Title: ANTI-TESTOSTERONE ANTIBODIES

Abstract: The present invention provides antibodies directed to the steroid testosterone and related molecules and uses of such antibodies. In particular, in some embodiments, there are provided fully human monoclonal antibodies directed to testosterone and related molecules. In further embodiments, nucleotide sequences encoding, and amino acid sequences comprising, heavy and light chain immunoglobulin molecules, particularly sequences corresponding to contiguous heavy and light chain sequences spanning the framework regions and/or complementarity determining regions (CDRs), specifically from FR1 through FR4 or CDR1 through CDR3.
ANTI-TESTOSTERONE ANTIBODIES

The present invention relates to antibodies to testosterone and related metabolites and/or precursor molecules and uses of such antibodies in the treatment of diseases.

Background
Testosterone is a steroid hormone belonging to the androgen group that is secreted from male testicular Leydig cells and female ovaries. The functions of testosterone are mediated by the androgen receptor (AR), which activates target gene expression upon binding of testosterone or dihydrotestosterone to the AR. Binding of these androgens to the AR enables the AR to interact with androgen-responsive elements in the sequences of genes whose expression is regulated by androgen signalling (McEwan, 2004 Endocr Relat Cancer 11: 281-293). Testosterone can also be converted to estradiol, which can then activate estrogen receptors which also activate the expression of target genes. In healthy individuals, testosterone has several vital functions, including the development and function of the male reproductive system, maintenance of energy levels, bone density, libido and the immune system. However, abnormal levels of serum testosterone (TES) and its closely related metabolites or derivatives, termed hyperandrogenism, are associated with a number of medical conditions, and so the accurate determination of TES concentration is crucial to correct diagnosis and subsequent monitoring of treatment.

The strong conservation of steroid structures within the animal kingdom also makes them difficult targets to raise monoclonal antibodies against using conventional approaches. In addition, the low molecular weight of testosterone and related androgens precludes the generation of specific antibodies by conventional immunisation routes, as they are too small to be recognised by the mammalian immune system.

However, the potential benefits of isolating high affinity, high specificity monoclonal antibodies against testosterone or any other steroid hormone are considerable. The diagnosis of several conditions requires the accurate determination of TES levels,
which vary from 10-35 nmol/L in men, 0.3-3.5 nmol/L in women, and <1.0 nmol/L in children before the onset of puberty (Braunwald et al., 1987, Harrison's Principles of Internal Medicine 2.McGraw-Hill, New York). Currently, methods of immunological quantification of TES are limited to the use of polyclonal sera from rabbits, but these are hampered by problems with the generation of sera of sufficient quality, and considerable batch to batch variation. These difficulties could be overcome by the availability of a monoclonal antibody, which would provide a constant source of well-defined material with minimal batch-to-batch variability.

In addition, monoclonal antibodies with specificities for testosterone could also be used therapeutically. In men, conditions associated with abnormal TES levels include testicular cancer, premature puberty, hypogonadism and problems with spermatogenesis, aggression and sociopathic tendencies. In addition, androgen signalling plays a key role in the development of prostate cancer, which accounts for about 40% of all cancers diagnosed in men in the US, representing over 1.8 million cases as of January 2002 (NCI SEER data http://seer.cancer.gov/). Whilst testosterone is important for normal growth and differentiation of the prostate gland, it is also important for tumor growth. However, most prostate cancers treated with androgen ablation and anti-androgen therapy progress from an androgen-dependent to an androgen-independent state, causing a high incidence of relapse. In women, excess TES is associated with hirsutism (excessive hair growth), virilism (development of male secondary sex characteristics in the female), infertility, and other conditions associated with hyperandrogenism e.g. polycystic ovarian syndrome. Additionally, excess androgen production in women has been linked to obesity and metabolic syndrome (Pasquali Fertil Steril, 2006. 85:1319-1340).

An additional potential use for anti-testosterone antibodies is in the lowering of excess androgens associated with acne in both men and women. It is even possible that topically applied antibodies could be used to prevent testosterone entering cells in the skin or hair follicles alone.

Treatment of some conditions related to excess TES can be treated by chemical or surgical castration, but these are drastic measures and more often, treatment is centred
on targeting androgen signalling, by blocking the production of testicular androgens and inhibiting the function of the AR. Steroidal or non-steroidal anti-androgens can be administered in order to compete for binding to the AR, and block its effects on transcription of downstream gene targets. One of the main drawbacks of these types of therapy is that mutations can accumulate within the hormone-binding region of the AR, enabling it to be activated by anti-androgens or other androgen structures in the absence of testosterone (Balk, 2002 Urology 60: 132-139; Feldman and Feldman 2001 Nat Rev Cancer 1: 34-45). Therefore anti-androgens can become ineffective over time, and can even exacerbate the condition that they were intended to treat, via an agonistic effect on AR-mediated gene transcription.

There remains a need for antibodies with sufficient specificities and affinities to be used as clinical diagnostic tools or therapeutic agents. The current invention describes methods for the isolation of anti-androgen antibodies with sufficient specificities and affinities to be used as clinical diagnostic tools or therapeutic agents.

Summary of the Invention
The invention described herein relates to monoclonal antibodies that bind testosterone and affect testosterone function. Accordingly, embodiments of the invention relate to human anti-testosterone monoclonal antibodies and anti-testosterone monoclonal antibody preparations with desirable properties from diagnostic and therapeutic perspectives.

In particular, one embodiment of the invention provides anti-testosterone antibodies having characteristics that provide therapeutic utility, including, for example, but not limited to, strong binding affinity for testosterone, the ability to neutralize testosterone and related metabolites in vitro, and the ability to produce prolonged neutralization of testosterone and related metabolites in vivo. Antibodies of the invention can be generated with different specificities by the use of testosterone derivatives conjugated to protein carriers at different positions on the steroid structure shown below. For example, as discussed below, antibodies with different related steroid specificities can be generated to testosterone conjugated to the 17-hydroxyl position or to the 3-position.
One aspect of the invention is a method of reducing circulating levels of testosterone associated with prostate cancer, including: identifying a patient in need of treatment for prostate cancer; and administering to the patient a therapeutically effective dose of a fully human monoclonal antibody that binds with an affinity to testosterone of less than 10 nM, and reduces the circulating levels of testosterone.

Another aspect of the invention is a method of effectively treating polycystic ovarian syndrome (PCOS) in a patient, including: identifying a patient in need of treatment for PCOS; and administering to the patient a therapeutically effective dose of a fully human monoclonal antibody that specifically binds to testosterone and related metabolites.

Another aspect of the invention is a method of effectively treating obesity or metabolic syndrome in a patient, including: identifying a patient in need of treatment for obesity or metabolic syndrome; and administering to the patient a therapeutically effective dose of a fully human monoclonal antibody that specifically binds to testosterone and related metabolites.

Another aspect of the invention is a method of effectively treating acne in a patient, including: identifying a patient in need of treatment for acne; and administering to the patient a therapeutically effective dose of a fully human monoclonal antibody that specifically binds to testosterone and related metabolites.

Another aspect of the invention is a method of effectively treating excessive aggression in a patient, including: identifying a patient in need of treatment for
excessive aggression; and administering to the patient a therapeutically effective dose of a fully human monoclonal antibody that specifically binds to testosterone and related metabolites.

5 Another aspect of the invention is an isolated or purified fully human monoclonal antibody that binds to testosterone and its biologically active metabolites with an affinity of less than 20nm.

Another aspect of the invention is a therapeutic composition for the treatment of hyperandrogenism, including a fully human monoclonal antibody that binds to testosterone and its metabolites in association with a therapeutically acceptable carrier.

Detailed Description

15 Embodiments of the invention described herein relate to fully human anti-testosterone antibodies and their uses. Such fully human antibodies have the advantage of improved pharmacokinetic and safety profiles relative to antibodies containing non-human sequences and, accordingly, immunogenicity in humans is not anticipated. Through use of a dual-antigen selection strategy combined with the screening technology described herein, monoclonal antibodies with rare affinity and specificity have been discovered that have utility in therapeutic applications, i.e. the antibodies of the invention are recombinant human antibodies. The two classes of anti-testosterone antibodies of the invention described herein have been found to preferentially and specifically bind to testosterone, and, depending on the selection strategy, to different clinically relevant testosterone metabolites.

According to a first aspect of the invention, there is provided a recombinant anti-testosterone antibody wherein the antibody comprises at least one heavy chain, or a testosterone-binding fragment thereof, comprising a variable heavy chain region selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9; and at least one light chain or a testosterone-binding fragment thereof, comprising a variable light chain region
selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12.

