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[Continued on next page]

(54) Title: METHODS FOR PROTECTING OVARIAN FUNCTION

(57) Abstract: Provided herein are methods for protecting ovarian function in a subject. The methods include administering to the subject an effective amount of an inhibitor of vascular endothelial growth factor (VEGF).

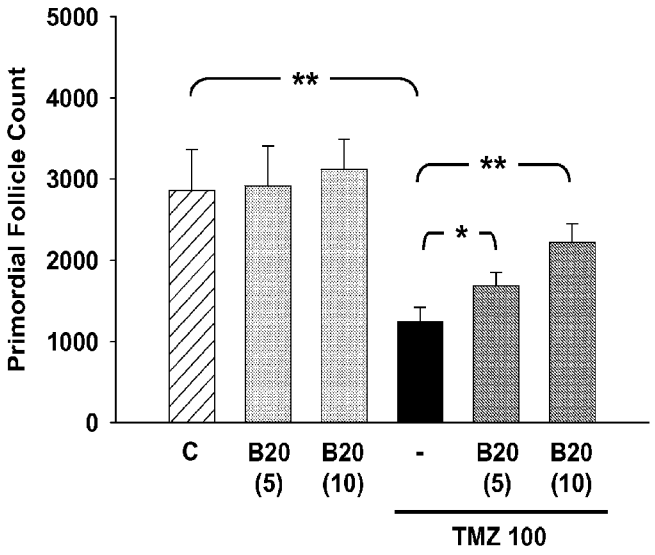


FIG. 1

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Methods for Protecting Ovarian Function

BACKGROUND OF THE INVENTION

[0001] As treatment for cancer has advanced, and as survivorship has improved over the last several decades, an important focus has been placed on post-treatment quality of life. Adverse reproductive health outcomes are one of the most significant results of cancer treatment. For example, chemotherapy has been shown to have detremental effects on ovarian function and it has been a goal to find ways to preserve fertility in premenopausal women.

BRIEF SUMMARY OF THE INVENTION

[0002] Provided herein, inter alia, are methods for protecting ovarian function in a subject. The methods include administering to the subject an effective amount of an inhibitor of vascular endothelial growth factor (VEGF).

[0003] The details of one or more embodiments are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] Figure 1 is a graph showing that an inhibitor of VEGF did not have any effect on primordial follicle count compared to control.

[0005] Figure 2 is a graph showing the effect of anti-cancer agent treatment on gonadotoxicity, which was attenuated by co-treatment with an inhibitor of VEGF.

DETAILED DESCRIPTION OF THE INVENTION

[0006] Obtaining treatments to protect ovarian function while administering anti-cancer agents can contribute to cancer care. As described herein, inhibiting angiogenesis may, by slowing down the follicular development, be protective if given with chemotherapy. Specifically, as

described herein, inhibitors of VEGF may preserve ovarian function while administering chemotherapy.

[0007] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, and complements thereof. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0008] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[0009] A particular nucleic acid sequence also implicitly encompasses "splice variants." Similarly, a particular protein encoded by a nucleic acid implicitly encompasses any protein encoded by a splice variant of that nucleic acid. "Splice variants," as the name suggests, are products of alternative splicing of a gene. After transcription, an initial nucleic acid transcript may be spliced such that different (alternate) nucleic acid splice products encode different polypeptides. Mechanisms for the production of splice variants vary, but include alternate splicing of exons. Alternate polypeptides derived from the same nucleic acid by read-through transcription are also encompassed by this definition. Any products of a splicing reaction, including recombinant forms of the splice products, are included in this definition. An example of potassium channel splice variants is discussed in Leicher, et al., J. Biol. Chem. 273(52):35095-35101 (1998).

[0010] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are near each other, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0011] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (see, e.g., NCBI web site or the like). Such sequences are then said to be "substantially identical." This definition also refers to, or may be applied to, the compliment of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

[0012] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default program parameters can be used, or alternative parameters can be designated. The sequence

comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0013] A "comparison window," as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology (Ausubel et al., eds. 1995 supplement)).

[0014] A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402 (1977) and Altschul et al., J. Mol. Biol. 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information, as known in the art. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0).

For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0015] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0016] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0017] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical

Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0018] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence with respect to the expression product, but not with respect to actual probe sequences.

[0019] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles.

[0020] The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L),

Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)).

[0021] The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acids, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10oC lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42oC, or, 5x SSC, 1% SDS, incubating at 65oC, with wash in 0.2x SSC, and 0.1% SDS at 65oC.

[0022] Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37oC, and a wash in 1X SSC at 45oC. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency. Additional guidelines for determining hybridization parameters are provided in numerous reference, e.g., and Current Protocols in Molecular Biology, ed. Ausubel, et al., John Wiley & Sons.

[0023] For PCR, a temperature of about 36°C is typical for low stringency amplification, although annealing temperatures may vary between about 32°C and 48°C depending on primer length. For high stringency PCR amplification, a temperature of about 62°C is typical, although high stringency annealing temperatures can range from about 50°C to about 65°C, depending on the primer length and specificity. Typical cycle conditions for both high and low stringency amplifications include a denaturation phase of 90°C to 95°C for 30 seconds to 2 minutes, an annealing phase lasting 30 seconds to 2 minutes, and an extension phase of about 72°C for 1 to 2 minutes. Protocols and guidelines for low and high stringency amplification reactions are provided, e.g., in Innis et al. (1990) PCR Protocols, A Guide to Methods and Applications, Academic Press, Inc. N.Y.).

[0024] An "inhibitory nucleic acid" is a nucleic acid (e.g. DNA, RNA, polymer of nucleotide analogs) that is capable of binding to a target nucleic acid (e.g. an mRNA translatable into PTPRS) and reducing transcription of the target nucleic acid (e.g. mRNA from DNA) or reducing the translation of the target nucleic acid (e.g.mRNA) or altering transcript splicing (e.g. single stranded morpholino oligo) relative to the absence of the inhibitor nucleic acid. A "morpholino oligo" may be alternatively referred to as a "morphlino nucleic acid" and refers to morpholine-containing nucleic acid nucleic acids commonly known in the art (e.g. phosphoramidate morpholinio oligo or a "PMO"). See Marcos, P., Biochemical and Biophysical Research Communications 358 (2007) 521-527. In some embodiments, the "inhibitory nucleic acid" is a nucleic acid that is capable of binding (e.g. hybridizing) to a target nucleic acid (e.g. an mRNA translatable into an RPTPS) and reducing translation of the target nucleic acid. The target nucleic acid is or includes one or more target nucleic acid sequences to which the inhibitory nucleic acid binds (e.g. hybridizes). Thus, an inhibitory nucleic acid typically is or includes a sequence (also referred to herein as an "antisense nucleic acid sequence") that is capable of hybridizing to at least a portion of a target nucleic acid at a target nucleic acid sequence. An example of an inhibitory nucleic acid is an antisense nucleic acid. Another example of an inhibitory nucleic acid is siRNA or RNAi (including their derivatives or pre-cursors, such as nucleotide analogs). Further examples include shRNA, miRNA, shmiRNA, or certain of their derivatives or pre-cursors. In some embodiments, the inhibitory nucleic acid is single stranded. In other embodiments, the inhibitory nucleic acid is double stranded.

