" as used herein.

**Antisense Oligonucleotides.** As used herein, the term "antisense oligonucleotide" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridizes under physiological conditions to DNA comprising a particular gene or to an mRNA transcript of that gene and, thereby, inhibits the transcription of that gene and/or the translation of that mRNA. In particular, by a "PSA-antisense oligonucleotide" and by a "probasin-antisense oligonucleotide" are meant oligonucleotides which hybridize under physiological conditions to the PSA gene/mRNA or probasin gene/mRNA and, thereby, inhibit transcription/translation of the PSA and probasin genes/mRNAs, respectively. The antisense molecules are designed so as to interfere with transcription or translation of PSA or probasin upon hybridization with the target. Those skilled in the art will recognize that the exact length of the antisense oligonucleotide and its degree of complementarity will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence. It is preferred that the antisense oligonucleotide be selected so as to hybridize selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any other sequence in the target cell under physiological conditions.

**Stringent hybridization conditions.** As used herein, the term "stringent hybridization conditions" means hybridization conditions from 30°C-60°C and from 5x to 0.1x SSC. Highly stringent hybridization conditions are at 45°C and 0.1x SSC. "Stringent hybridization conditions" is a term of art understood by those of ordinary skill in the art. For any given nucleic acid sequence, stringent hybridization conditions are those conditions of temperature and buffer solution which will permit hybridization of that nucleic acid sequence to its complementary sequence and not to substantially different sequences. The exact conditions which constitute "stringent" conditions, depend upon the length of the nucleic acid sequence and the frequency of occurrence of subsets of that sequence within other non-identical sequences. By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with identical sequences. Suitable ranges of such stringency conditions are described in Krause,
M.H. and S.A. Aaronson, *Methods in Enzymology*, 200:546-556 (1991). As used herein with respect to in vivo hybridization conditions, the term "physiological conditions" is considered functionally equivalent to the in vitro stringent hybridization conditions.

I. Design of PSA and Probasin Antisense Oligonucleotides

The present invention depends, in part, upon the discovery that the selective inhibition of the expression of PSA or probasin by antisense oligonucleotides in prostatic cells effectively inhibits cell growth and/or causes cell death.

Based upon SEQ ID NO.: 1 and SEQ ID NO.: 2, or upon allelic or homologous genomic or cDNA sequences, one of skill in the art can easily choose and synthesize any of a number of appropriate antisense molecules for use in accordance with the present invention. In order to be sufficiently selective and potent for PSA or probasin inhibition, such antisense oligonucleotides should comprise at least 10 and, more preferably, at least 15 consecutive bases which are complementary to the PSA or probasin mRNA transcripts. Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases. Although oligonucleotides may be chosen which are antisense to any region of the PSA or probasin genes or mRNA transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation or promoter sites. In addition, 3'-untranslated regions or telomerase sites may be targeted. Targeting to mRNA splicing sites has also been used in the art but may be less preferred if alternative mRNA splicing occurs. In addition, the PSA or probasin antisense is, preferably, targeted to sites in which mRNA secondary structure is not expected (see, e.g., Sainio et al. (1994)) and at which proteins are not expected to bind. With respect to probasin, an N-terminal antisense oligonucleotide may be targeted to either the first or the second initiation codon so as to interfere with translation of both forms or just the secreted form of probasin. Finally, although, SEQ ID NO.: 1 discloses a genomic DNA sequence and SEQ ID NO.: 2 discloses a cDNA sequence, one of ordinary skill in the art may easily derive the cDNA corresponding to the joined exons of SEQ ID NO.: 1 and may easily obtain the genomic DNA sequence corresponding to SEQ ID NO.: 2. Thus, the present invention also provides for antisense oligonucleotides which are complementary to the cDNA corresponding to SEQ ID NO.: 1 and the genomic DNA corresponding to SEQ ID NO.: 2. Similarly, antisense to allelic or homologous cDNAs and genomic DNAs are enabled without undue experimentation.
As will be understood by one of ordinary skill in the art, the antisense oligonucleotides of the present invention need not be perfectly complementary to the PSA or probasin genes or mRNA transcripts in order to be effective. Rather, some degree of mismatches will be acceptable if the antisense oligonucleotide is of sufficient length. In all cases, however, the oligonucleotides should have sufficient length and complementarity so as to hybridize to a PSA or probasin transcript under physiological conditions. Preferably, of course, mismatches are absent or minimal. In addition, although it is not recommended, the antisense oligonucleotides may have one or more non-complementary sequences of bases inserted into an otherwise complementary antisense oligonucleotide sequence. Such non-complementary sequences may “loop” out of a duplex formed by a PSA or probasin transcript and the bases flanking the non-complementary region. Therefore, the entire oligonucleotide may retain an inhibitory effect despite an apparently low percentage of complementarity. Of particular importance in this respect is the use of self-stabilized or hairpin oligonucleotides. Such oligonucleotides, or modified oligonucleotides, have a sequence at the 5’ and/or 3’ end which is capable of folding over and forming a duplex with itself. The duplex region, which is preferably at least 4-6 bases joined by a loop of 3-6 bases, stabilizes the oligonucleotide against degradation. These self-stabilized oligonucleotides are easily designed by adding the inverted complement of a 5’ or 3’ PSA or probasin sequence to the end of the oligonucleotide (see, e.g., Table 1, SEQ ID NO.: 5; Tang, J.-Y., et al. (1993) *Nucleic Acids Res.* 21:2729-2735).