The antibody may comprise at least one heavy chain of SEQ ID NO: 13 or SEQ ID NO: 15. Alternatively, antibody may comprise at least one light chain of SEQ ID NO: 14 or SEQ ID NO: 16. In other embodiments, the heavy chain may comprise the variable heavy chain regions SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3 and the light chain may comprise the variable light chain regions SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, or alternatively, the heavy chain may comprise the variable heavy chain regions SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9 and the light chain may comprise the variable light chain regions SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12.

One embodiment of the invention relates to antibodies that bind to the testosterone derived epitope that comprises testosterone, dihydrotestosterone and 17-beta estradiol (TDE), and other related steroids, and, as discussed below, it was discovered that antibodies, such as ARK2, ARK8 and ARK9 raised against testosterone conjugated via the 17-OH position, that bind to this epitope have therapeutic value for preventing such related steroids from binding the androgen receptor in human cells. As shown below these antibodies prevent activation of androgen receptor by DHT.

Antibody ARK8 has been deposited as XL1-Blue ARK-8 at NCIMB Ltd, Aberdeen, UK, under the terms of the Budapest Treaty as accession no. NCIMB 41412 on 11 July 2006.

The variable heavy chain (VH) and variable light chain (VL) regions of antibody ARK8 are shown in Figure 6(a) and (b), respectively. The complementarity determining regions (CDRs) are shown in bold and underlined.

The CDRs for the VH are:

CDR-H1: SYGMR

[SEQ ID NO: 1]
CDR-H2: TISSGGSTYYADSVKG [SEQ ID NO: 2]

CDR-H3: SRGTL [SEQ ID NO: 3]

5 The CDRs for the VL are:

CDR-L1: RSSQSLVYSDGNTYLN [SEQ ID NO: 4]

CDR-L2: KVSNRDS [SEQ ID NO: 5]

10 CDR-L3: MQGTHWPT [SEQ ID NO: 6]

One embodiment of the invention relates to antibodies that bind to the testosterone derived epitope that comprises testosterone and progesterone (TP), and other related steroids, as discussed below, it was discovered that antibodies, such as ARK17-1 and ARK17-9 raised against testosterone conjugated via the 3-O position, that bind to this epitope have therapeutic value for preventing such related steroids from binding the androgen receptor in human cells. As shown below these antibodies prevent activation of mutant androgen receptor (T877A) by progesterone.

20 Antibody ARK17-9 has been deposited as XL1-Blue ARK-17-9 at NCIMB Ltd, Aberdeen, UK, under the terms of the Budapest Treaty as accession no. NCIMB 41413 on 11 July 2006.

25 The variable heavy chain (VH) and variable light chain (VL) regions of antibody ARK17-9 are shown in Figure 7(a) and (b), respectively. The complementarity determining regions (CDRs) are shown in bold and underlined.

The CDRs for the VH are:

30 CDR-H1: SYWIG [SEQ ID NO: 7]

CDR-H2: IIYPGDSDTRYPSFQG [SEQ ID NO: 8]
CDR-H3: GSFRGFDY [SEQ ID NO: 9]

The CDRs for the VL are:

5

CDR-L1: GSSSNIGSNYYVY [SEQ ID NO: 10]

CDR-L2: RNNQRPS [SEQ ID NO: 11]

10

CDR-L3: AAWDDSLFPVV [SEQ ID NO: 12]

Still another embodiment of the invention relates to the synergistic effect in lowering testosterone and testosterone derived steroid levels in a mammal by the combined treatment of an antibody that binds to the TDE epitope in combination with a secondary antibody that binds to a different epitope of testosterone.

Accordingly, embodiments of the invention provide isolated antibodies, or fragments of those antibodies, that bind to testosterone. As known in the art, the antibodies can advantageously be, e.g., monoclonal, chimeric and/or human antibodies. Embodiments of the invention also provide cells for producing these antibodies.

In addition, embodiments of the invention provide for using these antibodies as a diagnostic or treatment for disease. For example, embodiments of the invention provide methods and antibodies for inhibition of testosterone associated with hyperandrogenism. Preferably, the antibodies are used to treat prostate cancer, acne, obesity, metabolic syndrome or polycystic ovarian syndrome. In association with such treatment, articles of manufacture comprising antibodies of the invention described herein are provided. Additionally, an assay kit comprising antibodies in accordance with the invention described herein is provided to screen for hyperandrogenism.

Antibodies of the invention described herein, such as anti-testosterone antibodies ARK8 and ARK 17-9, possess high affinity, significant neutralization potential, and sustained half-life and prolonged duration of action.
Definitions

Unless otherwise defined, scientific and technical terms used in connection with the invention described herein shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y. 1989). The nomenclatures utilized in connection with, and the laboratory 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 are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings: The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with all or a portion of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.
The term "isolated protein" referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the "isolated protein" (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, e.g. free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus. Preferred polypeptides in accordance with the invention comprise the human heavy chain immunoglobulin molecules and the human kappa light chain immunoglobulin molecules, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as the kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof.

The term "naturally occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally occurring.

The term "operably linked" as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter,
ribsomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for probes; although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

Peyman Chemical Reviews 90:543 (1990). An oligonucleotide can include a label for detection, if desired.

The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest will be at least 80%, and more typically with preferably increasing homologies of at least 85%, 90%, 95%, 99%, and 100%. Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. See M.O. Dayhoff, in Atlas of Protein Sequence and Structure, Vol. 5, 101-110 and Supplement 2 to Vol. 5, 1-10 (National Biomedical Research Foundation 1972). The two sequences or parts thereof are more preferably homologous if their, amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program. The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For
illustration, the nucleotide sequence "TATACT" corresponds to a reference sequence "TATAC" and is complementary to a "GTATA".

The following terms are used to describe the sequence relationships between two or more polynucleotide or amino acid sequences: "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity," and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 18 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8 amino acids in length, and often at least 48 nucleotides or 16 amino acids in length. Since two polynucleotides or amino acid sequences may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide or amino acid sequence) that is similar between the two molecules, and (2) may further comprise a sequence that is divergent between the two polynucleotides or amino acid sequences, sequence comparisons between two (or more) molecules are typically performed by comparing sequences of the two molecules over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window," as used herein, refers to a conceptual segment of at least 18 contiguous nucleotide positions or 6 amino acids wherein a polynucleotide sequence or amino acid sequence may be compared to a reference sequence of at least 18 contiguous nucleotides or 6 amino acid sequences and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions, deletions, substitutions, and the like (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. (U.S.A.) 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, (Genetics Computer Group, 575 Science Dr., Madison, Wis.), Geneworks, or
MacVector software packages), or by inspection, and the best alignment (i.e.,
resulting in the highest percentage of homology over the comparison window)
generated by the various methods is selected.

5 The term "sequence identity" means that two polynucleotide or amino acid sequences
are identical (i.e., on a nucleotide-by-nucleotide or residue-by-residue basis) over the
comparison window. The term "percentage of sequence identity" is calculated by
comparing two optimally aligned sequences over the window of comparison,
determining the number of positions at which the identical nucleic acid base (e.g., A,
T, C, G, U, or I) or residue occurs in both sequences to yield the number of matched
positions, dividing the number of matched positions by the total number of positions
in the comparison window (i.e., the window size), and multiplying the result by 100 to
yield the percentage of sequence identity. The terms "substantial identity" as used
herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein
the polynucleotide or amino acid comprises a sequence that has at least 85 percent
sequence identity, preferably at least 90 to 95 percent sequence identity, more usually
at least 99 percent sequence identity as compared to a reference sequence over a
comparison window of at least 18 nucleotide (6 amino acid) positions, frequently over
a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the
percentage of sequence identity is calculated by comparing the reference sequence to
the sequence which may include deletions or additions which total 20 percent or less
of the reference sequence over the comparison window. The reference sequence may
be a subset of a larger sequence.