[0025] An "antisense nucleic acid" is a nucleic acid (e.g. DNA, RNA or analogs thereof) that is at least partially complementary to at least a portion of a specific target nucleic acid (e.g. a target nucleic acid sequence), such as an mRNA molecule (e.g. a target mRNA molecule) (see, e.g., Weintraub, Scientific American, 262:40 (1990)), for example antisense , siRNA, shRNA, shmiRNA, miRNA (microRNA). Thus, antisense nucleic acids are capable of hybridizing to (e.g. selectively hybridizing to) a target nucleic acid (e.g. target mRNA). In some embodiments, the antisense nucleic acid hybridizes to the target nucleic acid sequence (e.g. mRNA) under stringent hybridization conditions. In some embodiments, the antisense nucleic acid hybridizes to the target nucleic acid (e.g. mRNA) under moderately stringent hybridization conditions. Antisense nucleic acids may comprise naturally occurring nucleotides or modified nucleotides such as, e.g., phosphorothioate, methylphosphonate, and -anomeric sugar-phosphate, backbonemodified nucleotides. An "anti-PTPRS antisense nucleic acid" is an antisense nucleic acid that is at least partially complementary to at least a portion of a target nucleic acid sequence, such as an mRNA molecule, that codes at least a portion of the PTPRS. In some embodiments, an antisense nucleic acid is a morpholino oligo. In some embodiments, a morpholino oligo is a single stranded antisense nucleic acid, as is know in the art. In some embodiments, a morpholino oligo decreases protein expression of a target, reduces translation of the target mRNA, reduces translation initiation of the target mRNA, or modifies transcript splicing. In some embodiments, the morpholino oligo is conjugated to a cell permeable moiety (e.g. peptide). Antisense nucleic acids may be single or double stranded nucleic acids.

[0026] In the cell, the antisense nucleic acids may hybridize to the target mRNA, forming a double-stranded molecule. The antisense nucleic acids, interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. The use of antisense methods to inhibit the in vitro translation of genes is well known in the art (Marcus-Sakura, Anal. Biochem., 172:289, (1988)). Antisense molecules which bind directly to the DNA may be used.

[0027] Inhibitory nucleic acids can be delivered to the subject using any appropriate means known in the art, including by injection, inhalation, or oral ingestion. Another suitable delivery system is a colloidal dispersion system such as, for example, macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions,

micelles, mixed micelles, and liposomes. An example of a colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. Nucleic acids, including RNA and DNA within liposomes and be delivered to cells in a biologically active form (Fraley, et al., Trends Biochem. Sci., 6:77, 1981). Liposomes can be targeted to specific cell types or tissues using any means known in the art. Inhibitory nucleic acids (e.g. antisense nucleic acids, morpholino oligos) may be delivered to a cell using cell permeable delivery systems (e.g. cell permeable peptides). In some embodiments, inhibitory nucleic acids are delivered to specific cells or tissues using viral vectors or viruses.

[0028] An "siRNA" refers to a nucleic acid that forms a double stranded RNA, which double stranded RNA has the ability to reduce or inhibit expression of a gene or target gene when the siRNA is present (e.g. expressed) in the same cell as the gene or target gene. The siRNA is typically about 5 to about 100 nucleotides in length, more typically about 10 to about 50 nucleotides in length, more typically about 15 to about 30 nucleotides in length, most typically about 20-30 base nucleotides, or about 20-25 or about 24-29 nucleotides in length, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. siRNA molecules and methods of generating them are described in, e.g., Bass, 2001, Nature, 411, 428-429; Elbashir et al., 2001, Nature, 411, 494-498; WO 00/44895; WO 01/36646; WO 99/32619; WO 00/01846; WO 01/29058; WO 99/07409; and WO 00/44914. A DNA molecule that transcribes dsRNA or siRNA (for instance, as a hairpin duplex) also provides RNAi. DNA molecules for transcribing dsRNA are disclosed in U.S. Patent No. 6,573,099, and in U.S. Patent Application Publication Nos. 2002/0160393 and 2003/0027783, and Tuschl and Borkhardt, Molecular Interventions, 2:158 (2002).

[0029] The siRNA can be administered directly or siRNA expression vectors can be used to induce RNAi that have different design criteria. A vector can have inserted two inverted repeats separated by a short spacer sequence and ending with a string of T's which serve to terminate transcription.

[0030] A "control" sample or value refers to a sample that serves as a reference, usually a known reference, for comparison to a test sample. For example, a test sample can be taken from a test condition, e.g., in the presence of a test compound, and compared to samples from known conditions, e.g., in the absence of the test compound (negative control), or in the presence of a

known compound (positive control). A control can also represent an average value gathered from a number of tests or results. One of skill in the art will recognize that controls can be designed for assessment of any number of parameters. For example, a control can be devised to compare therapeutic benefit based on pharmacological data (e.g., half-life) or therapeutic measures (e.g., comparison of side effects). One of skill in the art will understand which controls are valuable in a given situation and be able to analyze data based on comparisons to control values. Controls are also valuable for determining the significance of data. For example, if values for a given parameter (e.g., ovarian function parameter) are widely variant in controls, variation in test samples will not be considered as significant.

[0031] "Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Typically, the antigen-binding region of an antibody will be most critical in specificity and affinity of binding. In some embodiments, antibodies or fragments of antibodies may be derived from different organisms, including humans, mice, rats, hamsters, camels, etc. Antibodies of the invention may include antibodies that have been modified or mutated at one or more amino acid positions to improve or modulate a desired function of the antibody (e.g. glycosylation, expression, antigen recognition, effector functions, antigen binding, specificity, etc.).

[0032] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these light and heavy chains respectively.

[0033] Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an

antibody below the disulfide linkages in the hinge region to produce F(ab)'2, a dimer of Fab which itself is a light chain joined to VH-CH1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'2 dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (see Fundamental Immunology (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (see, e.g., McCafferty et al., Nature 348:552-554 (1990)).

[0034] For preparation of suitable antibodies of the invention and for use according to the invention, e.g., recombinant, monoclonal, or polyclonal antibodies, many techniques known in the art can be used (see, e.g., Kohler & Milstein, Nature 256:495-497 (1975); Kozbor et al., Immunology Today 4: 72 (1983); Cole et al., pp. 77-96 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985); Coligan, Current Protocols in Immunology (1991); Harlow & Lane, Antibodies, A Laboratory Manual (1988); and Goding, Monoclonal Antibodies: Principles and Practice (2d ed. 1986)). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity (see, e.g., Kuby, Immunology (3rd ed. 1997)). Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Patent 4,946,778, U.S. Patent No. 4,816,567) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized or human antibodies (see, e.g., U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, Marks et al., Bio/Technology 10:779-783 (1992); Lonberg et al., Nature 368:856-859 (1994); Morrison, Nature 368:812-13 (1994); Fishwild et al., Nature Biotechnology 14:845-51 (1996); Neuberger, Nature Biotechnology 14:826 (1996); and Lonberg & Huszar, Intern. Rev. Immunol. 13:65-93 (1995)). Alternatively,

phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty et al., Nature 348:552-554 (1990); Marks et al., Biotechnology 10:779-783 (1992)). Antibodies can also be made bispecific, i.e., able to recognize two different antigens (see, e.g., WO 93/08829, Traunecker et al., EMBO J. 10:3655-3659 (1991); and Suresh et al., Methods in Enzymology 121:210 (1986)). Antibodies can also be heteroconjugates, e.g., two covalently joined antibodies, or immunotoxins (see, e.g., U.S. Patent No. 4,676,980 , WO 91/00360; WO 92/200373; and EP 03089).