In one set of embodiments, the PSA and probasin antisense oligonucleotides of the invention may be composed of “natural” deoxyribonucleotides, ribonucleotides, or any combination thereof. That is, the 5’ end of one nucleotide and the 3’ end of another nucleotide may be covalently linked, as in natural systems, via a phosphodiester internucleoside linkage. These oligonucleotides may be prepared by art recognized methods which may be carried out manually or by an automated synthesizer.

In preferred embodiments, however, the antisense oligonucleotides of the invention also may include “modified” oligonucleotides. That is, the oligonucleotides may be modified in a number of ways which do not prevent them from hybridizing to their target but which enhance their stability or targeting to prostatic cells or which otherwise enhance their therapeutic effectiveness. The term “modified oligonucleotide” as used herein describes an oligonucleotide in which (1) at least two of its nucleotides are covalently linked via a synthetic internucleoside
linkage (i.e., a linkage other than a phosphodiester linkage between the 5' end of one nucleotide and the 3' end of another nucleotide) and/or (2) a chemical group not normally associated with nucleic acids has been covalently attached to the oligonucleotide.

Preferred synthetic internucleoside linkages are phosphorothioates, alkylphosphonates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidate, and carboxymethyl esters. Further, one or more of the 5'-3' phosphate group may be covalently joined to a low molecular weight (e.g., 15-500 Da) organic group. Such low molecular weight organic groups include lower alkyl chains or aliphatic groups (e.g., methyl, ethyl, propyl, butyl), substituted alkyl or aliphatic groups (e.g., aminoethyl, aminopropyl, aminohydroxyethyl, aminohydroxypropyl), a small saccharides or glycosyl groups. Other low molecular weight organic modifications include additions to the internucleoside phosphate linkages such as cholesteryl or diamine compounds with varying numbers of carbon residues between the amino groups and terminal ribose. Oligonucleotides with these linkages or other modifications can be prepared according to known methods (see, e.g., Agrawal and Goodchild (1987); Agrawal et al. (1988); Uhlmann et al. (1990); Agrawal et al. (1992); Agrawal (1993); and U.S. Pat. No. 5,149,798).

The term “modified oligonucleotide” also encompasses oligonucleotides with a covalently modified base and/or sugar. For example, modified oligonucleotides include oligonucleotides having backbone sugars which are covalently attached to low molecular weight organic groups other than a hydroxyl group at the 3' position and other than a phosphate group at the 5' position. Thus modified oligonucleotides may include a 2'-O-alkylated ribose group such as a 2'-O-methylated ribose. In addition, modified oligonucleotides may include sugars such as arabinose instead of ribose. Alternatively, the modified oligonucleotides may be branched oligonucleotides. Unoxidized or partially oxidized oligonucleotides having a substitution in one or more nonbridging oxygen per nucleotide in the molecule are also considered to be modified oligonucleotides.

Also considered as modified oligonucleotides are oligonucleotides having prostate-targeting, nuclease resistance-conferring, or other bulky substituents and/or various other structural modifications not found in vivo without human intervention. The androgen receptor and other hormonal receptor sites on prostate cells allow for targeting antisense oligonucleotides specifically or particularly to prostatic cells. Attachment of the antisense oligonucleotides by a
molecular "tether" (e.g., an alkyl chain) to estramustine, emcylt or estracyt (Sheridan and Tew (1991)), for example, may provide prostatic targeting and the possibility of covalent alkylation of host prostatic DNA. Estramustine targets particularly to the ventral prostate (Forsgren, et al. (1979)). Similarly, one may covalently attach androgen, estrogen, androgen or estrogen derivatives, or other prostate cell ligands to antisense oligonucleotides using tethers and conjugating linkages for prostatic targeting. Finally, one may of course covalently attach other chemotherapeutic agents (e.g., dexamethasone, vinblastine, etoposide) to the antisense oligonucleotides for enhanced effect.