25 As used herein, the twenty conventional amino acids and their abbreviations follow
conventional usage. See Immunology - A Synthesis (2d ed., Golub, E.S. and Gren,
acids) of the twenty conventional amino acids, unnatural amino acids such as [alpha]-,
[alpha]-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other
unconventional amino acids may also be suitable components for polypeptides of the
invention described herein. Examples of unconventional amino acids include: 4-
hydroxyproline, [gamma]-carboxyglutamate, [epsilon]-N,N,N-trimethyllysine,
[epsilon]-N-acytlylsine, O-phosphoserine, N-acetylseryne, N-formylmethionine, 3-
methylhistidine, 5-hydroxylysine, [sigma]-N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

Similarly, unless specified otherwise, the left-hand end of single-stranded polynucleotide sequences is the 5' end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences"; sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity, and most preferably at least 99 percent sequence identity. Preferably, residue positions that are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.
As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the invention described herein, providing that the variations in the amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar=glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. More preferred families are: serine and threonine are aliphatic-hydroxy family; asparagine and glutamine are an amide-containing family; alanine, valine, leucine and isoleucine are an aliphatic family; and phenylalanine, tryptophan, and tyrosine are an aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie et al., Science 253:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.
Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, ed., W. H. Freeman and Company, New York 1984); Introduction to Protein Structure (Branden, C. and Tooze, J. eds., Garland Publishing, New York, N.Y. 1991); and Thornton et al, Nature 354:105 (1991). [0069] The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally occurring sequence deduced, for example, from a full-length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, preferably at least 14 amino acids long, more preferably at least 20 amino acids long, usually at least 50 amino acids long, and even more preferably at least 70 amino acids long. The term "analog" as used herein refers to polypeptides which are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and which has at least one of the following properties: (1) specific binding to a testosterone epitope, under suitable binding conditions, (2) ability to block appropriate testosterone binding, or (3) ability to inhibit testosterone dependent cell growth in vitro or in vivo. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally occurring sequence. Analogs typically are at least 20 amino acids long, preferably at
least 50 amino acids long or longer, and can often be as long as a full-length naturally occurring polypeptide.

Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics." Fauchere, J. Adv. Drug Res. 15:29 (1986); Veber and Freidinger, TINS p.392 (1985); and Evans et al, J. Med. Chem. 30:1229 (1987). Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity), such as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: -CH2NH-, -CH2S-, -CH2-CH2-, -CH=CH-(cis and trans), -COCH2-, -CH(OH)CH2-, and -CH2SO-, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Giersch Ann. Rev. Biochem. 61:387 (1992); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

"Antibody" or "antibody fragment(s)" refer to an intact antibody, or a binding fragment thereof that competes with the intact antibody for specific binding. Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Binding fragments include Fab, Fab', F(ab')2, Fv, and single-chain antibodies (scFv). An antibody other than a "bispecific" or "bifunctional" antibody is understood to have each of its binding sites identical. An antibody substantially inhibits adhesion of a receptor to a counterreceptor when an excess of antibody reduces the quantity of receptor bound to counterreceptor by at
least about 20%, 40%, 60% or 80%, and more usually greater than about 85% (as measured in an in vitro competitive binding assay).

The term "epitope" includes any determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as a steroid, amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is <1 μM, preferably <100 nM and most preferably <20 nM or < 10 nM.

The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

"Active" or "activity" for the purposes herein refers to form(s) of testosterone (or metabolites) which retain a biological and/or an immunological activity of native or naturally occurring testosterone, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by native or naturally occurring testosterone other than the ability to induce the production of an antibody against an antigenic epitope possessed by native or naturally occurring testosterone and an "immunological" activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by native or naturally occurring testosterone.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

"Mammal" refers to any animal classified as a mammal, including humans, other primates, such as monkeys, chimpanzees and gorillas, domestic and farm animals, and zoo, sports, laboratory, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs,
goats, rabbits, rodents (for example rats or mice), etc. For purposes of treatment, the mammal is preferably human.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter-ions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONIC™.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an "F(ab')2" fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment that contains a complete antigen-recognition and binding site of the antibody. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, for example, even a single variable domain (e.g., the VH or VL portion of the Fv dimer or half of an Fv comprising only three CDRs specific for an antigen) may have the ability to recognize and bind antigen, although, possibly, at a lower affinity than the entire binding site.
A Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. F(ab')2 antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

"Solid phase" means a non-aqueous matrix to which the antibodies described herein can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phases can comprise the well of an assay plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

The term "liposome" is used herein to denote a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as an anti-testosterone antibody or fragment) to a mammal. The components of the liposomes are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

The term "small molecule" is used herein to describe a molecule with a molecular weight below about 500 Daltons.

As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but
are not limited to, the following: radioisotopes or radionuclides (e.g., $^3\text{H}$, $^{14}\text{C}$, $^{15}\text{N}$, $^{35}\text{S}$, $^{90}\text{Y}$, $^{99}\text{Tc}$, $^{111}\text{In}$, $^{125}\text{I}$, $^{131}\text{I}$), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, [beta]-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)).

As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

The term "patient" includes human and veterinary subjects.

"Anti-testosterone" means an antibody that has the ability to bind to testosterone or its metabolites generated by a natural biological process.
The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50 to 70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N. Y. (1989)). The variable regions of each light/heavy chain pair form the antibody-binding site. Thus, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDRI, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. 1991) (1987), or Chothia and Lesk, J. Mol. Biol. 196:901-17 (1987); Chothia et al., Nature 342:878-83 (1989).

A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai and Lachmann, Clin. Exp. Immunol. 79: 315-21 (1990); Kostelny et al, J. Immunol. 148:1547-53 (1992). Production of bispecific antibodies can be a relatively labor intensive process compared with production of conventional antibodies and yields and degree of purity are generally lower for
Bispecific antibodies. Bispecific antibodies do not exist in the form of fragments having a single binding site (e.g., Fab, Fab', and Fv).

**Human Antibodies and Humanization of Antibodies**

Human antibodies avoid certain of the problems associated with antibodies that possess murine or rat variable and/or constant regions. The presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. In order to avoid the utilization of murine or rat derived antibodies, fully human antibodies can be isolated from libraries of variable domain fragments displayed on phage fused to a coat protein or preferably a DNA binding domain (McGregor & Robins 2001 Jul 15;294(2):108-17 and WO 99/11785). As discussed above in connection with human antibody generation, there are advantages to producing antibodies with reduced immunogenicity. This can be accomplished with techniques of humanization and display techniques using appropriate libraries. It will be appreciated that murine antibodies or antibodies from other species can be humanized or primatized using techniques well known in the art. See e.g., Winter and Harris, Immunol Today 14:43-46 (1993) and Wright et at, Crit, Reviews in Immunol. 12:125-168 (1992). References to a human antibody therefore include fully human antibodies isolated from a library, as well as antibodies humanized by any one or more of the techniques referred to above.

The antibody of interest may be engineered by recombinant DNA techniques to substitute the CH1, CH2, CH3, hinge domains, and/or the framework domain with the corresponding human sequence. Also, the use of Ig cDNA for construction of chimeric immunoglobulin genes is known in the art (Liu et at, P.N.A.S. 84:3439 (1987) and J. Immunol. 139:3521 (1987)). mRNA is isolated from a hybridoma or other cell producing the antibody and used to produce cDNA. The cDNA of interest may be amplified by the polymerase chain reaction using specific primers.

Alternatively, a library is made and screened to isolate the sequence of interest. The DNA sequence encoding the variable region of the antibody is then fused to human constant region sequences. The sequences of human constant regions genes may be found in Kabat et at, "Sequences of Proteins of Immunological Interest," N.I.H.
publication no. 91-3242 (1991). Human C region genes are readily available from known clones. The choice of isotype will be guided by the desired effector functions, such as complement fixation, or activity in antibody-dependent cellular cytotoxicity. Preferred isotypes are IgGl, IgG3 and IgG4. Either of the human light chain constant regions, kappa or lambda, may be used. The chimeric, humanized antibody is then expressed by conventional methods.

More preferably, human antibodies or antibodies from other species can be generated through display-type technologies, including, without limitation, DBDx display, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules can be subjected to additional maturation, such as affinity maturation, as such techniques are well known in the art. Wright and Harris, supra., Hanes and Pluckthun, PNAS USA 94:4937-4942 (1997) (ribosomal display), Parmley and Smith, Gene 73:305-318 (1988) (phage display), Scott, TIBS 17:241-245 (1992), Cwirla et al, PNAS USA 87:6378-6382 (1990), Russell et al, Nucl Acids Res. 21:1081-1085 (1993), Hoogenboom et al, Immunol. Reviews 130:43-68 (1992), Chiswell and McCafferty, TIBTECH 10:80-84 (1992). If display technologies are utilized to produce antibodies that are not human, such antibodies can be humanized as described above. Additionally, human antibodies can be generated by the use of transgenic mice such as described by Green et al Nature Genetics 7:13-21 (1994).