[0035] Methods for humanizing or primatizing non-human antibodies are well known in the art (e.g., U.S. Patent Nos. 4,816,567; 5,530,101; 5,859,205; 5,585,089; 5,693,761; 5,693,762; 5,777,085; 6,180,370; 6,210,671; and 6,329,511; WO 87/02671; EP Patent Application 0173494; Jones et al. (1986) Nature 321:522; and Verhoyen et al. (1988) Science 239:1534). Humanized antibodies are further described in, e.g., Winter and Milstein (1991) Nature 349:293. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers (see, e.g., Morrison et al., PNAS USA, 81:6851-6855 (1984), Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); Morrison and Oi, Adv. Immunol., 44:65-92 (1988), Verhoeyen et al., Science 239:1534-1536 (1988) and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992), Padlan, Molec. Immun., 28:489-498 (1991); Padlan, Molec. Immun., 31(3):169-217 (1994)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. For example, polynucleotides comprising a first sequence coding for humanized immunoglobulin framework regions and a second sequence set coding for the desired immunoglobulin complementarity determining regions can be produced synthetically or by combining appropriate cDNA and genomic DNA segments. Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells.

[0036] A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity. The preferred antibodies of, and for use according to the invention include humanized and/or chimeric monoclonal antibodies.

[0037] Techniques for conjugating therapeutic agents to antibodies are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery"in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review" in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

[0038] The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions requires an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with the selected antigen and not with other proteins. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Using

Antibodies, A Laboratory Manual (1998) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

[0039] As used herein, the term "pharmaceutically acceptable" is used synonymously with "physiologically acceptable" and "pharmacologically acceptable". A pharmaceutical composition will generally comprise agents for buffering and preservation in storage, and can include buffers and carriers for appropriate delivery, depending on the route of administration.

[0040] As used herein, the term "cancer" refers to all types of cancer, neoplasm, or malignant tumors found in mammals, including leukemia, carcinomas and sarcomas. Exemplary cancers include cancer of the brain, breast, cervix, colon, head & neck, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus and Medulloblastoma. Additional examples include, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine and exocrine pancreas, and prostate cancer.

[0041] The term "leukemia" refers broadly to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Leukemia is generally clinically classified on the basis of (1) the duration and character of the disease-acute or chronic; (2) the type of cell involved; myeloid (myelogenous), lymphoid (lymphogenous), or monocytic; and (3) the increase or non-increase in the number abnormal cells in the blood-leukemic or aleukemic (subleukemic). The P388 leukemia model is widely accepted as being predictive of in vivo anti-leukemic activity. It is believed that a compound that tests positive in the P388 assay will generally exhibit some level of anti-leukemic activity in vivo regardless of the type of leukemia being treated. Accordingly, the present application includes a method of treating leukemia, and, preferably, a method of treating acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophylic leukemia, blast cell

leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, multiple myeloma, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, and undifferentiated cell leukemia.

[0042] The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas which can be treated with a combination of antineoplastic thiol-binding mitochondrial oxidant and an anticancer agent include a chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma.

[0043] The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas which can be treated with a combination of antineoplastic thiol-binding mitochondrial oxidant and an anticancer agent include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna

melanoma, malignant melanoma, nodular melanoma, subungal melanoma, and superficial spreading melanoma.

[0044] The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas which can be treated with a combination of antineoplastic thiol-binding mitochondrial oxidant and an anticancer agent include, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniforni carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes,

transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, and carcinoma villosum.

[0045] As used herein, the terms "metastasis," "metastatic," and "metastatic cancer" can be used interchangeably and refer to the spread of a proliferative disease or disorder, e.g., cancer, from one organ or another non-adjacent organ or body part. Cancer occurs at an originating site, e.g., breast, which site is referred to as a primary tumor, e.g., primary breast cancer. Some cancer cells in the primary tumor or originating site acquire the ability to penetrate and infiltrate surrounding normal tissue in the local area and/or the ability to penetrate the walls of the lymphatic system or vascular system circulating through the system to other sites and tissues in the body. A second clinically detectable tumor formed from cancer cells of a primary tumor is referred to as a metastatic or secondary tumor. When cancer cells metastasize, the metastatic tumor and its cells are presumed to be similar to those of the original tumor. Thus, if lung cancer metastasizes to the breast, the secondary tumor at the site of the breast consists of abnormal lung cells and not abnormal breast cells. The secondary tumor in the breast is referred to a metastatic lung cancer. Thus, the phrase metastatic cancer refers to a disease in which a subject has or had a primary tumor and has one or more secondary tumors. The phrases non-metastatic cancer or subjects with cancer that is not metastatic refers to diseases in which subjects have a primary tumor but not one or more secondary tumors. For example, metastatic lung cancer refers to a disease in a subject with or with a history of a primary lung tumor and with one or more secondary tumors at a second location or multiple locations, e.g., in the breast.

[0046] By "therapeutically effective dose or amount" herein is meant a dose that produces effects for which it is administered. The exact dose and formulation will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, Pharmaceutical Dosage Forms (vols. 1-3, 1992); Lloyd, The Art, Science and Technology of Pharmaceutical Compounding (1999); Remington: The Science and Practice of Pharmacy, 20th Edition, Gennaro, Editor (2003), and Pickar, Dosage Calculations (1999)).

[0047] The term "pharmaceutically acceptable salts" or "pharmaceutically acceptable carrier" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present application contain relatively acidic functionalities, base

addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present application contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, e.g., Berge et al., Journal of Pharmaceutical Science 66:1-19 (1977)). Other pharmaceutically acceptable carriers known to those of skill in the art are suitable for compositions of the present application.

[0048] A "subject," "individual," or "patient," is used interchangeably herein, which refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Tissues, cells and their progeny of a biological entity obtained in vitro or cultured in vitro are also encompassed.

[0049] "VEGF" refers to vascular endothelial growth factor, a signaling molecule, which includes a sub-family of growth factors involved in vasculogenesis and angiogenesis. See, e.g., Shibuya, *J. Biochem.* 153(1):13-9 (2013) and Takahashi, *Biol. Pharm. Bull.*, 34(12):1785-8 (2011), which are incorporated by reference herein in their entireties. Optionally, the term VEGF refers to VEGF and includes all variants, isoforms, and homologs thereof. Optionally, the term VEGF refers to VEGF-A and includes all variants, isoforms and homologs thereof. The amino acid sequence of VEGF-A thereof can be found, for example, at UniProtKB/Swiss-Prot Accession Nos. P15692 (human) and Q00731 (mouse) and at GenBank Accession Nos. NP_001020537 (human) and NP_001020421, which are incorporated by reference herein in their