The most preferred modified oligonucleotides are hybrid or chimeric oligonucleotides in which some but not all of the phosphodiester linkages, bases or sugars have been modified. Hybrid modified antisense oligonucleotides may be composed, for example, of stretches of ten 2'-O-alkyl nucleotides or ten phosphorothioate synthetic linkages at the 5' and/or 3' ends, and a segment of seven unmodified oligodeoxynucleotides in the center, or of similar terminal segments of alkyl phosphonates, with central P=S or P=O oligonucleotides (Agrawal, et al. (1990); Metelev, et al. (1994)). The currently most preferred modified oligonucleotides are 2'-O-methylated hybrid oligonucleotides. Since degradation occurs mainly at the 3' end, secondarily at the 5' end, and less in the middle, unmodified oligonucleotides located at this position can activate RNase H, and yet are degraded slowly. Furthermore, the T_m of such a 27-mer is approximately 20°C higher than that of a 27-mer all phosphorothioate oligodeoxynucleotide.

This greater affinity for the targeted genomic area can result in greater inhibiting efficacy. Obviously, the number of synthetic linkages at the termini need not be ten and synthetic linkages may be combined with other modifications, such as alkylation of a 5' or 3' phosphate, or 2'-O-alkylation. Thus, merely as another example, one may produce a modified oligonucleotide with the following structure, where B represents any base, R is an alkyl, aliphatic or other substituent, the subscript S represents a synthetic (e.g. phosphorothioate) linkage, and each n is an independently chosen integer from 1 to about 20:

\[
\begin{align*}
\text{OH} \\
5'(B_S)_n\text{BBBB- ... -BBB(B}_S)_n\text{B}\quad \text{P=O}^3 \\
\text{O-R}
\end{align*}
\]
II. Products and Methods of Treatment for BPH and Prostate Cancer

The methods of the present invention represent new and useful additions to the field of benign prostate hyperplasia or prostate cancer therapy. In particular, the methods of the present invention are especially useful for late stage prostate cancer in which metastases have occurred and in which the cells have become resistant to estrogen or anti-androgen therapy. The methods may, however, also be used in benign prostate hyperplasia or early stage prostate cancer and may provide a substitute for more radical procedures such as transurethral resection, radical prostatectomy, or physical or chemical castration. The products of the present invention include the isolated antisense oligonucleotides described above. As used herein, the term “isolated” as applied to an antisense oligonucleotide means not covalently bound to and physically separated from the 5’ and 3’ sequences which flank the corresponding antisense sequence in nature.

Administration of the PSA or probasin antisense oligonucleotides may be oral, intravenous, parenteral, cutaneous or subcutaneous. For BPH or when the site of a prostatic tumor is known, the administration also may be localized to the prostate or to the region of the tumor by injection to or perfusion of the site.

PSA or probasin antisense oligonucleotides may be administered as part of a pharmaceutical composition. Such a pharmaceutical composition may include the antisense oligonucleotides in combination with any standard physiologically and/or pharmaceutically acceptable carriers which are known in the art. The compositions should be sterile and contain a therapeutically effective amount of the antisense oligonucleotides in a unit of weight or volume suitable for administration to a patient. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. The term "physiologically acceptable" refers to a non-toxic material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier will depend on the route of administration. Physiologically and pharmaceutically acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials which are well known in the art. The pharmaceutical composition of the invention may also contain other active factors and/or agents which inhibit prostate cell growth or increase cell death. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect or to minimize side-effects caused.
The pharmaceutical composition of the invention may be in the form of a liposome in which the PSA or probasin antisense oligonucleotides are combined, in addition to other pharmaceutically acceptable carriers, with amphiphatic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which are in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. No. 4,235,871; U.S. Pat. No. 4,501,728; U.S. Pat. No. 4,837,028; and U.S. Pat. No. 4,737,323.

The pharmaceutical composition of the invention may further include compounds such as cyclodextrins and the like which enhance delivery of oligonucleotides into cells. When the composition is not administered systemically but, rather, is injected at the site of the target cells, cationic detergents (e.g. Lipofectin) may be added to enhance uptake.

When a therapeutically effective amount of PSA or probasin antisense oligonucleotides is administered orally, the oligonucleotides will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder may contain from about 5 to 95% of the PSA and/or probasin antisense oligonucleotides and preferably from about 25 to 90% of the oligonucleotides. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition may contain from about 0.5 to 90% by weight of a PSA and/or probasin antisense oligonucleotide and preferably from about 1 to 50% of the oligonucleotide.