Antibody fragments, such as Fv, F(ab')2 and Fab may be prepared by cleavage of the intact protein, e.g., by protease or chemical cleavage. Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the F(ab')2 fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

Consensus sequences of H and L J regions may be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.
Expression vectors include plasmids, retroviruses, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter, including retroviral LTRs, e.g., SV-40 early promoter, (Okayama et al, Mol. Cell. Bio. 3:280 (1983)), Rous sarcoma virus LTR (Gorman et al, P.N.A.S. 79:6777 (1982)), and moloney murine leukemia virus LTR (Grosschedl et al, Cell 41:885 (1985)). Also, as will be appreciated, native Ig promoters and the like may be used.

Regarding the importance of specificity and affinity to the therapeutic utility of anti-testosterone antibodies, it will be understood that one can generate anti-testosterone antibodies, and assess such antibodies for binding affinity or specificity. One approach that can be utilized is to take the heavy chain cDNA from an antibody, prepared as described herein and found to have good affinity to testosterone, and combine it with the light chain cDNA from a second antibody, and also found to have good affinity to testosterone, to produce a third antibody. The affinities or specificities of the resulting third antibodies can be measured by methods known in the art, and those with desirable affinities or specificities isolated and characterized. For example, based on the high binding affinity of anti-testosterone antibodies ARK8 and ARK17-9 antibodies, heavy chain cDNA from ARK8 can be linked to the light chain cDNA from ARK17-9 and the resulting antibody can be assayed for binding. Alternatively, the light chain of any of the antibodies described herein can be used as a tool to aid in the generation of a heavy chain that when paired with the light chain will exhibit a high affinity for testosterone, or vice versa. For example, the light chain or the light chain variable region of ARK8 can be expressed with a library of heavy chains or heavy chain variable regions. These heavy chain variable regions in this library could be isolated from naive animals, isolated from hyperimmune animals, generated
artificially from libraries containing variable heavy chain sequences that differ in the CDR regions, or generated by any other methods that produce diversity within the CDR regions of any heavy chain variable region gene (such as random or directed mutagenesis). These CDR regions, and in particular CDR3, may be a significantly different length or sequence identity from the heavy chain initially paired with anti-testosterone antibody ARK8. The resulting library could then be screened for high affinity binding to testosterone to generate a therapeutically relevant antibody molecule with similar properties as ARK8 antibody (high affinity and neutralization). A similar process using the heavy chain or the heavy chain variable region can be used to generate a therapeutically relevant antibody molecule with a unique light chain variable region. Furthermore, the novel heavy chain variable region, or light chain variable region, can then be used in a similar fashion as described above to identify a novel light chain variable region, or heavy chain variable region, that allows the generation of a novel antibody molecule.

Another combinatorial approach that can be utilized is to perform mutagenesis on germ line heavy and/or light chains that are demonstrated to be utilized in the antibodies of the invention described herein, particularly in the complementarity determining regions (CDRs). The affinities of the resulting antibodies can be measured as described herein and those with desirable dissociation constants isolated and characterized. Upon selection of a preferred binder, the sequence or sequences encoding the same may be used to generate recombinant antibodies as described above. Appropriate methods of performing mutagenesis on an oligonucleotide are known to those skilled in the art and include chemical mutagenesis, for example, with sodium bisulfite, enzymatic misincorporation, and exposure to radiation. It is understood that the invention described herein encompasses antibodies with substantial identity, as defined herein, to the antibodies explicitly set forth herein, whether produced by mutagenesis or by any other means. Further, antibodies with conservative or non-conservative amino acid substitutions, as defined herein, made in the antibodies explicitly set forth herein, are included in embodiments of the invention described herein.
In the preferred embodiment, the properties of anti-testosterone antibodies ARK8 and ARK17-9 include, for example, high affinity of the antibodies for testosterone, specificity for a neutralizing epitope on testosterone or orthologous steroids, and the ability to neutralize androgen receptor activity in testosterone responsive cells. The examples are illustrative of the many possible means under the current art to use the sequences of the invention to aid in the generation of an antibody with similar properties to anti-testosterone antibodies ARK8 and ARK17-9. Any improvements to the current art or any generation of unique antibodies through future or conventional technology with the aforementioned properties ascribed to anti-testosterone antibodies ARK8 and ARK17-9 are deemed to be "functionally equivalent" to anti-testosterone antibodies ARK8 and ARK17-9 and thereby are included in embodiments of the invention described herein.

As will be appreciated, antibodies in accordance with the invention described herein can be expressed in various cell lines. Sequences encoding particular antibodies can be used for transformation of a suitable mammalian host cell. Transformation can be accomplished by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455. The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have
high expression levels and produce antibodies with constitutive testosterone binding properties.

**Diagnostic Use**

Antibodies in accordance with the invention described herein are useful for diagnostic assays, and, particularly, in vitro assays, for example, for use in determining the level of circulating testosterone in the bloodstream. It is possible to determine the presence and/or severity of hyperandrogenism in a subject based on expression levels of the testosterone antigen. Patient samples, preferably blood, and more preferably blood serum, are taken from subjects diagnosed as being at various stages in the progression of hyperandrogenism, and/or at various points in the therapeutic treatment of the disease. The concentration of the testosterone antigen present in the blood samples is determined using a method that specifically determines the amount of the antigen that is present. Such a method includes an ELISA method in which, for example, antibodies of the invention may be conveniently immobilized on an insoluble matrix, such as a polymer matrix. Using a population of samples that provides statistically significant results for known levels of progression or therapy, a range of concentrations of the antigen that may be considered characteristic of each level is designated.

In order to determine the degree of hyperandrogenism in a subject under study, or to characterize the response of the subject to a course of therapy, a sample of blood is taken from the subject and the concentration of the testosterone antigen present in the sample is determined. The concentration so obtained is used to identify in which range of concentrations the value falls. The range so identified correlates with a level of disease progression or a level of therapy identified in the various populations of diagnosed subjects, thereby providing a level in the subject under study.

**Therapeutic Use**

In accordance with embodiments of the invention described herein and based on the activity of the antibodies that are produced and characterized herein with respect to testosterone, the design of other therapeutic modalities beyond antibody moieties is facilitated. Such modalities include, without limitation, advanced antibody
therapeutics, such as bispecific antibodies, immunotoxins, and radiolabeled therapeutics, generation of peptide therapeutics, gene therapies, particularly intrabodies, antisense therapeutics, and small molecules.

5 In connection with the generation of advanced antibody therapeutics, where complement fixation is a desirable attribute, it may be possible to sidestep the dependence on complement for cell killing through the use of bispecifics, immunotoxins, or radiolabels. In connection with immunotoxins, antibodies can be modified to act as immunotoxins utilizing techniques that are well known in the art. See e.g., Vitetta Immunol Today 14:252 (1993). See also U.S. Patent No. 5,194,594. In connection with the preparation of radiolabeled antibodies, such modified antibodies can also be readily prepared utilizing techniques that are well known in the art. See e.g., Junghans et al. in Cancer Chemotherapy and Biotherapy 655-686 (2d edition, Chafner and Longo, eds., Lippincott Raven (1996)).

15 Biologically active anti-testosterone antibodies in accordance with the invention described herein may be used in a sterile pharmaceutical preparation or formulation to reduce the level of serum testosterone thereby effectively treating pathological conditions where, for example, serum testosterone is abnormally elevated. Such conditions include, for instance, hyperandrogenism conditions such as prostate cancer, PCOS, obesity, acne and metabolic syndrome. The anti-testosterone antibody preferably possesses adequate affinity to potently suppress testosterone to within the target therapeutic range, and preferably has an adequate duration of action to allow for infrequent dosing. Preferably, in all hyperandrogen patient populations, a prolonged duration of action of greater than two days, preferably three to five days, more preferably seven to ten days, will allow for less frequent and more convenient dosing schedules by alternate parenteral routes such as subcutaneous or intramuscular injection. A preferred antibody of the invention has a KD of 10nM, a half-life exceeding 1.5 days, preferably 2 to 4 days, more preferably 6 to 10 days and suppresses the levels of circulating testosterone by greater than 25%, preferably by greater than 60%, 65%, or 70%, more preferably by greater than 75%, 80% or 85%. In a preferred embodiment, an antibody demonstrating a suppression of greater than 75% for greater than 1.5 days is provided.
Biologically active anti-testosterone antibodies of the invention may be employed alone or in combination with other therapeutic agents. For example, current approved therapy for prostate cancer consists of (1) LHRH analogues, and, (2) androgen ablation therapy consisting of steroid based anti-androgens or castration. The above-mentioned peptide LHRH analogues could be used in combination with antibodies of the invention described herein in a therapeutic regime.