entireties. The nucleic acid sequence of VEGF-A can be found, for example, at GenBank Accession Nos. NM_001025366 (human) and NM_001025250 (mouse), which are incorporated by reference herein in their entireties. Additional sequences for the types of VEGF proteins include for VEGF-A, UniProtKB/Swiss-Prot Accession Nos. B5BU86, H0Y2S8, H0Y407, H0Y414, H0Y462, H0Y8N2, H3BLW7, O60720, O75875, Q074Z4, Q16889, Q5UB46, Q6P0P5, Q96KJ0, Q96L82, Q96NW5, Q9H1W8, Q9H1W9, Q9UH58, and Q9UL23, for VEGF-B, Accession Nos. P49765 and Q16528, for VEGF-C, Accession Nos. P49767 and B2R9Q8, for VEGF-D, Accession Nos. O43915 and B2R7Z3, and for placenta growth factor (PLGF) Accession Nos. P49763, Q07101, Q9BV78, and Q9Y6S8. VEGF-A has 17 isoforms produced by alternative promoter usage, alternative splicing and alternative initiation. These isoforms include isoform VEGF206 (Accession No. P15692-1), isoform VEGF189 (Accession No. P15692-2), isoform VEGF183 (Accession No. P15692-3), isoform VEGF165 (Accession No. P15692-4), isoform VEGF148 (Accession No. P15692-5), isoform VEGF145 (Accession No. P15692-6), isoform VEGF165B (Accession No. P15692-8), isoform VEGF121 (Accession No. P15692-9), isoform VEGF111 (Accession No. P15692-10), isoform L-VEGF165 (Accession No. P15692-11), isoform L-VEGF121 (Accession No. P15692-12), isoform L-VEGF189 (Accession No. P15692-13), isoform L-VEGF206 (Accession No. P15692-14), VEGF-A isoform 15 (Accession No. P15692-15), VEGF-A isoform 16 (Accession No. P15692-16), VEGF-A isoform 17 (Accession No. P15692-17), VEGF-A isoform 18 (Accession No. P15692-18). VEGF-B has 2 isoforms produced by alternative splicing. These isoforms are isoform VEGF-B186 (Accession No. P49765-1) and isoform VEGF-B167 (Accession No. P49765-2). PLGF has 3 isoforms produced by alternative splicing. These isoforms are isoform PlGF-3 (Accession No. P49763-1), isoform PlGF-1 (Accession No. PlGF-131; P49763-2) and isoform PlGF-2 (Accession No. PlGF-152; P49763-3).

[0050] As used herein, "an inhibitor of VEGF" is a molecule (e.g. antibody, nucleic acid, inhibitory nucleic acid, synthetic chemical, small chemical molecule) that directly or indirectly inhibits the expression or activity of VEGF *in vitro*, *ex vivo*, or *in vivo,* i.e, when administered to a subject in a therapeutically effective dose or amount relative to the absence of the inhibitor. Optionally, the inhibitor is an agent that binds VEGF. An agent preferentially binds to a molecule, for example, when the binding to the targeted molecule is greater than the binding to other molecules of a similar form. In some embodiments, the preferential binding is 1.1-fold,

1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, 1000-fold, 2000-fold, 3000-fold, 4000-fold, 5000-fold, 6000-fold, 7000-fold, 8000-fold, 9000-fold, 10000 fold, 100,000-fold, 1,000,000-fold greater. By way of example, an agent binds VEGF, a nucleic acid (e.g. RNA or DNA) encoding VEGF, or a protein of VEGF when a binding assay or experiment (e.g. gel electrophoresis, chromatography, immunoassay, radioactive or non-radioactive labeling, immunoprecipitation, activity assay, etc.) reveals only an interaction or primarily an interaction with a single VEGF, a nucleic acid (e.g. RNA or DNA) of VEGF, or a protein of VEGF. An agent may also inhibit VEGF, a nucleic acid (e.g. RNA or DNA) of VEGF, or a protein of VEGF by decreasing or increasing the amount of VEGF in a cell or organism relative to the absence of the agent, or decreasing the interaction between VEGF and another molecule, e.g., a physiological or natural ligand. A person having ordinary skill in the art, using the guidance provided herein, may easily determine whether an agent inhibits VEGF in a cell or organism by binding VEGF, decreasing or increasing the amount of VEGF, or inhibiting the interaction of VEGF with another molecule.

[0051] Many chemotherapeutic agents, particularly alkylating agents such as cyclophosphamide, are toxic to the egg cells (oocytes) in the ovaries. If the number of remaining oocytes in the ovaries reaches a critically low point during treatment, women experience ovarian failure, which means the ovaries stop functioning during or shortly after cancer treatment.

[0052] Provided herein are methods for protecting ovarian function in a subject. Optionally, the subject is a cancer patient. The methods include administering to the subject an effective amount of an inhibitor of vascular endothelial growth factor (VEGF). Optionally, the methods further include selecting a subject with cancer or selecting a subject exposed to an anti-cancer agent. Optionally, the subject is to be exposed to an anti-cancer agent.

[0053] The ovary is an ovum- or egg-producing reproductive organ, often found in pairs as part of the vertebrate female reproductive system. Ovaries of vertebrate females produce a single fertilizable egg approximately every three to five weeks in a menstrual cycle. Ovarian function in every menstrual cycle involves the formation and maturation of the dominant follicle in the ovary, followed by the follicle rupture and the release of the egg (ovulation). The

periodically recurring development of ovarian follicles, in preparation for the periodically recurring ovulation, is called folliculogenesis. Most of the follicles remain resting but, at the beginning of every menstrual cycle, a group or cohort of follicles are recruited to grow; only one of these will mature and will normally ovulate, with the rest of the group succumbing to atresia (death). As used herein, "protecting ovarian function" includes, but is not limited to, preventing ovarian damage, e.g., by preventing cell death and/or suppressing one or more of the steps of normal ovarian function. Optionally, protecting ovarian function includes slowing cell division (e.g., follicle or egg cell division) or slowing egg development. For example, in the provided methods, ovarian function is protected by slowing follicle formation, recruitement and/or maturation. Ovarian reserve and activity can be assessed to evaluate ovarian function. In humans, ovarian reserve can be assessed by measuring serum ovarian reserve markers (i.e. anti-mullerian hormone, inhibin B and follicle stimulating hormone) and antral follicle count (by ultrasound). Such methods are known and are described in, for example, Rosen MP et al. Fertil Steril. 2012 Jan;97(1):238-43, which is incorporated by reference herein in its entirety. In animals, ovarian reserve can be determined by counting primordial and primary follicles in both ovaries (in histologic sections). Such methods are known and are described in, for example, Smith BJ et al. Reprod Toxicol 1991, 5: 379–383, which is incorporated by reference herein in its entirety. In humans, ovarian activity can be evaluated by assessing the number of growing follicles (by ultrasound) and by measuring the serum estradiol levels. In animals, ovarian activity can be determined by counting growing follicles (i.e. secondary, preantral, antral and graafian follicles) in both ovaries (in histologic sections) or by assessing the average number of pups in each mating cycle. Such methods are known and described in, for example, Muskhelishvili L et al. Toxicol Pathol. 2002 May-Jun;30(3):400-2, which is incorporated by reference herein in its entirety. Thus, ovarian function can be measured using methods including blood assays, histology assays, x-rays and ultrasound. For example, ovarian function can be assessed by measuring hormone levels or by assessing follicle count by ultrasound.

[0054] As used herein the protect, protecting, protection, preserve, and preserving refers to a method of reducing the effects of one or more symptoms of a disease or condition, e.g., reducing the effects of anti-cancer agents in a subject. Thus, protection includes a method of reducing the effects of anti-cancer agents in a subject by protecting and preserving ovarian function in the subject. Thus in the disclosed method, protecting can refer to a 10%, 20%, 30%, 40%, 50%,

60%, 70%, 80%, 90%, or 100% reduction in the severity of a condition, e.g., ovarian failure, or symptom of the condition, e.g., egg cell or oocyte death or loss. Optionally, protecting refers to at least a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% reduction in the severity of the condition, e.g., ovarian failure, or symptom of the condition, e.g., egg cell or oocyte death or loss. Thus, ovarian function is protected if there is a 10% reduction in the negative effects of anti-cancer agents on ovarian function. Alternatively, ovarian function is protected or preserved if there is a 10% increase in ovarian function in a subject as compared to a control, e.g., in the absence of the inhibitor of VEGF. Thus, increase can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent increase in between 10% and 100% as compared to native or control levels. It is understood that protecting ovarian function does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition.