When a therapeutically effective amount of a PSA or probasin antisense oligonucleotide is administered by intravenous, cutaneous or subcutaneous injection, the oligonucleotides will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or
subcutaneous injection should contain, in addition to the antisense oligonucleotides, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or another vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

In preferred embodiments, when the target cells are readily accessible, administration of the antisense oligonucleotides is localized to the region of the targeted cells in order to maximize the delivery of the antisense and to minimize the amount of antisense needed per treatment. Thus, in one preferred embodiment, administration is by direct injection at or perfusion of the site of the targeted cells, such as a tumor. Alternatively, the antisense oligonucleotides may be adhered to small particles (e.g., microscopic gold beads) which are impelled through the membranes of the target cells (see, e.g., U.S. Pat. No. 5,149,655).

In another series of embodiments, a recombinant gene is constructed which encodes a PSA or probasin antisense oligonucleotide and this gene is introduced within the targeted cells on a vector. Such a PSA or probasin antisense gene may, for example, consist of the normal PSA or probasin sequence, or a subset of the normal sequences, operably joined in reverse orientation to a promoter region. An operable antisense gene may be introduced on an integration vector or may be introduced on an expression vector. In order to be most effective, it is preferred that the antisense sequences be operably joined to a strong eukaryotic promoter which is inducible or constitutively expressed.

In all of the above-described methods of treatment, the PSA and/or probasin antisense oligonucleotides are administered in therapeutically effective amounts. As used herein, the term "therapeutically effective amount" means that amount of antisense which, under the conditions of administration, including mode of administration and presence of other active components, is sufficient to result in a meaningful patient benefit, i.e., the killing or inhibition of the growth of target cells.

The amount of PSA and/or probasin antisense oligonucleotides in the pharmaceutical composition of the present invention will depend not only upon the potency of the antisense but also upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of antisense with which to treat each individual patient. Initially, the attending physician
will administer low doses of the inhibitor and observe the patient's response. Larger doses of antisense may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. In preferred embodiments, it is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 1.0 µg to about 100 mg of oligonucleotide per kg body weight.

The duration of intravenous therapy using the pharmaceutical compositions of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. Because a bolus of oligonucleotides, particularly highly negatively-charged phosphorothioate modified oligonucleotides, may have adverse side effects (e.g., rapid lowering of blood pressure), slow intravenous administration is preferred. Thus, intravenous administration of therapeutically effective amounts over a 12-24 hour period are contemplated. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

The following examples of the use of PSA and probasin antisense are presented merely to illustrate some of the oligonucleotides, including modified oligonucleotides, that may be employed according to the present invention. The particular oligonucleotides used, therefore, should not be construed as limiting of the invention but, rather, as indicative of the wide range of oligonucleotides which may be employed. As will be obvious to one of ordinary skill in the art in light of the present disclosure, a great many equivalents to the presently disclosed antisense oligonucleotides and disclosed methods are now available. In particular, other antisense oligonucleotides substantially complementary to subsets of SEQ ID NO.: 1 or SEQ ID NO.: 2, and chemical modifications of the same which do not prevent hybridization under physiological conditions, are contemplated as equivalents of the examples presented below. In general, the use of prostate specific antisense oligonucleotides is contemplated as a method of selectively inhibiting the growth of or killing prostatic cells.

**Experimental Examples**

Three permanent cell lines of human prostatic cancer were grown in monolayer culture: LNCaP, PC3-1435, and DU145, all obtained from the American Type Culture Collection. The LNCaP cells grow as stellate cells in a monolayer, retain hormone sensitivity and, of particular importance, secrete PSA into the tissue culture medium (Oesterling (1995)). Cells were grown
in Dulbecco's medium supplemented with 10 percent fetal calf serum, glutamate, pyruvate, penicillin and streptomycin, in 25-150 cm flasks, incubated at 37°C in 6 percent CO₂-air.

A number of PSA and probasin antisense oligonucleotides were tested for their inhibitory effect on prostatic cells. The base sequences of these oligonucleotides are disclosed as SEQ ID NO.: 3 through SEQ ID NO.: 9. SEQ ID NO.: 3 is antisense to positions 92-118 of the probasin gene (SEQ ID NO.: 1). SEQ ID NO.: 4 is antisense to a region upstream of the probasin gene at positions 76-99. SEQ ID NO.: 5 is a self-stabilized or hairpin oligonucleotide. The first 21 bases are complementary to positions 80-100 of the probasin gene. The remaining eight are identical to positions 84-91 of the gene, allowing formation of a 3' hairpin. SEQ ID NO.: 6 is another self-stabilized antisense oligonucleotide. The first 21 bases of this oligonucleotide are complementary to positions 92-112 of the probasin gene. The remaining eight are identical to positions 96-103 of the gene, allowing for formation of a 3' hairpin. SEQ ID NO.: 7 and SEQ ID NO.: 8 are antisense sequences corresponding to positions 401-427 and 384-410 of the PSA gene.