When used for in vivo administration, the antibody formulation should be sterile. This is readily accomplished, for example, by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The antibody ordinarily will be stored in lyophilized form or in solution. Therapeutic antibody compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having an adapter that allows retrieval of the formulation, such as a stopper pierceable by a hypodermic injection needle.

The route of antibody administration is in accord with known methods, e.g., injection or infusion by intravenous, subcutaneous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intrathecal, inhalation or intralesional routes, or by sustained release systems as noted below. The antibody is preferably administered continuously by infusion, by bolus injection, or by subcutaneous injection. An effective amount of antibody to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer antibody until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays or by the assays described herein.

Antibodies in accordance with the invention may be prepared in a mixture with a pharmaceutically acceptable carrier. This therapeutic composition can be administered intravenously or through the nose or lung, preferably as a liquid or powder aerosol (lyophilized). The composition may also be administered parenterally or
subcutaneously as desired. When administered systemically, the therapeutic composition should be sterile, pyrogen-free and in a parenterally acceptable solution having due regard for pH, isotonicity, and stability. These conditions are known to those skilled in the art.

Briefly, dosage formulations of the compounds of the invention described herein are prepared for storage or administration by mixing the compound having the desired degree of purity with physiologically acceptable carriers, excipients, or stabilizers. Such materials are non-toxic to the recipients at the dosages and concentrations employed, and include buffers such as TRIS HCl, phosphate, citrate, acetate and other organic acid salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) peptides such as polyarginine, proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidinone; amino acids such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium and/or nonionic surfactants such as TWEEN, PLURONICS or polyethylene glycol.

Sterile compositions for injection can be formulated according to conventional pharmaceutical practice as described in Remington’s Pharmaceutical Sciences (18th ed., Mack Publishing Company, Easton, PA 1990). For example, dissolution or suspension of the active compound in a vehicle such as water or naturally occurring vegetable oil like sesame, peanut, or cottonseed oil or a synthetic fatty vehicle like ethyl oleate or the like may be desired. Buffers, preservatives, antioxidants and the like can be incorporated according to accepted pharmaceutical practice.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, films or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer et al, J. Biomed Mater. Res., 15:167-277 (1981) and Langer, Chem. Tech., 12:98-105 (1982), or poly(vinylalcohol)), polylactides (U.S. Pat. No.
3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al, Biopolymers, 22:547-56 (1983)), non-degradable ethylene-vinyl acetate (Langer et al., supra), degradable lactic acid-glycolic acid copolymers such as the LUPRON Depot(TM) (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37 degrees Celsius, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.


The dosage of the antibody formulation for a given patient will be determined by the attending physician taking into consideration various factors known to modify the action of drugs including severity and type of disease, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors. Therapeutically effective dosages may be determined by either in vitro or in vivo methods.
An effective amount of the antibody of the invention to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage might range from about 0.001 mg/kg to up to 100 mg/kg or more, depending on the factors mentioned above. Desirable dosage concentrations include 0.001 mg/kg, 0.005 mg/kg, 0.01 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.5 mg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30mg/kg, 35mg/kg, 40mg/kg, 45mg/kg, 50mg/kg, 55mg/kg, 60mg/kg, 65mg/kg, 70mg/kg, 75mg/kg, 80mg/kg, 85mg/lkg, 90mg/kg, 95mg/kg, and 100mg/kg or more. Typically, the clinician will administer the therapeutic antibody until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays or as described herein.

Preferred features for the second and subsequent aspects of the invention are as for the first aspect mutatis mutandis.

The present invention will now be further described with reference to the following Examples which are present for the purposes of illustration only. Reference is also made herein to a number of Figures in which:

FIGURE 1 shows the steroid binding specificity of soluble scFv antibodies raised against 3-O conjugated testosterone demonstrated by competition ELISA against a diverse collection of testosterone and metabolites.

FIGURE 2 shows the steroid binding specificity of soluble scFv antibodies raised against 17-OH conjugated testosterone demonstrated by competition ELISA against a diverse collection of testosterone and metabolites.

FIGURE 3 shows testosterone–wild type androgen receptor–luciferase activation assay in PC3 cells demonstrating in vitro prevention of androgen receptor depending on antibody specificity. All antibodies prevent testosterone activation of androgen receptor.
FIGURE 4 shows dihydro-testosterone--wild type androgen receptor-luciferase activation assay in PC3 cells demonstrating in vitro prevention of androgen receptor depending on antibody specificity. 3-O conjugation selection antibodies prevent androgen receptor activation.

FIGURE 5 shows progesterone--T877A mutant androgen receptor-luciferase activation assay in PC3 cells demonstrating in vitro prevention of androgen receptor depending on antibody specificity. 17-OH conjugation selection antibodies prevent androgen receptor activation.

FIGURE 6 shows CDR sequences of clone ARK8 in bold font and underline. Figure 6(a) shows the variable heavy chain regions [SEQ ID NO: 13] and Figure 6(b) shows the variable light chain regions [SEQ ID NO: 14].

FIGURE 7 shows CDR sequence of clone ARK17-9 in bold font and underline. Figure 7(a) shows the variable heavy chain regions [SEQ ID NO: 15] and Figure 6(b) shows the variable light chain regions [SEQ ID NO: 16].

Example 1. Testosterone Conjugate Preparation
Prior to biopanning of the naive library the testosterone hapten was conjugated to two carrier proteins, bovine serum albumen (BSA) and thyroglobulin (TG). In addition testosterone was also biotinylated with Biotin PEO-Amine (Pierce).

Both the conjugation and biotinylation of the testosterone hapten was achieved through the condensation of primary amides of the conjugate proteins with the carboxylic acid of the two testosterone derivatives testosterone 3-O-carboxymethyl amine (Sigma) and testosterone 17-hemisuccinate (Steraloids). This results in a zero-length cross-link between the two molecules via a direct covalent bond.

The formation of the amide linkage between the carboxyl of the testosterone derivative and the amine of the conjugate proteins BSA and TG was mediated using N,N'-Dicyclohexyl carbodiimide (DCC) in N,N'-dimethylformidine (DMF). DCC
reacts with the carboxylates to form an active ester intermediate o-acylisourea, the primary amine of the conjugate protein reacts with this intermediate forming the testosterone conjugate and a dicyclohexylisourea by-product. However o-acylisourea in the presence of water will rapidly hydrolyse in the absence of the amide of the conjugate protein regenerating the carboxyl group. N-Hydroxysuccinimide hydrolyses relatively slowly in water compared to the o-acylisourea ester. On addition to the reaction mix the N-Hydroxysuccinimide attacks the carboxyl of the ester creating a more stable intermediate, under nucleophilic attack from the amide of the conjugate the carboxyl is released to for a stable amide bond. With the addition of this second more stable intermediaed efficiency of the conjugation reaction can be increased 20-fold.

Both conjugate proteins BSA and TG (1 mg ml-1) dissolved in 5 ml sterile water, (Baxter) stirred at room temperature 1 hr. Forty times molar excess to conjugate protein (BSA and TG) dissolved in 1 ml dimethylformamide (Aldrich) together with equimolar N-Hydroxysuccinimide (NHS), equimolar 1-hydroxy benzotriazole (HOBr) and 10% excess Dicyclohexyl carbodiimide (DCC) stirred vigorously at room temperature for 3.5 hr. Two hundred and fifty microlitres of the activated testosterone ester added very slowly to the conjugate protein stirred slowly 23 hr at 4°C. Conjugates dialysed against 4 l PBS 3 hr dialysed again for 24 hr against fresh PBS. The number of haptens conjugated to BSA was determined though MALDI-TOF mass spectroscopy.

Both testosterone derivatives were biotinylated to biotin PEO-amine (Pierce) as with BSA and TG however testosterone was added in a 5 molar excess. The biotinylated testosterone was vacuum dried and successful biotinylation determined using LC/MS.

The O-acylisourea intermediate can also undergo spontaneous rearrangement to an inactive N-acylisourea product. To prevent this rearrangement 1-hydroxy benzotriazole (HOBr) was added in equimolar concentrations to DCC o-acylisourea rearranges to form an active ester secondary intermediate. The reaction must take place in the absence of any free amino acids, o-acylisourea in the presence of small amino acids react to form an azlactone. The azlactone ring can open in the presence of
the primary amine of the conjugate proteins resulting in a conjugate with a short link between the conjugate protein and the testosterone. This link could be selective in the antibody binding.

