CTLA4 ANTIBODY AND AROMATASE INHIBITOR COMBINATION TREATMENT FOR BREAST CANCER

Background of the Invention

Each year, more than 180,000 women are diagnosed with breast cancer in the United States. If current breast cancer rates remain constant, each woman born today has a one-in-ten chance of developing breast cancer at a median onset age of about 60 to 65.

Approximately two-thirds of post-menopausal breast cancer patients have estrogen-dependent disease thereby rendering them amenable to antiestrogen therapy. Estrogens promote growth and proliferation of specific target cells, such as breast epithelium and estrogen-dependent breast carcinoma cells (Brueggemeier *Amer J Therapeutics* **8**:333-344 (2001)). Thus, systemic adjuvant treatment therapy, administered in addition to primary treatment of localized breast cancer (*e.g.*, breast-conserving surgery and radiation or mastectomy) has focused on agents that block the estrogen receptor on tumor cells. Such antihormonal agents include tamoxifen (NOVALDEX D, SOLTAMOX, TAMOFEN), which inhibits binding of estrogen with the estrogen receptor, fulvestrant (FASLODEX), which degrades the receptor, as well as aromatase inhibitors (*e.g.*, anastrozole (ARIMIDEX), letrozole (FEMARA), and exemestane (AROMASIN)), which block aromatase enzyme catalysis of androgens to estrogens thereby decreasing the amount of hormone ligand available to bind with the receptor.

Despite advances in hormonal or other breast cancer therapies, it is estimated that 40,921 women will die of recurrent or advanced breast cancer in 2004 (American Cancer Society, Cancer Statistics (2004)). Currently, there is no curative treatment available for patients with extensive disease and therapy is essentially palliative. Thus, novel therapies, such as combining antihormonal agent with immunotherapy, are important in the treatment of breast cancer. More specifically, one cancer immunotherapy approach targets cytotoxic T lymphocyte-associated antigen 4 (CTLA4; CD152), which is a cell surface receptor expressed on activated T cells. Binding of CTLA4 to its natural ligands, B7.1 (CD80) and B7.2 (CD86), delivers a negative regulatory signal to T cells, and blocking this negative signal results in enhanced T cell immune function and antitumor activity in animal models (Thompson and Allison *Immunity* **7**:445-450 (1997); McCoy and LeGros *Immunol.& Cell Biol.* **77**:1-10 (1999)). Several studies have demonstrated that CTLA4 blockade using antibodies markedly enhances T cell-mediated killing of tumors and can induce antitumor immunity (Leach et al., *Science* 271:1734-1736 (1996); Kwon et al. *Proc. Natl. Acad. Sci. USA* **94**:8099-8103 (1997); Kwon et al., *Natl. Acad. Sci. USA* **96**:15074-15079 (1999)).

Although use of anti-CTLA4 antibodies to induce an anti-tumor response holds great promise in the treatment of cancer, and despite the promise of antihormonal therapies in the treatment of breast cancer, there is a need to develop novel therapies to treat breast cancer. The present invention meets this need.

Summary of the Invention

The invention includes a method for the treatment of breast cancer in a patient in need of such treatment. The method comprises administering to the patient a therapeutically effective amount of an anti-CTLA4 antibody, or antigen-binding portion thereof, in combination with a therapeutically effective amount of an aromatase inhibitor.

In one aspect, the aromatase inhibitor is at least one inhibitor selected from the group consisting of anastrozole, letrozole and exemestane. In another aspect, the aromatase inhibitor is exemestane.

In one aspect, the therapeutically effective amount of a human anti-CTLA4 antibody amount ranges from about 1 mg/kg to 40 mg/kg.

In a further aspect, the therapeutically effective amount of a human anti-CTLA4 antibody amount ranges from about 3 mg/kg to 15 mg/kg.

In yet another aspect, the therapeutically effective amount of exemestane ranges from about 25 mg per day to 200 mg per day.

In a further aspect, the therapeutically effective amount of exemestane is about 25 mg per day.

In one aspect, the treatment is selected from the group consisting of a neoadjuvant therapy, an adjuvant therapy, a first line treatment, a second line treatment, and a third line treatment.

In another aspect, the antibody is selected from a group consisting of a non-human mammalian antibody, a chimeric antibody, and a human antibody.

In one aspect, the antibody is a human anti-CTLA4 antibody.