Table 1 shows some of the antisense oligonucleotides tested. The numbers at the left of each sequence correspond to the sequence numbers in the sequence listing. Antisense oligonucleotides with unmodified or natural internucleoside linkages (P=O) and oligonucleotides with all phosphorothioate synthetic linkages (P=S) were tested. In addition, modified oligonucleotides were tested in which just the terminal two phosphodiester linkages at each end had been replaced by phosphorothioate synthetic linkages (shown as a subscript S between nucleotides in Table 1) and/or in which small aliphatic chemical groups (e.g., 2-hydroxy-3-amino-propyl) were added to the 3' terminal phosphate.

Growth of the PC3-1435 cell line in tissue culture monolayers was consistently inhibited by addition of phosphorothioate-modified oligodeoxynucleotides targeted against the PSA or probasin genes and incubation for 24-48 hours thereafter. As the concentration of modified oligonucleotides is decreased from the 10-20 μM level, most effective inhibition occurs with specific antisense oligodeoxynucleotides at the 2-5 μM level, as contrasted with mismatched oligodeoxynucleotides (see Tables 2 and 3).

While the effects on cell growth (i.e. cell numbers) are readily manifest, visual substage microscopy of wells revealed additional features of the inhibition events using PSA antisense oligonucleotides against PC3-1435 cells. The first evidence of antisense inhibition is rupture of
the monolayer fabric. The stellate cells in a confluent culture lose contact with their neighbors, round up individually or in clumps, become pyknotic, and cease growing, as examined on successive days. There is an early loss of adhesiveness to the floor of the plastic wells. These changes are more severe (see Table 4) than those measured by \(^3\)H-thymidine incorporation into DNA, in other words more drastic than the impairment of DNA synthesis.

PSA protein was measured in the tissue culture medium in which LNCaP cells were grown in multi-well plates. This provided a quantitative assay, using a \(^{125}\)I-anti-PSA labeled antibody sandwich assay. We measured PSA levels in tissue cultures in as little as 2.5 \(\mu\)l of incubation medium by means of the iodinated antibody \(^{125}\)I-technique. The labeled antibody is available from Hybritech (San Diego, CA). Table 5 shows the results from two negative controls (no treatment), two positive controls employing an arbitrary oligonucleotide which is antisense to a portion of the HIV genome, and the SEQ ID NO.: 8 modified oligonucleotide of Table 1. Using this sandwich assay, the PSA output into the tissue culture medium of LNCaP cells was reduced 51 percent at the 5 \(\mu\)M level, and 45 percent at the 2.5 \(\mu\)M level at a 48 hour time period when the PSA gene was targeted by antisense oligonucleotides.

Each of the above-mentioned references and patents is hereby incorporated by reference.

**TABLE 1**

**Antisense Oligonucleotides**

<table>
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<tr>
<th>Sequence</th>
<th>Target</th>
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<tbody>
<tr>
<td>#3 (5')CTT-TTT-GAG-ATT-CTT-GTC-TGT-CAT-CAT(3')</td>
<td>Probasin, P=S</td>
</tr>
<tr>
<td>#3 (5')CTT-TTT-GAG-ATT-CTT-GTC-TGT-CAT-CAT(3')</td>
<td>Probasin, P=O</td>
</tr>
<tr>
<td>#4 (5')GTC-ATC-ATA-CTG-GAG-ACA-CCT-AGC(3')</td>
<td>Probasin-</td>
</tr>
<tr>
<td></td>
<td>upstream, P=S</td>
</tr>
<tr>
<td>#5 (5')TGT-CAT-CAT-ACT-GGA-GAC-ACC-TCT-CCA-GT(3')</td>
<td>Probasin 3'end</td>
</tr>
<tr>
<td></td>
<td>hairpin, P=S</td>
</tr>
<tr>
<td>#6 (5')GAG-ATT-CTT-GTC-TGT-CAT-CAT-TGA-CAG-AC(3')</td>
<td>Probasin 3'end</td>
</tr>
<tr>
<td></td>
<td>hairpin, P=S</td>
</tr>
<tr>
<td>#7 (5')GGT-GAG-GAA-GAC-AAC-CGG-GAC-CCA-CAT(3')</td>
<td>PSA, P=S</td>
</tr>
<tr>
<td>#8 (5')GGA-CCC-ACA-TGG-TGA-CAC-AGC-TCT-CCG(3')</td>
<td>PSA, P=S</td>
</tr>
</tbody>
</table>
### TABLE 2