5 Example 2. Biopanning

It is difficult to raise antibodies against haptns smaller than 1000Da as they are too small to be recognised the immune system. Testosterone is a small hapten (288da) after conjugation to a carrier protein can be used to screen large naive antibody libraries through the technique of DBDx phage display (McGregor & Robins 2001 Jul 15;294(2):108-17) and biopanning.

A human synthetic antibody library, HuHAP 2 containing 2x108 unique human antibodies derived from multiple natural frameworks with synthetic diversity introduced into CDR3 of the heavy chain was used to pan against the two testosterone and biotinylated conjugates.

Testosterone-3-BSA conjugate (17 hapten per BSA) was immobilised on the surface of a 75 mm x 12 mm immunotube, (Nunc) 4 ml (1μg ml-1) incubated at 37°C 1hr. The tube washed 3 times PBS and blocked with 2% marvel-PBS (2%MPBS) and incubated at 37°C 1hr. Four milliliters of 2% MPBS containing the phagemid particles of the HuHAP2 library and incubated at room temperature (RT) rotating 30 min and for a further 90 min standing. The tube was washed 10 times 0.1% PBS-Tween (PBST) and a further 10 times PBS. Unbound phage was eluted with the addition of 100 mM triethylamine (TEA) and rotated for 10 min and quenched with the addition of 0.5 ml 1 M 2-amino-2-hydroxymethylpropane-1,3-diol (Tris pH 7.4). Half of the eluted phage (0.75ml) was infected into 9.25 ml of exponential phase TG1 cells in 2 x TY broth by incubation for 30 min at 37°C.

The infected TG1 culture was centrifuged at 4000 rpm (Anmita PK120R) for 10 min and the supernatant discarded. The pellet was resuspended in 0.5 ml 2 x TY and plated onto a 140 mm large bio-dish, (Sterilin) containing ampicillin (100 μg ml-1) and 1% glucose and incubated overnight at 30°C.
One millilitre of 2 x TY added to the incubated bio-dish and the cells loosened with a sterile glass spreader. One hundred millilitres of 2 x TY-Amp100-1% Glucose was inoculated with 100 of the scraped bacteria and incubated (shaking) in a baffled flask at 37°C until OD600 0.5 Ten millilitres of the culture was infected with M13K07 helper phage at a ratio of 1:20 and incubated in a 50 ml tube (Nunc) without shaking at for 30 min.

Testosterone-BSA was used as the conjugate for pan one and the phage eluted with triethylamine, (TEA) for pan two biotinylated testosterone was immobilised on streptavidin coated beads panning done in solution and again phage eluted with TEA. For pan three testosterone 3-TG was used a conjugate however on this occasion the phage were eluted with free testosterone.

Results from a polyclonal ELISA shows slight enrichment of phage after both pans one and two, after pan three however there has been a considerable enrichment of phage. After 96 well monoclonal ELISA three positive clones were identified against both conjugates but negative against the conjugate proteins. Further to this another 96 random colonies were picked for a monoclonal ELISA this resulted in 22 positive clones however fingerprint analysis and sequencing of the total 22 positives confirmed three individual clones (ARK2, ARK8 & ARK9).

In addition to the testosterone conjugated to the three position (A-Ring) a second panning strategy was undertaken with the testosterone conjugated and biotinylated to the 17 position (D-Ring). Panning with the new conjugate a previously however pan one with testosterone 17-BSA, pan two testosterone 17-TG and pan three with biotinylated testosterone-17. Phage from pans one and two were both eluted with TEA however pan three was eluted with free testosterone. Polyclonal ELISA indicated enrichment by pan 2 and a considerable enrichment by pan 3.

From pan three 96 clones were picked at random and used in a monoclonal ELISA from this twenty three clones positive to both BSA and TG conjugate were identified. As previously fingerprint analysis and sequencing confirmed two individual clones (ARK17-1 & ARK17-2).
Example 3. Antibody specificity

Competition ELISA was used to investigate if the addition of excess free testosterone to show the all five antibodies were selective for testosterone. In addition a number of different steroids were also used to show any cross reactivity within the steroid family. Competition ELISA was used to investigate if the addition of excess free testosterone plus Dihydrotestosterone (DHT), Dihydroepiandrosterone (DHEA), 17-α-estradiol, 17-β-estradiol, Esterol, Progesterone, and Esterone to show the specificity of all five scAbs against these steroids.

Ninety six well ELISA plate coated with 100 μl per well testosterone BSA conjugate (1 μg ml-1), testosterone TG conjugate (1 μg ml-1) and BSA (1 μg ml-1), TG (1 μg ml-1) alone as a control and incubated at 37°C for 1 hr. The plate was washed three times PBST and three times PBS. Blocked with 2% MPBS incubated at 37°C for 1 hr, washed three times PBST and three times PBS. Seventy five microlitres of scAb added to each well in addition 75 μl with each steroid hormone (2 mM), 75μl PBS added as a control and incubated at room temperature 1 hr, this was repeated with all 5 scAbs. Plate washed as previously 1/2000 dilution of anti-human kappa light chain peroxydase (Sigma), incubated at room temperature 1 hr, washed with PBST four times and PBS three times. One hundred microlitres of TMB substrate added to each well and blue colour allowed to develop, reaction quenched with 50 μl of 1M sulphuric acid, plate read at 420 nm.

The ELISA demonstrates that clones ARK2, ARK8 and ARK9 recognise testosterone but do not recognise progesterone however there is cross reactivity with 5α-dihydrotestosterone (DHT) and some cross reactivity with 17β-estradiol (Figure 1). The competition ELISA was repeated using the ARK17-1 and ARK17-9 clones isolated from the testosterone-17 conjugates. As with the previous three clones they indeed recognise testosterone however in contrast do not recognise DHT or 17β-estradiol, but do recognize progesterone (Figure 2).

Specificity of the 5 antibodies is summarised below in Table 2:
<table>
<thead>
<tr>
<th>Steroid</th>
<th>ARK2</th>
<th>ARK8</th>
<th>ARK9</th>
<th>ARK17-1</th>
<th>ARK17-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Dihydrotestosterone</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>DHEA</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>17-alpha-estradiol</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>17-beta-estradiol</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Esterol</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Progesterone</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Esterone</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

**Example 4. In vitro activity**

**Cell line and Media**

COS7 (monkey kidney cells) were cultured in Greiner Bio-one 250 ml (surface area 75cm2) tissue culture flasks with 12 ml of 90% (v/v) Dulbecco’s modified Eagle’s medium (D-MEM) with 4 mM L-glutamine adjusted to contain 1.5 g/l sodium bicarbonate and 4.5 g/l glucose (Gibco, Invitrogen) supplemented with 10% (v/v) carbon stripped fetal bovine serum (biowest). The cell line was incubated in a sterile Jencons Millenium 5% CO2 incubator at 37°C. The cells were sub-cultured every seven days and the media replaced every three days.

**Lipofectamine 2000 Transient transfection of androgen receptor and luciferase vectors**

Twenty four hours prior to transfection the COS7 cells were washed twice with 10 ml of sterile PBS and incubated for 5 minutes in 3 ml trypsin : EDTA (2.5 g/l) at 37°C to detach the cells from the surface of the flask. Nine millilitres of complete medium was added and gently aspirated several times until the cells were uniformly suspended, transferred to a sterile Falcon tube and centrifuged for 3 minutes at 3000 rpm. The supernatant discarded and the cells re-suspended in 12 ml of fresh medium. The cells were counted on a haemocytometer under a Wilovert light microscope to estimate the density of the suspension. The cells diluted in fresh medium to a density of 1.5x10^5 cells ml-1 and 2 ml fractions seeded into individual wells of a 6 well plate, (NUNC).
The cells were allowed the cells to adhere to the plate surface by incubating at 37°C overnight or until the cells were 90% confluent.