[0055] As used herein, the terms higher, increases, elevates, or elevation refer to increases above a control. As used herein, the terms low, lower, reduces, or reduction refer to any decrease below a control. By way of example, a control includes the condition, e.g., ovarian function or failure, or symptom of the condition, e.g., egg cell or oocyte death or loss, prior to, or in the absence of, addition of an agent such as a chemotherapeutic agent or radiotherapy or such as a VEGF inhibitor. Thus, for example, a reduction in the negative effects of cancer therapy or anti-cancer agents on ovarian function refers to a decrease of one or more of the effects or symptoms thereof as compared to ovarian function in the absence of an inhibitor of VEGF. Similarly, an increase in ovarian function refers to an increase in ovarian function or a symptom thereof as compared to ovarian function in the absence of an inhibitor of VEGF. Thus, the reduction or increase can be or can be at least a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction on increase in between as compared to native or control levels.

[0056] Angiogenesis is regulated by the balance of angiogenic stimuli and angiogenic inhibitors that are produced in the target tissue and at distant sites. Vascular endothelial growth factor-A (VEGF) is a primary stimulant of angiogenesis. Angiogensis inhibitors, e.g., inhibitors of VEGF, and methods of using angiogenesis inhibitors are know. Existing VEGF inhibitors target the VEGF pathway in various ways including by direct inhibition of VEGF protein (e.g., anti-VEGF monoclonal antibodies), by prevention of VEGF receptor binding (e.g., VEGF

receptor antagonists), and by inhibition of VEGF receptor function through inhibition of tyrosine kinase (e.g., tyrosine kinase inhibitors (TKIs)). Thus, inhibitory anti-VEGF receptor antibodies, soluble receptor constructs, antisense strategies, RNA aptamers against VEGF and low molecular weight VEGF receptor tyrosine kinase (RTK) inhibitors have all been used for use in interfering with VEGF signaling. See, e.g., Siemeister et al., Cancer Metastasis Rev., 17(2):241-8 (1998); U.S. Patent No. 8,394,943; and Hurwitz et al., N. Engl. J. Med. 350:2335-2342 (2004); and Cook and Figg, CA Cancer J. Clin. 60(4):222-43 (2010)), which are all incorporated by reference herein in their entireties. Thus, inhibitors of VEGF include, but are not limited to, small molecules, nucleic acids, polypeptides or antibodies. Optionally, the inhibitor slows follicular development. Optionally, the VEGF is VEGF-A.

[0057] Optionally, the inhibitor is a peptide or polypeptide. Suitable polypeptides that inhibit VEGF include, but are not limited to, aflibercept (VEGF-Trap), interferon-alpha, platelet factor-4, thrombospondin and prolactin. Aflibercept is a recombinant fusion protein with VEGF-binding portions from the extracellular domains of human VEGF receptors 1 and 2, fused to the Fc portion of the human IgG1 immunoglobulin. See, e.g., Stewart, Inflamm. Allergy Drug Targets, 10(6):497-508 (2011), which is incoporated by reference herein in its entirety. Aflibercept (V-Trap; Eylea™; Regeneron Pharmaceuticals, Tarrytown, NY, USA) acts as a decoy receptor irreversibly binding to circulating VEGF-A, VEGF-B, and placental growth factors. Inhibitory peptides may also include dominant negative mutants of a VEGF. Dominant negative mutations (also called antimorphic mutations) have an altered phenotype that acts antagonistically to the wild-type or normal protein. Thus, dominant negative mutants of VEGF act to inhibit the normal VEGF protein. Such mutants can be generated, for example, by site directed mutagenesis or random mutagenesis. Proteins with a dominant negative phenotype can be screened for using methods known to those of skill in the art, for example, by phage display.

[0058] Optionally, the inhibitor is an antibody. Antibodies that inhibit VEGF are known and others can be generated as described herein and tested for their desired activity using *in vitro* assays, or by analogous methods, after which their *in vivo* therapeutic or prophylactic activities are tested according to known testing methods. See, for example, U.S. Patent No. 8,394,943, which is incorporated by reference herein in its entirety. Suitable inhibitory VEGF antibodies also include, but are not limited to, bevacizumab, ranibizumab, and ramucirumab. Bevacizumab

(Avastin™; Genentech/Roche, South San Francisco, CA, USA) is a recombinant, humanized VEGF-A neutralizing, monoclonal antibody. Ranibizumab (Lucentis™; Genentech) is a monoclonal antibody fragment derived from bevacizumab that binds VEGF-A receptors with enhanced affinity and inhibits vasculogenesis. Ramucirumab (IMC-1121B) is a fully human monoclonal antibody (IgG1) directed against VEGFR2 and works as a receptor antagonist blocking the binding of VEGF to VEGFR2, thus, mediating the majority of the downstream effects of VEGF in angiogenesis.

[0059] Optionally, the inhibitor is a nucleic acid. Suitable nucleic acid inhibitors include, but are not limited to, antisense molecules, aptamers, ribozymes, triplex forming molecules, siRNA, miRNA, and external guide sequences. Optionally, thenucleic acid inhibitor is a ribozyme. Optionally, the nucleic acid inhibitor is an aptamer. For example, the inhibitor of VEGF can be pegaptanib (Macugen™; OSI Pharmaceuticals, Melville, NY, USA), a pegylated anti-vascular endothelial growth factor (VEGF) aptamer that binds to the 165 isoform of VEGF. Nucleic acid inhibitors of VEGF are known and can be made using the nucleic acid sequences of VEGF, which can be found, for example, at GenBank Accession Nos. NM_001025366 and NM_001025250. See, e.g., U.S. Patent Nos, 7,345,027; 8,227,444; and 8,470,792, which are incorporated by reference herein in their entireties. In addition, antisense molecules that inhibit VEGF can be designed and made using standard nucleic acid synthesis techniques or obtained from a commercial entity, e.g., Regulus Therapeutics (San Diego, CA). Further, examples of known VEGF antisense oligonucleotides include, but are not limited to:

5′-TGGCTTGAAGATGTACTCGAT-3′ ((SEQ ID NO:1); Xin GH et al. Int J Ophthalmol. 2012;5(4):440-7, which is incorporated by reference herein in its entirety);

5`-AAGAAGCCCAGCAAGTGCAT-3` ((SEQ ID NO:2); for VEGF-C; Peng C et al. Asian Pac J Cancer Prev. 2011;12(8):2097-9, which is incoporated by reference herein in its entirety);

5′-GGGCTCCTCTCCCTTCTG-3′ ((SEQ ID NO:3); Fu YB et al. Chin Med J (Engl). 2011 May;124(10):1573-5, which is incoporated by reference herein in its entirety);

5′-GTTCATGGTTTCGGAGGC-3′ ((SEQ ID NO:4); Fu YB et al. Chin Med J (Engl). 2011 May;124(10):1573-5); and

5′-AATCGGTCTTTCCGGTGG-3′ ((SEQ ID NO:5); Fu YB et al. Chin Med J (Engl). 2011 May;124(10):1573-5).