**3H-thymidine incorporation into DNA PC3-1435**

human prostate cancer tissue culture

<table>
<thead>
<tr>
<th>Genes Targeted</th>
<th>Concentration (µM)</th>
<th>CPM†</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no oligo)</td>
<td>--</td>
<td>38,000</td>
<td>0</td>
</tr>
<tr>
<td>Probasin (P = S)</td>
<td>20</td>
<td>13,700</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>18,000</td>
<td>52</td>
</tr>
<tr>
<td>Mismatch (P = S)</td>
<td>20</td>
<td>20,000</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>27,000</td>
<td>30</td>
</tr>
</tbody>
</table>

† Averages of 3 separate wells

### TABLE 3

Comparisons of degree of inhibition of DNA synthesis in human PC3-1435 prostate cancer tissue cultures for one target and a mix of targets

<table>
<thead>
<tr>
<th>Genes targeted</th>
<th>Concentration (µM)</th>
<th>CPM †</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no oligo)</td>
<td>--</td>
<td>14,700</td>
<td>0</td>
</tr>
<tr>
<td>Mismatch</td>
<td>20</td>
<td>6,990</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10,750</td>
<td>27</td>
</tr>
<tr>
<td>Mix*</td>
<td>20</td>
<td>4,930</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6,054</td>
<td>59</td>
</tr>
</tbody>
</table>

* Mix: Probasin, PSA. PSA upstream, PSA farther upstream.

5 µM each at 20 µM total; 1.25 µM each at 5 µM total.

† Averages of 3 separate wells.
TABLE 4
Morphological Comparison of Treated and Control Cells

<table>
<thead>
<tr>
<th>Gene Target</th>
<th>Concentration μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA gene (P=S)</td>
<td>20   10  5  2</td>
</tr>
<tr>
<td>Mismatch (P=S)</td>
<td>4+   3+  2-1/2+ 1+</td>
</tr>
<tr>
<td></td>
<td>1-1/2+ 1/2+ 0  0</td>
</tr>
</tbody>
</table>

Observation 24 hours after oligonucleotide addition. Damage: 4+ devastating; 3+ severe; 2+ serious; 1+ visible; 1/2+ slight; 0 none

TABLE 5
PSA Levels in Media of Cultured LNCaP Cells 24 hours after Oligodeoxynucleotide Phosphorothioate Treatment

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Description</th>
<th>CPM</th>
<th>PSA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No treatment</td>
<td>11,940</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>No treatment</td>
<td>11,389</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>+ Control 5 μM</td>
<td>8,311</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>+ Control 1 μM</td>
<td>8,892</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>PSA antisense 5 μM</td>
<td>5,765</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>PSA antisense 1 μM</td>
<td>6,375</td>
<td>17</td>
</tr>
</tbody>
</table>
References

Forsgren et al. (1979) Cancer Res. 39:5155-5164.
(1) GENERAL INFORMATION:

(i) APPLICANT: WORCESTER FOUNDATION FOR BIOLOGICAL RESEARCH INC.

(ii) TITLE OF INVENTION: ANTISENSE OLIGONUCLEOTIDE CHEMOTHERAPY
FOR BENIGN HYPERPLASIA OR CANCER OF THE PROSTATE

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: WOLF, GREENFIELD & SACKS, P.C.
(B) STREET: 600 ATLANTIC AVENUE
(C) CITY: BOSTON
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02210

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
(viii) ATTORNEY/AGENT INFORMATION:
   (A) NAME: TWOMEY, MICHAEL J.
   (B) REGISTRATION NUMBER: 38,349
   (C) REFERENCE/DOCKET NUMBER: W0461/7029

(ix) TELECOMMUNICATION INFORMATION:
   (A) TELEPHONE: 617-720-3500
   (B) TELEFAX: 617-720-2441

10 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 5873 base pairs
   (B) TYPE: nucleic acid

15   (C) STRANDEDNESS: double
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

20 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
25   (A) ORGANISM: HOMO SAPIENS
   (G) CELL TYPE: LYMPHOID
   (H) CELL LINE: GM 607

(ix) FEATURE:
30   (A) NAME/KEY: TATA_signal
   (B) LOCATION: 332..338
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   (B) LOCATION: 355..365
   (D) OTHER INFORMATION: /note= "TRANSCRIPTIONAL START REGION"