Five micro litres of Lipofectamine 2000 (Invitrogen) was diluted in 250 μl of serum free D-MEM and incubated at room temperature for 5 minutes. Meanwhile 1.5 μg of pSVAR0 (androgen receptor vector) and 1.5 μg of GRE2 ‘TATA’ (luciferase reporter vector) were incubated together in 250 μl of serum free D-MEM. The DNA and Lipofectamine media mixes were combined and incubated at room temperature for 20 minutes to allow the lipid-DNA complexes to form. After incubation 500 μl of the mix was added to each well and gently swirled to mix and incubated for a further 24 hours at 37°C. After incubation the medium was replaced with 1 ml of medium containing the respective anti-testosterone antibody (for the wells with no antibody was substituted with PBS, a microcystin antibody was used as a non-specific control). After a 1 hour incubation at 37°C 1 ml of media was added containing testosterone to a final concentration of 1nM, (medium with 0.1% ethanol as a control), the cells were then incubated at 37°C for a further 24 hours.

After incubation the cells were harvested and measured for luciferase activity. The cells were washed twice with 1 ml PBS and scraped off the plate in a further 1 ml of PBS and transferred to a sterile 1.7 ml eppendorf and centrifuged at 3000 rpm. The supernatant discarded and the cell re-suspended in 40 μl of 0.1 M KH2PO4 (pH 7.8) by vortexing. The cells were lysed by freeze-thaw three times in liquid nitrogen and to 37°C in a hot block. The lysed cells were centrifuged at 13,000 rpm for 1 minute to pellet the debris and the supernatant transferred to a fresh Eppendorf tube.

The Luciferase activity was subsequently measured in a Berthold Lumat LB9501 luminometer. Five micro litres of each sample was added to 350 μl of luciferase assay buffer (15 mM MgSO4•7H2O, 30 mM GlyGly pH 7.8 and 2nM Na2 ATP) and placed in a 5 ml, 75 x 12 mm luminometer tube (Sarstedt). After insertion inside the measuring chamber of the luminometer 100 μl of 0.5 mM luciferin (Molecular Probes), dissolved in 30 mM pH 7.8 GlyGly was injected into the sample. The light emission produced from the reaction was measure for 10 seconds by the luminometer photodetector. The data obtained for each sample was recorded as relative light units.
(RLU) and the luciferase activity for each sample was normalised for protein levels by dividing the raw RLU by the sample protein concentration determined using a Bradford assay. All samples were analysed in triplicate.

5 The luciferase assay demonstrates the ability of all five antibodies to prevent testosterone activating the androgen receptor in cultured human cells (Figure 3).

References

Hemminki et al Protein Eng. 11:311-319.

Hemminki et al Immunotechnology 4: 59-69
CLAIMS

1. A recombinant anti-testosterone antibody wherein the antibody comprises at least one heavy chain, or a testosterone-binding fragment thereof, comprising a variable heavy chain region selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9; and at least one light chain or a testosterone-binding fragment thereof, comprising a variable light chain region selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12.

2. A recombinant anti-testosterone antibody as claimed in claim 1, wherein the antibody comprises at least one heavy chain of SEQ ID NO: 13 or SEQ ID NO: 15.

3. A recombinant anti-testosterone antibody as claimed in claim 1 or claim 2, wherein the antibody comprises at least one light chain of SEQ ID NO: 14 or SEQ ID NO: 16.

4. A recombinant anti-testosterone antibody as claimed in claim 1, wherein the heavy chain comprises the variable heavy chain regions SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3 and the light chain comprises the variable light chain regions SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

5. A recombinant anti-testosterone antibody as claimed in claim 1, wherein the heavy chain comprises the variable heavy chain regions SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9 and the light chain comprises the variable light chain regions SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12.

7. A recombinant anti-testosterone antibody that binds to testosterone and/or its biologically active metabolites with an affinity of less than 20nM.

8. A recombinant anti-testosterone antibody as claimed in claim 7, wherein the biologically active metabolites of testosterone are dihydrotestosterone and 17-beta estradiol (TDE),
9. A recombinant anti-testosterone antibody raised against testosterone conjugated via the 17-OH position on the testosterone molecule.

10. A recombinant anti-testosterone antibody as claimed in claim 7, wherein the biologically active metabolite of testosterone is progesterone.

11. A recombinant anti-testosterone antibody raised against testosterone conjugated via the 3-O position on the testosterone molecule.

12. A recombinant anti-testosterone antibody as claimed in claim 1 which is antibody clone ARK8 deposited as accession no. NCIMB 41412.

13. A recombinant anti-testosterone antibody as claimed in claim 1 which is antibody clone ARK17-9 deposited as accession no. NCIMB 41413.

14. A pharmaceutical composition comprising a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13.

15. A recombinant anti-testosterone antibody as claimed in any of claims 1 to 13 for use in neutralizing the activity of testosterone in vivo.

16. A method of neutralizing the activity of testosterone in vivo, comprising the administration of a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13 to a subject.

17. A recombinant anti-testosterone antibody as claimed in any of claims 1 to 13 for use in reducing circulating levels of testosterone in vivo.

18. A method of reducing circulating levels of testosterone associated with prostate cancer, comprising the administration to a subject of a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13.
19. A method of treating polycystic ovarian syndrome (PCOS) in a subject, comprising the administration to the subject of a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13.

20. A method of treating obesity or metabolic syndrome in a subject, comprising the administration to the subject of a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13.

21. A method of treating acne in a subject, comprising the administration to the subject of a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13.

22. A method of treating aggressive behaviour in a subject, comprising the administration to the subject of a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13.

23. A method of treating hyperandrogenism in a subject, comprising the administration to the subject of a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13.

24. A method for lowering testosterone and testosterone derived steroid levels in a mammal, comprising the separate, simultaneous or sequential administration of a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13 and a secondary antibody that binds to a different epitope of testosterone.

25. The use of a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13 in the manufacture of a medicament for the treatment of prostate cancer, hypogonadism, sociopathic behaviour disorder, polycystic ovarian syndrome (PCOS), acne, hyperandrogenism, obesity, metabolic syndrome.

26. A kit comprising a recombinant anti-testosterone antibody as claimed in any one of claims 1 to 13 for screening for hyperandrogenism.
27. A nucleic acid molecule having a sequence of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 or SEQ ID NO: 16, or a sequence complementary or homologous thereto, or a fragment thereof.

28. A vector comprising a nucleic acid molecule of claim 27.

29. A host cell transformed with a vector of claim 28.

30. A peptide having an amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 or SEQ ID NO: 16 or a sequence homologous thereto, or a fragment thereof.
FIG. 2

ARKit-1

ARKit-9

Clone

1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000

A450

T: BSA
T: TG
BSA
Testosterone
DHT
17 beta-Estradiol
17 alpha-Estradiol
Estradiol
DHEA
Progestosterone
Estrone
(a) Clone ARK 8 Variable Heavy chain [SEQ ID NO: 13]

QVQLKESGCGVQPGRSLRLSCAACAGTGAGCTGACAGAATCTGGGGAAGCTGAGTGCCCTGAGACTATCTGCTGCA
GTCCACGTCAGACTTCTCAGAACCACCCCTCGACCAAGGACCTGACCTGGAGAGGAGACTGACATCTGATAGAGACG
T

ASGFTFSSYGMRWVQRTPDKRLEGCCCTGTGATGACTATGGCATGCCTGCCAGACCAAGAAGAGCTGGAG
CGGAGACCTAATGTGAATCTAGATCTCGAGAACCACCGCAGGTCTGGATAGTACCTGACCT

WVATISSGGSTYYADSVKGRFTI
TTGGCTCCACACCATTAGTGGGTGTAGCAGTAATACGAGACTCTGAGAGGCGCGTTGACCCATAC
ACCCAGCTTGATATCACTGACCCACCTCGATGATGATCAGCTGACCACCTCTGCAGGGCCAAAGTTAG

SRDNSDKMNLYLMQMNLSRLAEDTAV
TCCAGAGATATCCCCAGACAGTCGTATCTCGCAAAATGACAGCTGAGAGCCAGAGACACGCGCTG
AGGTCTCCTAATAGGTCTAGCTGACGTGTTCTGAGCTAACCTCTGCGATCTGCCTGCTGCGGCAC

YYCARSRGTLWQGTLVTVSA
TATTACTGTCAAGATCGCCGCGGACCTCTTGAGGCGCCAGAGATACGCTGACCTGACCTGCA
ATAATGACACGTTCTAGCGCCGCTGAGAACCACCGGTCTCCATGAGACAGAGACAG

(b) Clone ARK 8 Variable Light chain [SEQ ID NO: 14]

DIVMTQSPSLPVTLQGASISC
GATATTGTGATGACTCAGTCTCACTCTCCAGCAGCTACAGTGACACCGCCACCCCTGACTCCTGTGC
CTATAACACTGACTGACTGAGGCTAGGTGAGGACGGAGGCAGGTGACACTGCTCGAGCCGAGAGTAGAGGACG