In another aspect, the anti-CTLA4 antibody, or antigen-binding portion thereof, is at least one antibody selected from the group consisting of:

(a) a human antibody having a binding affinity for CTLA4 of about 10^{-8} or greater, and which inhibits binding between CTLA4 and B7-1, and binding between CTLA4 and B7-2;

(b) a human antibody having an amino acid sequence comprising at least one human CDR sequence that corresponds to a CDR sequence from an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and 10D1;

(c) a human antibody having the heavy and light chain amino acid sequences of an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, and 12.9.1.1;

(d) a human antibody having the amino acid sequences of a heavy chain variable region and a light chain variable region of an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and 10D1;

(e) an antibody, or antigen-binding portion thereof, that competes for binding with CTLA4 with at least one antibody having the heavy and light chain amino acid sequences of an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and 10D1; and

(f) an antibody, or antigen-binding portion thereof, that cross-competes for binding with CTLA4 with at least one antibody having the heavy and light chain amino acid sequences of an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and 10D1.

In another aspect, the antibody is a human antibody having the heavy and light chain amino acid sequences of antibody 11.2.1.

In yet another aspect, the antibody comprises a heavy chain and a light chain wherein the amino acid sequences of the heavy chain variable region of the heavy chain and the light chain variable region of the light chain are selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:3 and the amino acid sequence of SEQ ID NO:9;

(b) the amino acid sequence of SEQ ID NO:15 and the amino acid sequence of SEQ ID NO:21 ;

(c) the amino acid sequence of SEQ ID NO:27 and the amino acid sequence of SEQ ID NO:33;

(d) the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:1 and the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:7;

(e) the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:13 and the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:19;

(f) the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:25 and the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:31;

(g) the amino acid sequence of a heavy chain variable region and a light chain variable region of antibody 10D1.

In one aspect, the antibody, or antigen-binding portion thereof, is an antibody selected from the group consisting of:

(a) an antibody having a heavy chain variable region comprising the amino acid sequences set forth in SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and further having a light chain variable region comprising the amino acid sequences set forth in SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12;

(b) an antibody having a heavy chain variable region comprising the amino acid sequences set forth in SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18, and further having a light chain variable region comprising the amino acid sequences set forth in SEQ ID NO:22, SEQ ID NO:23 and SEQ ID NO:24;

(c) an antibody having a heavy chain variable region comprising the amino acid sequences set forth in SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, and further having a light chain variable region comprising the amino acid sequences set forth in SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36; and

(d) an antibody having a heavy chain variable region comprising the amino acid sequences of the heavy chain CDR1, CDR2, and CDR3 of antibody 10D1, further having a light chain variable region comprising the amino acid sequences of the light chain CDR1, CDR2, and CDR3 of antibody 10D1.

In another aspect, the method further comprises administering to the patient at least one agent selected from the group consisting of an alkylating agent, a folate antagonist, a pyrimidine antagonist, an

anthracycline antibiotic, a platinum compound, a taxane, a vinca alkaloid, a camptothecin analog, a toll-like receptor stimulating agent, a heat shock protein-based tumor vaccine, an antigen presenting cell-based therapy, a mammalian target of rapamycin inhibitor, an erbB2 inhibitor, an EGFR inhibitor, a VEGF inhibitor, a VEGFR inhibitor, an angiogenesis inhibitor, an antibody, an immunomodulator, a selective estrogen receptor modulator, a cytokine, a tumor vaccine, an antiproliferative agent, an immune costimulatory molecule, and a cytokine.

The invention includes a pharmaceutical composition for the treatment of breast cancer comprising: a therapeutically effective amount of an anti-CTLA4 antibody; a therapeutically effective amount of an aromatase inhibitor; and a pharmaceutically acceptable carrier.

In one aspect, the aromatase inhibitor is at least one aromatase inhibitor selected from the group consisting of anastrozole, letrozole, and exemestane.

In a further aspect, the aromatase inhibitor is exemestane.

The invention includes a use of an amount of an anti-CTLA4 antibody in the preparation of a composition for the treatment of breast cancer in a patient wherein the treatment further comprises administering to the patient an amount of an aromatase inhibitor.

In one aspect, the aromatase inhibitor is exemestane and further wherein the antibody is a human antibody having the heavy and light chain variable region amino acid sequences of antibody 11.2.1.

Brief Description of the Drawings

The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention there are shown in the drawings embodiment(s) which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown.