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   (B) LOCATION: 401..446

(ix) FEATURE:
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   (B) LOCATION: 1688..1847

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   (B) LOCATION: 3477..3763

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   (B) LOCATION: 3907..4043

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5873

(2) INFORMATION FOR SEQ ID NO:2:

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30  (A) LENGTH: 776 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: RATTUS NORVEGICUS

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 41..574

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 41..91

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(A) NAME/KEY: mat_peptide
(B) LOCATION: 92..571

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Ala Ala Ser Ser Val Glu Lys Ile Asn Glu Gly Ser Pro Leu Arg Thr
10 15 20

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PCT/US96/15123
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776

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25 (A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

(ix) FEATURE:

(A) NAME/KEY: misc_feature

(B) LOCATION: 1..27

(D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS 92-118 OF SEQ ID NO.: 2."

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(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES
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   (A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

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   (A) NAME/KEY: misc_feature
   (B) LOCATION: 1..24
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   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

25

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

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   (A) NAME/KEY: misc_feature
   (B) LOCATION: 1..21
   (D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS
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   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

20  (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

25  (vi) ORIGINAL SOURCE:
   (A) ORGANISM: SYNTHETIC Oligonucleotide

(ix) FEATURE:
30  (A) NAME/KEY: misc_feature
   (B) LOCATION: 1..21
OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS 92-112 OF SEQ ID NO.: 2"

SEQUENCE DESCRIPTION: SEQ ID NO:6:

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INFORMATION FOR SEQ ID NO:7:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:
(A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS 401-427 OF SEQ ID NO.: 1."
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(2) INFORMATION FOR SEQ ID NO:8:

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(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

20 (vi) ORIGINAL SOURCE:
(A) ORGANISM: SYNTHETIC OLIGONUCLEOTIDE

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /note= "ANTISENSE TO POSITIONS 384-410 OF SEQ ID NO.: 1."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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27
We claim:

1. A method for treating a patient diagnosed as having benign prostatic hyperplasia or a prostatic cancer comprising
   administering to said patient a therapeutically effective amount of a composition
   comprising an antisense oligonucleotide which selectively hybridizes to a gene or mRNA sequence of said patient;
   wherein said antisense inhibits expression of said gene or mRNA sequence; and
   wherein said gene or mRNA sequence is selected from the group consisting of a PSA and a probasin gene or mRNA sequence.

2. A method as in claim 1 wherein said oligonucleotide is selected from the group consisting of
   (a) oligonucleotides comprising at least 10 consecutive bases from SEQ ID NO.: 1;
   (b) oligonucleotides comprising at least 10 consecutive bases from the joined exons of SEQ ID NO.: 1; and
   (c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or (b) under physiological conditions.

3. A method as in claim 1 wherein said oligonucleotide is selected from the group consisting of
   (a) oligonucleotides comprising at least 20 consecutive bases from SEQ ID NO.: 1;
   (b) oligonucleotides comprising at least 20 consecutive bases from the joined exons of SEQ ID NO.: 1; and
   (c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or (b) under physiological conditions.

4. A method as in claim 1 wherein said oligonucleotide is selected from the group consisting of
(a) oligonucleotides comprising at least 10 consecutive bases from SEQ ID NO.: 2;
(b) oligonucleotides comprising at least 10 consecutive bases from a genomic sequence corresponding to SEQ ID NO.: 2; and
(c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or
(b) under physiological conditions.

5. A method as in claim 1 wherein said oligonucleotide is selected from the group consisting of
(a) oligonucleotides comprising at least 20 consecutive bases from SEQ ID NO.: 2;
(b) oligonucleotides comprising at least 20 consecutive bases from a genomic sequence corresponding to SEQ ID NO.: 2; and
(c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or
(b) under physiological conditions.

10  6. A method as in claim 1 wherein said oligonucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, SEQ ID NO.: 6, SEQ ID NO.: 7, and SEQ ID NO.: 8.

7. A method as in claim 1 wherein said oligonucleotide is a modified oligonucleotide.

8. A method as in claim 7 wherein said oligonucleotide is a modified oligonucleotide including at least one synthetic internucleoside linkage.

9. A method as in claim 8 wherein said synthetic internucleoside linkage is selected from the group consisting of phosphorothioates, alkylphosphonates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidates, and carboxymethyl esters.

10. A method as in claim 7 wherein said oligonucleotide is a modified oligonucleotide having at least one low molecular weight organic group covalently bound to a phosphate group of said oligonucleotide.
11. A method as in claim 7 wherein said oligonucleotide is a modified oligonucleotide having at least one low molecular weight organic group covalently bound to a 2′ position of a ribose of said oligonucleotide.