RSQSLVYSDBGNTYLNWFQQRPG
AGGTCTAGTCAGAGCCCTGCTATACAGGTGGAAACACTACCTACTTGGATGTCTAGCAAGAGGCGAGGGCT
TCCAGATCTGGAGCTGAGATCTGAGACAGATCTACGGGCTTGGATGAACTTACCAAGAAGTGCCTCCTCCGTCG

QSPRLKIVSNRDSGVPDRTFSG
CAATCTCCAGAGACGCTAATTATAGGTCTAAGGCCTGAGCTGACGACAGATTCTACCCAGG
GTGAGAGTTCTGCGGATGAAATTATTCACCAAGATGGCCTGAGACACCGGAGGGAGCTGCTCATAGTGCG

SGSGTDFTLKIISRVEAEADVGY
AGTGAGTCAGGAAGCTGAGTTACAGCAGCTGAGGCTGAGGATGTGTTGCTGTAATTAC
TCACCGAGCTCAGCTCAGCTAAGGCTGACTTCTAGCTCTCCACCTCCGGACTCTCATTACCAACCAATATG

CMQGTHWPTFGQGTKLEIKR
TGCAATGCAAGATCGACAGTGCTGCGCGCAAGGGCGACAGATGGGAATCTACCAACGT
ACGGTACGGTCCATGCTGAGAGCTGAGGCTGAGGATGTGTTGCTGTAATTAC
TCACCGAGCTCAGCTCAGCTAAGGCTGACTTCTAGCTCTCCACCTCCGGACTCTCATTACCAACCAATATG

FIG. 6
(a) Clone ARK 17-9 Variable Heavy chain [SEQ ID NO: 15]

Q V Q L L Q S A A E V K K P G E S L K I S C K
CAGTGTGACTGTGTGAGTGGAGACTGAGGAGAGGAGCTCAGATCTCTGCTAG
GTCCACATTAGACAGTAGTCTGCTCTCCACTTTTTTCCCGGCCCCTCAGAGACTTCT
G S G Y S F T S Y W I G W V R Q M P G K G L E
GGTCCGAGACCTAGGCTTACGAGCTTACGAGGTGGAGCAGGAGCAGAAGCGCCGAGAGC
CAAGACACTAGTCTGAAATGATGATAGCAGGACAGTACTAGTCAAGGAGCTCCGGG
W M G I I Y P G D S D T R Y S P S F Q G Q V T
TGGATGGGATGTCATCTATCTCTGATCTGATATCACACATAGCAGCCCCTCTCTGAGCT
ACCTAAGCTATGATAGAAGGACAGTACATATGCTATGCTGACGAGAAGTTGCCCAGG
I S A D K S I S T A Y L Q W S S L K A S D T A
ATCTGACGCAGACAGCTTCGACAGAGCTCAGACGCGCTTACGAGAAGCGCCGAGAGC
TAGATGCTGCTGTACGAGAGAGAGAGAGAGCTTGAGCGCAGACGCGCTTACGAGAAGCG
V Y Y C A R G S F R G F D Y W G Q G T L V T V
GTGTTTTACTCTGCAAGAGGTCTTTTGTGTTTTGACTATATGGGCAAGGATCTCCCGCT
CAACTATGACAGCAGCTTCCAGAAAGACACCCAAACTGATAACCCCGGCTATGGGACAG

(b) Clone ARK 17-9 Variable Light chain [SEQ ID NO: 16]

Q S V V T Q P P S A G T G P Q R V T I S C S
CAGTCTGCTGACTGAGGAGGAGAGGAGCTCAGATCTCTGCTAG
GTCCACATTAGACAGTAGTCTGCTCTCCACTTTTTTCCCGGCCCCTCAGAGACTTCT
G S S S N I G S N Y V Y W Y Q Q L P G T A P K
GGAGCAGCTTCACATCGGAGTAATATGATATTAGTACGAGGAGCAGTCGGCCAGGACAGC
CICCAGCAGCAGCTTGAGGTGTAGCTTCATATATACGATGAGGAGCTCAGAAGGAGGT
L L I Y R N Q R P S G V P D R F S G S K S G
CTCTCCATCATATAGGATATAATAGGACGGTCCAGCTGCTAGGATCTCCAGGAGGATCTCCG
GAGGAGTAGATATATCCTTATTAGTATCGGCGGAGTCACCAGGAGTGCTGCTGAGGGTTCCG
T S A S L A I S G L Q S E D E A D Y Y C A A W
ACCTCAGCCTTCCCTGGGCTCATGAGGGGTCTCGATGAGGAGCTGATATATACGCTGACGAT
TGGAGTGAGAGGAGGAGGATGCTCAGGAGATGAGCTTACCGAGGAGTCGGCAGCTCTCAGAAGG
D D S L F P V V F G G G T K L T V L G
GATGACAGCCTGTCTCTCTGCTATCTGCGGAGGAGCAAGCCTGACGCTCTAGGT
CTACTGTCGAGAACAAGGACACATAAGCCGCTCCCTGGTCTCGACTGGCAGATCCA

FIG. 7

SUBSTITUTE SHEET (RULE 26)
INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 6, line 22.

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet.

Name of depositary institution
NCIMB Ltd

Address of depositary institution (including postal code and country)
Ferguson Building
Craigstone Estate
Bucksburn
Aberdeen
AB21 9YA, GB

Date of deposit
11 July 2006

Accession Number
NCIMB 41412

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet.

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

In respect of all designated States to which such action is possible and to the extent that it is legally permissible under the law of the designated State, is is requested that a sample of the deposited biological material be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), UK Patent Rules 1995, Schedule 2, Paragraph 3, Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated State.

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

For receiving Office use only
This sheet was received with the international application

Authorized officer
J. R. Lloyd-Thompson

For International Bureau use only
This sheet was received by the International Bureau on:

Authorized officer
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

Haptoens Ltd
Polworth Building
Foresterhill
Aberdeen
AB25 2ZD

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page

NAME AND ADDRESS OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM

<table>
<thead>
<tr>
<th>Identification reference given by the DEPOSITOR:</th>
<th>Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>NCIMB 41412</td>
</tr>
<tr>
<td>XL1 – Blue ARK-8</td>
<td></td>
</tr>
</tbody>
</table>

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

- [ ] a scientific description
- [X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 11 July 2006 (date of the original deposit)

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd.,
Address: Ferguson Building, Craigstone Estate, Buckburn, Aberdeen, AB21 9YA, Scotland, UK

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s):

[Signature]

Date: 19 July 2006

Where Rule 6/4(d) applies, such date is the date on which the status of International Depositary Authority was acquired.

Form BP/4 (sole page)
INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL
(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 1, line 21

B. IDENTIFICATION OF DEPOSIT

Name of depositary institution
NCIMB Ltd

Address of depositary institution (including postal code and country)
Ferguson Building
Crailstone Estate
Bucksburn
Aberdeen
AB21 9YA, GB

Date of deposit 11 July 2006
Accession Number NCIMB 41413

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

In respect of all designated States to which such action is possible and to the extent that it is legally permissible under the law of the designated State, is is requested that a sample of the deposited biological material be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. EPC Rule 28(4), UK Patent Rules 1995, Schedule 2, Paragraph 3, Australian Regulation 3.25(3) and generally similar provisions mutatis mutandis for any other designated State.

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

Form PCT/RO/134 (July 1998; reprint January 2004)
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page

NAME AND ADDRESS OF DEPOSITOR

| Haptogen Ltd   | INTERNATIONAL FORM                                      |
| Polworth Building | RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT              |
| Foresterhill   | issued pursuant to Rule 7.1 by the                      |
| Aberdeen       | INTERNATIONAL DEPOSITARY AUTHORITY identified at the    |
| AB25 2ZD       | bottom of this page                                     |

I. IDENTIFICATION OF THE MICROORGANISM

| Identification reference given by the DEPOSITOR: | Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: |
| Escherichia coli | NCIMB 41413 |
| XL1 – Blue ARK -17-9 | |

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

- [ ] a scientific description
- [x] a proposed taxonomic designation
  (Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 11 July 20056 (date of the original deposit)

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

| Name: NCIMB Ltd., | Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s): |
| Address: Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB21 9YA Scotland, UK |
| | Terence Dansee |
| Date: 19 July 2006 | |

1 Where Rule 6/4(d) applies, such date is the date on which the status of International Depositary Authority was acquired.

Form BP/4 (sole page)