[0060] Optionally, the inhibitor is a small molecule. Exemplary small molecule inhibitors of VEGF include, but are not limited to, itraconazole, pazopanib, sorafenib, sunitinib, axitinib, vadetanib, cabozantinib, suramin, cediranib (AZD2171), nintedanib (BIBF 1120), brivanib (BMS-582664), linifanib (ABT-869), ABT-751 (N-[2-[(4-Hydroxyphenyl)amino]-3-pyridinyl]-4-methoxybenzenesulfonamide), plinabulin (NPI-2358), and semaxanib (SU5416). Optionally, the VEGF inhibitor is a tyrosine kinase inhibitor. Kinases are enzymes that exert their biological functions by transferring a phosphate group from high energy donor molecules (such as adenosine triphosphate [ATP]) by phosphorylation resulting in functional changes in target proteins including transcription factors. There are hundreds of human kinases including around 30 tyrosine kinases subdivided into receptor tyrosine kinases and cytoplasmic tyrosine kinases. Tyrosine kinase inhibitors (TKIs) are small molecules which can pass through the plasma membrane and interfere with intracellular tyrosine kinase activity such as those of the VEGFRs. TKIs can be divided into three groups: type I TKIs competitively inhibit binding of ATP in the active conformation of the kinase (eg, sunitinib [Sutent™; Pfizer, New York, NY, USA]); type II TKIs indirectly compete with ATP by binding to the inactive conformation of the kinase (eg, sorafenib [Nexavar™; Bayer, Leverkusen, Germany]); type III are "covalent inhibitors" that covalently bind cysteines to specific sites on the kinase (eg, vandetanib [Capreslsa™; AstraZeneca, London, UK]).

[0061] Anti-cancer agents and methods for using anti-cancer agents are known. See, e.g., Physician's Drug Handbook, 12th Edition, Lippincott, Williams & Wilkins, (2007) or Physician's Cancer Chemotherapy Drug Manual 2013, by Chu and DeVita, Jones & Bartlett Learning, LLC, (2013). Optionally, the provided methods further include the step of administering an anti-cancer agent to the subject. Anti-cancer agents include, but are not limited to, alkylating agents, anthracyclines, taxanes, epothilones, histone deacetylase inhibitors, inhibitors of Topoisomerase I or II, kinase inhibitors, antibodies, nucleotide analogs and precursor analogs, platinum compounds, retinoids and vinca alkaloids or derivatives thereof. Optionally, the anti-cancer agent is a platinum compound. Optionally, the anti-cancer agent is selected from the group

consisting of temozolomide, cyclophosphamide, mechlorethamine, melphalan, chlorambucil, carmustine, cisplatin, carboplatin, and oxaliplatin.

[0062] Combinations of agents, e.g., a VEGF inhibitor or anti-cancer agent, may be administered either concomitantly (e.g., as an admixture), separately but simultaneously (e.g., via separate intravenous lines into the same subject), or sequentially (e.g., one of the compounds or agents is given first followed by the second). Thus, the term combination is used to refer to either concomitant, simultaneous, or sequential administration of two or more agents. Optionally, the inhibitor is administered before, during and/or after the anti-cancer agent. Optionally, the inhibitor is administered before, during, or before and during the anti-cancer agent.

[0063] According to the methods provided herein, the subject is administered an effective amount of the agent (e.g., VEGF inhibitor or anti-cancer agent). The terms effective amount and effective dosage are used interchangeably. The term effective amount is defined as any amount necessary to produce a desired physiologic response. Effective amounts and schedules for administering the agent may be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for administration are those large enough to produce the desired effect in which one or more symptoms of the disease or disorder are affected (e.g., reduced or delayed). The dosage should not be so large as to cause substantial adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex, type of disease, the extent of the disease or disorder, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. Dosages can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products.

[0064] Therapeutically effective amount, as used herein, refers to that amount of a therapeutic agent sufficient to reduce or ameliorate one or more symptoms of a disease or disorder. Optionally, an effective amount, as used herein, refers to that amount of an agent that preserves or protects ovarian function. For example, for the given parameter (e.g., ovarian function parameter), a therapeutically effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Therapeutic efficacy

can also be expressed as "-fold" increase or decrease. For example, a therapeutically effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control.

[0065] The provided compositions may be administered to a subject or contacted with cells as described in the methods herein at a dosage of between about 1 mg/kg body weight to 1000 mg/kg body weight, or about 10 mg/kg body weight to about 500 mg/kg body weight, or about 50 mg/kg body weight to about 300 mg/kg body weight, or about 100 mg/kg body weight to about 200 mg/kg body weight. The dosage that can be used in the provided methods can be any amount between 1 mg/kg body weight to 1000 mg/kg body weight inclusive. Optionally, the dosage of inhibitor of VEGF is 5 mg/kg to 10 mg/kg. Optionally, the dosage of anti-cancer agent is 100 mg/kg to 300 mg/kg.

[0066] Provided herein are compositions including the inhibitors and anti-cancer agents provided herein. The compositions are, optionally, suitable for formulation and administration *in vitro* or *in vivo*. Optionally, the compositions comprise one or more of the provided agents and a pharmaceutically acceptable carrier. Suitable carriers and their formulations are described in Remington: *The Science and Practice of Pharmacy, 21st Edition*, David B. Troy, ed., Lippicott Williams & Wilkins (2005). By pharmaceutically acceptable carrier is meant a material that is not biologically or otherwise undesirable, i.e., the material is administered to a subject without causing undesirable biological effects or interacting in a deleterious manner with the other components of the pharmaceutical composition in which it is contained. If administered to a subject, the carrier is optionally selected to minimize degradation of the active ingredient and to minimize adverse side effects in the subject.

[0067] The inhibitors and anti-cancer agents are administered in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, intracavity, transdermal, oral, topical, intratumoral, parenteral, or inhalation routes. Thus, the compositions are administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated.

[0068] The compositions for administration will commonly comprise an agent as described herein (e.g. inhibitor of VEGF) dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like.

These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs.

[0069] Solutions of the active compounds as free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms.

[0070] Oral formulations can include excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders. In some embodiments, oral pharmaceutical compositions will comprise an inert diluent or assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 75% of the weight of the unit, or preferably between 25-60%. The amount of active compounds in such compositions is such that a suitable dosage can be obtained

[0071] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered and the liquid diluent first rendered isotonic with sufficient saline or glucose. Aqueous solutions, in particular, sterile aqueous media, are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. For example, one

dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion

[0072] Sterile injectable solutions can be prepared by incorporating the active compounds or constructs in the required amount in the appropriate solvent followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium. Vacuum-drying and freeze-drying techniques, which yield a powder of the active ingredient plus any additional desired ingredients, can be used to prepare sterile powders for reconstitution of sterile injectable solutions. The preparation of more, or highly, concentrated solutions for direct injection is also contemplated. DMSO can be used as solvent for extremely rapid penetration, delivering high concentrations of the active agents to a small area.

[0073] The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials. Thus, the composition can be in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. Thus, the compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include, but are not limited to, powder, tablets, pills, capsules and lozenges.

[0074] Provided herein are kits for protecting ovarian function in a subject. Optionally, the kit for protecting ovarian function in a subject comprises one or more doses of an effective amount of an angiogenesis inhibitor, e.g., a VEGF inhibitor. Optionally, the composition is present in a container such as a vial or packet. Optionally, the kit comprises one or more additional agents. Thus, for example, the kit further includes an additional or second therapeutic agent, e.g., an anti-cancer agent. The additional or second therapeutic agent may be included in a composition comprising the inhibitor or formulated as a second composition. Optionally, the kit comprises a means of administering the compositions, such as, for example, a syringe, needle, tubing, catheter, patch, and the like. The kit may also comprise formulations and/or materials requiring sterilization and/or dilution prior to use. Optionally, the provided kits include instructions for use.