12. A method as in claim 7 wherein said oligonucleotide is a modified oligonucleotide having covalently attached thereto a compound selected from the group consisting of androgen, androgen derivatives, estrogen, estrogen derivatives, estramustine, emcyt and estracyt.

13. A method as in claim 1 wherein said oligonucleotide is administered intravenously at a dosage between 1.0 µg and 100 mg per kg body weight of said patient.

14. A pharmaceutical composition comprising
   a sterile pharmaceutically acceptable carrier; and
   a therapeutically effective amount of an isolated antisense oligonucleotide which
   selectively hybridizes to a gene or mRNA sequence of a patient;
   wherein said antisense inhibits expression of said gene or mRNA sequence; and
   wherein said gene or mRNA sequence is selected from the group consisting of a PSA and
   a probasin gene or mRNA sequence.

15. A composition as in claim 14 wherein said oligonucleotide is selected from the group consisting of
   (a) oligonucleotides comprising at least 10 consecutive bases from SEQ ID NO.: 1;
   (b) oligonucleotides comprising at least 10 consecutive bases from the joined exons of
       SEQ ID NO.: 1; and
   (c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or (b) under physiological conditions.

16. A composition as in claim 14 wherein said oligonucleotide is selected from the group consisting of
   (a) oligonucleotides comprising at least 20 consecutive bases from SEQ ID NO.: 1;
   (b) oligonucleotides comprising at least 20 consecutive bases from the joined exons of
SEQ ID NO.: 1; and
(c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or (b) under physiological conditions.

17. A composition as in claim 14 wherein said oligonucleotide is selected from the group consisting of
(a) oligonucleotides comprising at least 10 consecutive bases from SEQ ID NO.: 2;
(b) oligonucleotides comprising at least 10 consecutive bases from a genomic sequence corresponding to SEQ ID NO.: 2; and
(c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or (b) under physiological conditions.

18. A composition as in claim 14 wherein said oligonucleotide is selected from the group consisting of
(a) oligonucleotides comprising at least 20 consecutive bases from SEQ ID NO.: 2;
(b) oligonucleotides comprising at least 20 consecutive bases from a genomic sequence corresponding to SEQ ID NO.: 2; and
(c) oligonucleotides that hybridize to the complements of the oligonucleotides of (a) or (b) under physiological conditions.

19. A composition as in claim 14 wherein said oligonucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NO.: 3, SEQ ID NO.: 4, SEQ ID NO.: 5, SEQ ID NO.: 6, SEQ ID NO.: 7, SEQ ID NO.: 8, and SEQ ID NO.: 9.

20. A composition as in claim 14 wherein said oligonucleotide is a modified oligonucleotide.

21. A composition as in claim 14 wherein said oligonucleotide is a modified oligonucleotide including at least one synthetic internucleoside linkage.

22. A composition as in claim 21 wherein said synthetic internucleoside linkage is selected from the group consisting of phosphorothioates, alkylphosphonates, phosphorodithioates,
phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidates, and carboxymethyl esters.

23. A composition as in claim 20 wherein said oligonucleotide is a modified oligonucleotide having at least one low molecular weight organic group covalently bound to a phosphate group of said oligonucleotide.

24. A composition as in claim 20 wherein said oligonucleotide is a modified oligonucleotide having at least one low molecular weight organic group covalently bound to a 2' position of a ribose of said oligonucleotide.

25. A composition as in claim 20 wherein said oligonucleotide is a modified oligonucleotide having covalently attached thereto a compound selected from the group consisting of androgen, androgen derivatives, estrogen, estrogen derivatives, estramustine, emcyt and estracyt.

26. A pharmaceutical kit comprising the pharmaceutical composition of claim 14 in a pharmaceutically acceptable carrier for intravenous administration.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/11 A61K31/70 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Relevant to claim No.</th>
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<td>WO 94 16738 A (HEKTEN INST FOR MEDICAL RESEA) 4 August 1994</td>
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<td>SHERIDAN, V. &amp; TEW, K.: &quot;Mechanism based chemotherapy for prostate cancer&quot;</td>
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[X] Further documents are listed in the continuation of box C.  [X] Patent family members are listed in annex.

1 Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another document or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"Z" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"V" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

1 Date of the actual completion of the international search
14 February 1997

Date of mailing of the international search report
26.02.97

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer
Andres, S
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INTERNATIONAL SEARCH REPORT

Box I  Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(3)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:
   Please see Further Information sheet enclosed.

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II  Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.
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