[0075] Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods

and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutations of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a method is disclosed and discussed and a number of modifications that can be made to a number of molecules including the method are discussed, each and every combination and permutation of the method, and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed, it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each such combination or subset of combinations is specifically contemplated and should be considered disclosed.

[0076] Publications cited herein and the material for which they are cited are hereby specifically incorporated by reference in their entireties.

[0077] A number of embodiments have been described. Nevertheless, it will be understood that various modifications may be made. Accordingly, other embodiments are within the scope of the claims.

EXAMPLE

Example 1. Anti-Vegf Antibody Treatment Protects Ovarian Function *In Vivo*

[0078] Angiogenesis is required for follicular development. The FDA reports that Bevacizumab (Avastin® (Genentech, Inc., San Francisco, CA), anti-VEGF antibody inhibiting angiogenesis, causes ovarian failure in humans. However, it is proposed that inhibiting angiogenesis may not be gonadotoxic, but by slowing down the follicular development, can be protective if given with chemotherapy.

[0079] **Materials and Methods:** Seven-week nude female mice (n=4-6 mice/group) received either vehicle (control), 4 doses of anti-VEGF antibody (B20, 5 or 10 mg/kg) every 3 days, single dose of alkylating chemotherapy agent (temozolomide (TMZ), 10 to 300 mg/kg) or B20

with TMZ (100 mg/kg). Four weeks after initiating treatment, both ovaries were obtained. Paraffin embedded samples were serially sectioned and ovarian reserve was assessed by counting primordial and primary follicles. The groups were compared with ANOVA and $p<0.05$ defined as significant.

[0080] **Results**: B20 treatment alone at 5 mg/kg (2918±489; mean±SEM) or at 10 mg/kg (3114±376) did not have any effect on primordial follicle count compared to control (2857±510) (FIG. 1 and Table 1). TMZ 100 and 300 mg/kg treatments had the highest gonadotoxicity and resulted in 56±3% (1243±176) and 60±3% (1154±192) decrease in primordial follicle number compared to control, retrospectively ($p<0.05$) (FIG. 2 and Table 1). TMZ-induced gonadotoxicity was attenuated with B20 co-treatment in a dose dependent manner, and 10 mg/kg B20 resulted in 79±12% (2220±228) higher primordial follicle count compared to TMZ 100 mg/kg alone ($p<0.05$) (FIG. 2 and Table 1). Similar findings were obtained in primary follicle counts across the treatment groups.

Table 1. Follicle Counts in Mice

	Control (n=6)	**B20 5 (n=5)**	**TMZ 100 (n=5)**	**TMZ+B20 5 (n=4)**	**TMZ+ B20 10 (n=3)**
	2570	**2705**	**1308**	**2132**	**2520**
	1425	**2165**	**1240**	**1716**	**1772**
	2560	**4250**	**1084**	**1528**	**2368**
	4570	**3815**	**1508**	**1352**	
	4150	**1656**	**1076**		
	1868				
Average	**2857.2**	**2918.2**	**1243.2**	**1682.0**	**2220.0**

[0081] In contrast to FDA report, anti-VEGF antibody treatment does not have a negative effect on ovarian reserve and may ameliorate the gonadotoxicity of alkylating chemotherapeutic agents.

WHAT IS CLAIMED IS:

1. A method for protecting ovarian function in a cancer patient comprising administering to the cancer patient an effective amount of an inhibitor of vascular endothelial growth factor (VEGF).

2. The method of claim 1, further comprising administering an anti-cancer agent to the subject.

3. A method for protecting ovarian function in a subject comprising selecting a subject with cancer and administering to the subject an effective amount of an inhibitor of vascular endothelial growth factor (VEGF) wherein administration protects ovarian function in the subject.

4. The method of claim 3, wherein the subject is to be exposed to an anti-cancer agent.

5. A method for protecting ovarian function in a subject comprising selecting a subject exposed to an anti-cancer agent and administering to the subject an effective amount of inhibitor of vascular endothelial growth factor (VEGF) wherein administration protects ovarian function in the subject.

6. The method of any one of claims 1-5, wherein the VEGF is VEGF-A.

7. The method of any one of claims 1-6, wherein the inhibitor is selected from the group consisting of a small molecule, a nucleic acid, a polypeptide or an antibody.

8. The method of any one of claims 1-6, wherein the inhibitor is an antibody.

9. The method of any one of claims 1-6, wherein the inhibitor is a nucleic acid.

10. The method of claim 9, wherein the nucleic acid is a ribozyme.

11. The method of any one of claims 1-6, wherein the inhibitor is bevacizumab.

12. The method of any one of claims 1-6, wherein the inhibitor is pegaptanib, alfibercept, or ranibizumab.

13. The method of any one of claims 1-12, wherein the anti-cancer agent is an alkylating agent.

14. The method of any one of claims 1-12, wherein the anti-cancer agent is selected from the group consisting of temozolomide, cyclophosphamide, mechlorethamine, melphalan, chlorambucil, carmustine, cisplatin, carboplatin, and oxaliplatin.

15. The method of any one of claims 1-12, wherein the anti-cancer agent is temozolomide.

16. The method of any one of claims 2-15, wherein the inhibitor is administered before, during and/or after the anti-cancer agent.

17. The method of claim 16, wherein the inhibitor is administered before the anti-cancer agent.

18. The method of claim 16, wherein the inhibitor is administered during the anti-cancer agent.

19. The method of claim 16, wherein the inhibitor is administered before and during the anti-cancer agent administration.

20. The method of any one of claims 1-19, wherein the inhibitor of VEGF forms part of a pharmaceutical composition, wherein the pharmaceutical composition comprises a pharmaceutically acceptable excipient.

21. The method of any one of claims 1-20, wherein the inhibitor slows follicular development.

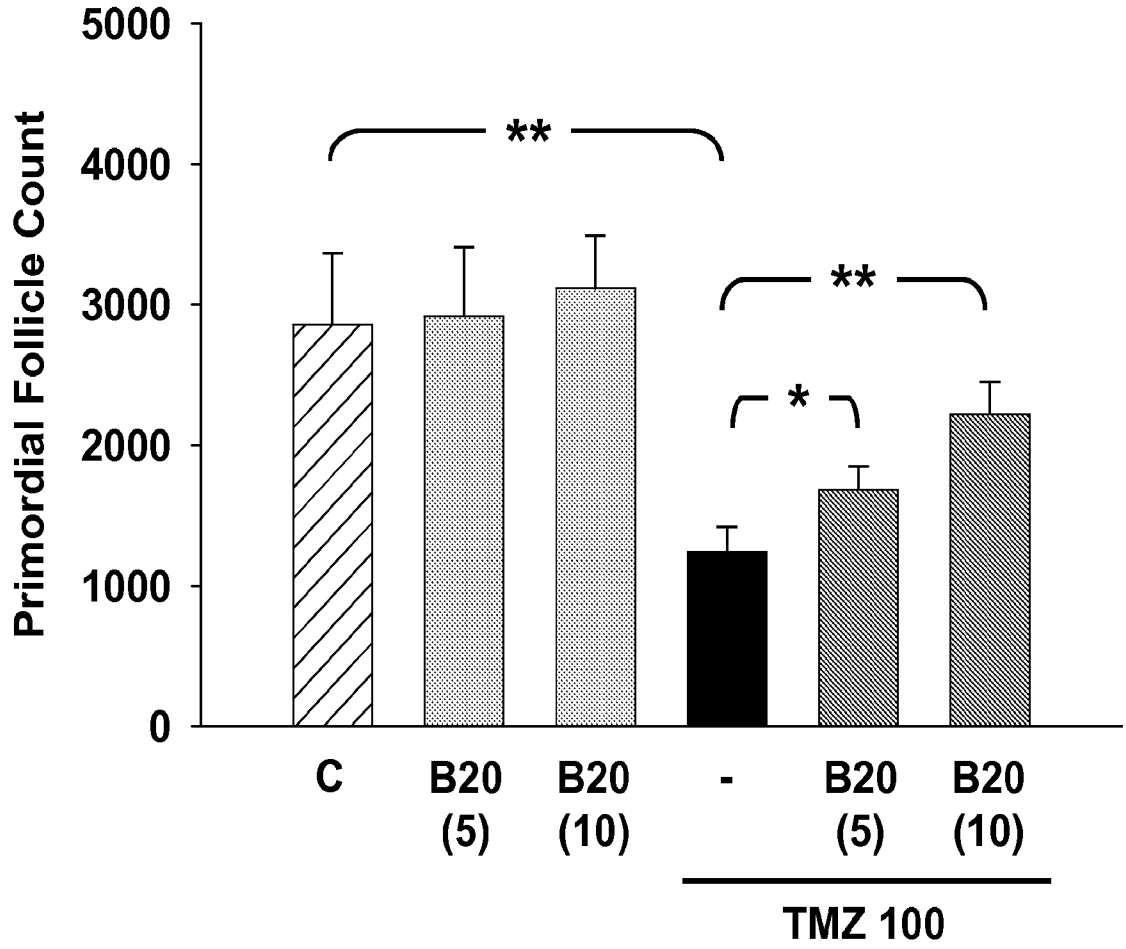

Primordial Follicle Count
5000
4000
3000
2000
1000
0
**
**
*
C
B20 (5)
B20 (10)
-
B20 (5)
B20 (10)
TMZ 100

FIG. 1

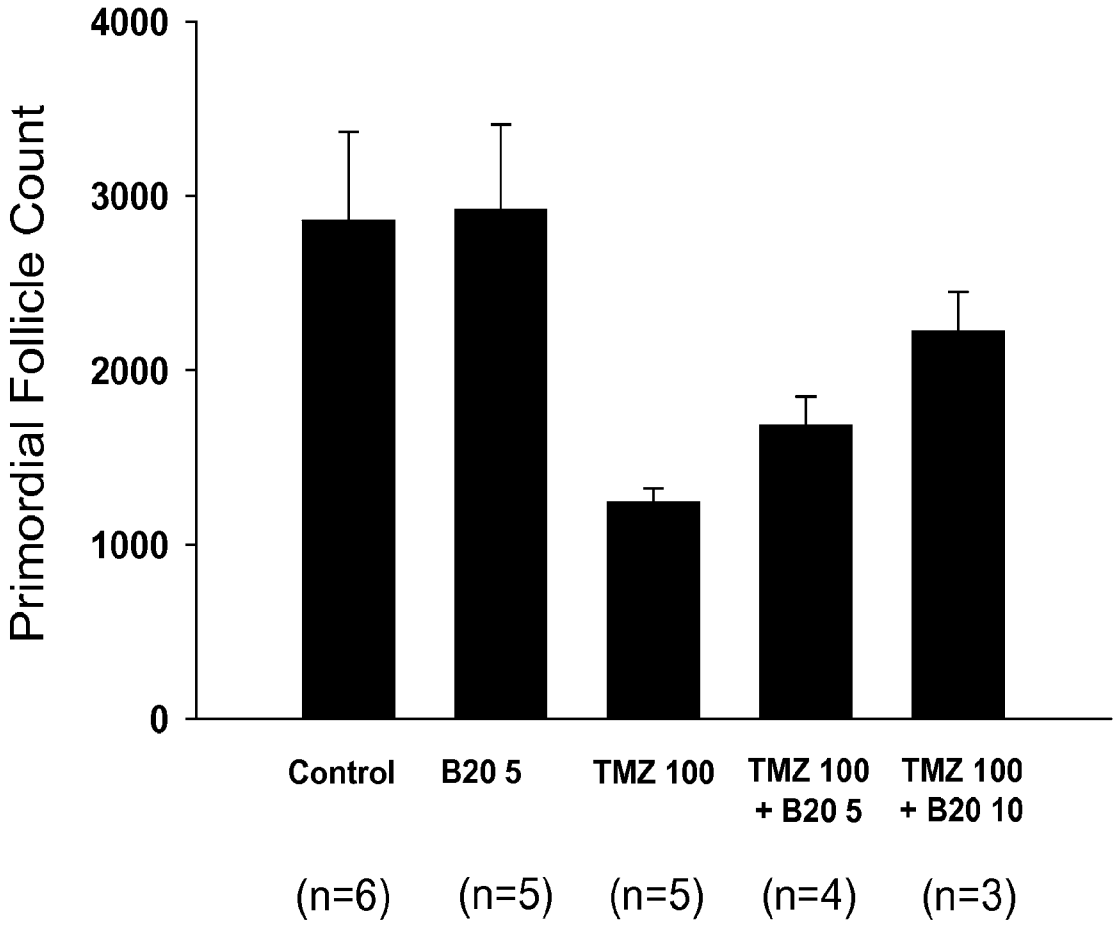

Primordial Follicle Count
4000
3000
2000
1000
0
Control
B20 5
TMZ 100
TMZ 100 + B20 5
TMZ 100 + B20 10
(n=6)
(n=5)
(n=5)
(n=4)
(n=3)

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/042174

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395 (2014.01)
CPC - A61K 39/3955 (2014.09)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/337, 39/395; A61P 15/08, 35/00; C07K 16/22 (2014.01)
USPC - 424/130.1, 133.1; 514/8.1, 19.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 31/337, 2039/507, 31/4745, 31/704, 39/3955, 45/06; C07K 16/22 (2014.09)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase,Google Patents, Google, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2010/0254999 A1 (FERRARA et al) 07 October 2010 (07.10.2010) entire document	1-5 ---- 6
Y	FARGHALY et al. "Ovarian Cancer - Basic Science Perspective," 17 February 2012 (17.02.2012), Pgs. 335-354. entire document	6
A	TEOH et al. "Antiangiogenic Therapies in Epithelial Ovarian Cancer," Cancer Control, 01 January 2011 (01.01.2011), Vol. 18, No. 1, Pgs. 31-43. entire document	1-6
A	LENGYEL et al. "Ovarian Cancer Development and Metastasis," The American Journal of Pathology, 01 September 2010 (01.09.2010), Vol. 177, No. 3, Pgs. 1053-1064. entire document	1-6
A	SCHMIDT et al. "Combining Bevacizumab with Radiation or Chemoradiation for Solid Tumors: A Review of the Scientific Rationale, and Clinical Trials," Curr Angiogenes, 01 September 2012 (01.09.2012), Vol. 3, No. 3, Pgs. 169-179. entire document.	1-6

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2014	19 NOV 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/042174

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Specifically, SEQ ID NO: 1 was searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/042174

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-21
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.