In the drawings:

Figure 1, comprising Figures 1A-1D, shows the nucleotide and amino acid sequences for anti-CTLA4 antibody 4.1.1. Figure 1A shows the full length nucleotide sequence for the 4.1.1 heavy chain (SEQ ID NO:1). Figure 1B shows the full length amino acid sequence for the 4.1.1 heavy chain (SEQ ID NO:2), and the amino acid sequence for the 4.1.1 heavy chain variable region (SEQ ID NO:3) designated between brackets "[ ]". The amino acid sequence of each 4.1.1 heavy chain CDR is underlined. The CDR sequences are as follows: CDR1: GFTFSSHGMH (SEQ ID NO:4); CDR2: VIWYDGRNKYYADSV (SEQ ID NO:5); and CDR3: GGHFGPFDY (SEQ ID NO:6). Figure 1C shows the nucleotide sequence for the 4.1.1 light chain (SEQ ID NO:7). Figure 1D shows the amino acid sequence of the full length 4.1.1 light chain (SEQ ID NO:8), and the variable region as indicated between brackets "[ ]" (SEQ ID NO:9). The amino acid sequence of each CDR is indicated as follows: CDR1: RASQSISSSFLA (SEQ ID NO:10); CDR2: GASSRAT (SEQ ID NO:11); and CDR3: CQQYGTSPWT (SEQ ID NO:12).

Figure 2, comprising Figures 2A-2D, shows the nucleotide and amino acid sequences for anti-CTLA4 antibody 4.13.1. Figure 2A shows the full length nucleotide sequence for the 4.13.1 heavy chain (SEQ ID NO:13). Figure 2B shows the full length amino acid sequence for the 4.13.1 heavy chain (SEQ ID NO:14), and the amino acid sequence for the 4.13.1 heavy chain variable region (SEQ ID NO:15)

designated between brackets "[ ]". The amino acid sequence of each 4.13.1 heavy chain CDR is underlined. The CDR sequences are as follows: CDR1: GFTFSSHGIH (SEQ ID NO:16); CDR2: VIWYDGRNKDYADSV (SEQ ID NO:12); and CDR3: VAPLGPLDY (SEQ ID NO:18). Figure 2C shows the nucleotide sequence for the 4.13.1 light chain (SEQ ID NO:19). Figure 2D shows the amino acid sequence of the full length 4.13.1 light chain (SEQ ID NO:20), and the variable region as indicated between brackets "[ ]" (SEQ ID NO:21). The amino acid sequence of each CDR is indicated as follows: CDR1: RASQSVSSYLA (SEQ ID NO:22); CDR2: GASSRAT (SEQ ID NO:23); and CDR3: CQQYGRSPFT (SEQ ID NO:24).

Figure 3, comprising Figures 3A-3D, shows the nucleotide and amino acid sequences for anti-CTLA4 antibody 11.2.1. Figure 3A shows the full length nucleotide sequence for the 11.2.1 heavy chain (SEQ ID NO:25). Figure 3B shows the full length amino acid sequence for the 11.2.1 heavy chain (SEQ ID NO:26), and the amino acid sequence for the 11.2.1 heavy chain variable region (SEQ ID NO:27) designated between brackets "[ ]". The amino acid sequence of each 11.2.1 heavy chain CDR is underlined. The CDR sequences are as follows: CDR1: GFTFSSYGMH (SEQ ID NO:28); CDR2: VIWYDGSNKYYADSV (SEQ ID NO:29); and CDR3: DPRGATLYYYYYGMDV (SEQ ID NO:30). Figure 3C shows the nucleotide sequence for the 11.2.1 light chain (SEQ ID NO:31). Figure 3D shows the amino acid sequence of the full length 11.2.1 light chain (SEQ ID NO:32), and the variable region as indicated between brackets "[ ]" (SEQ ID NO:33). The amino acid sequence of each CDR is indicated as follows: CDR1: RASQSINSYLD (SEQ ID NO:34); CDR2: AASSLQS (SEQ ID NO:35); and CDR3: QQYYSTPFT (SEQ ID NO:36).

Detailed Description Of The Invention

The invention relates to anti-CTLA4 antibodies used in combination with at least one aromatase inhibitor, *e.g.*, anastrozole, letrozole, and exemestane, to treat breast cancer in a patient in need of such treatment. The invention further relates to treatment of breast cancer by combination of the antibody-aromatase inhibitor combination with another agent or agents.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art.

The methods and techniques of the present invention are generally performed according to methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Such references include, *e.g.*, Sambrook and Russell, *Molecular Cloning, A Laboratory Approach*, Cold Spring Harbor Press, Cold Spring Harbor, NY (2001), Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (2002), and Harlow and Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as

commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology--A Synthesis* (2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference.

A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson, *Methods Mol. Biol.* **243**:307-31 (1994).

Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al., *Science* **256**:1443-45 (1992), herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs comprising substitutions, deletions, and/or insertions can include various muteins of a sequence other than the specified peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the specified sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts, *e.g.*, outside of the CDRs). A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized

polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al., *Nature* **354**:105 (1991), which are each incorporated herein by reference.

Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, Genetics Computer Group (GCG available from Genetics Computer Group, Inc.), also referred to as the Wisconsin Package, is an integrated software package of over 130 programs for accessing, analyzing and manipulating nucleotide and protein sequences. GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters to determine sequence similarity, homology and/or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG version 6.1, version 9.1, and version 10.0.

Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* **183**:63-98 (1990); Pearson, *Methods Mol. Biol.* **132**:185-219 (2000)). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters. *See, e.g.*, Altschul et al., *J. Mol. Biol.* **215**:403-410 (1990); Altschul et al., *Nucleic Acids Res.* **25**:3389-402 (1997); herein incorporated by reference.

An "aromatase," as the term is used herein, refers to an enzyme (*e.g.*, 450_{arom}) that catalyzes the aromatization of the A ring of an androgen substrate (mainly androstenedione) to estrone.

"Aromatase inhibitor" means a compound or substance that when contacted with an aromatase, detectably inhibits the formation of the A ring of an androgen substrate compared with the level of such aromatization in the absence of the compound or substance. Any assay for assessing the level of enzymatic activity by an aromatase can be used to assess whether there is inhibition and/or the level thereof. An aromatase inhibitor includes, but is not limited to, a steroidal and a non-steroidal inhibitor such as, *inter alia*, exemestane, anastrozole, letrozole, fadrozole, vorozole, and formestane, and the term encompasses any aromatase inhibitor whether now known or identified in the future.

An intact "antibody" comprises at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. See generally, *Fundamental Immunology*, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). Each heavy chain is comprised of a heavy chain variable region (HCVR or V_H) and a heavy chain constant region (C_H). The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (LCVR or V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs

and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD (1987 and 1991)), or Chothia & Lesk, *J. Mol. Biol.* **196**:901-917 (1987); Chothia et al., *Nature* **342**:878-883 (1989).

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., CTLA4). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_H1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv)); see e.g., Bird et al. *Science* 242:423-426 (1988) and Huston et al. *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988)). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger et al. *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993); Poljak et al. *Structure* 2:1121-1123 (1994)).

Still further, an antibody or antigen-binding portion thereof may be part of larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov et al. *Human Antibodies and Hybridomas* 6:93-101 (1995)) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov et al. *Mol. Immunol.* 31:1047-1058 (1994)). Other examples include where one or more CDRs from an antibody are incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to an antigen of interest, such as CTLA4. In such embodiments, the CDR(s) may be incorporated as part of a larger polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently. Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

Where an "antibody" is referred to herein with respect to the present invention, it should be understood that an antigen-binding portion thereof may also be used. An antigen-binding portion competes with the intact antibody for specific binding. See generally, Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. In some embodiments, antigen-binding portions include Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an antibody that is sufficient to confer specific antigen binding to the polypeptide. In embodiments having one or more binding sites, the binding sites may be identical to one another or may be different.

The terms "human antibody" or "human sequence antibody", as used interchangeably herein, include antibodies having variable and constant regions (if present) derived from human germline immunoglobulin sequences. The human sequence antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human antibody", as used herein, is not intended to include "chimeric" antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences (i.e., "humanized" or PRIMATIZED™ antibodies).

The term "chimeric antibody" as used herein means an antibody that comprises regions from two or more different antibodies. In one embodiment, one or more of the CDRs are derived from a human anti-CTLA4 antibody. In another embodiment, all of the CDRs are derived from a human anti-CTLA4 antibody. In another embodiment, the CDRs from more than one human anti-CTLA4 antibodies are combined in a chimeric human antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-CD40 antibody, a CDR2 from the light chain of a second human anti-CTLA4 antibody and a CDR3 and CDR3 from the light chain of a third human anti-CTLA4 antibody, and the CDRs from the heavy chain may be derived from one or more other anti-CD40 antibodies. Further, the framework regions may be derived from one of the same anti-CTLA4 antibodies or from one or more different human(s).

Moreover, as discussed previously herein, chimeric antibody includes an antibody comprising a portion derived from the germline sequences of more than one species.

"Glycoform" refers to a complex oligosaccharide structure comprising linkages of various carbohydrate units. Such structures are described in, *e.g.*, *Essentials of Glycobiology* Varki et al., eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1999), which also provides a review of standard glycobiology nomenclature. Such glycoforms include, but are not limited to, G2, G1, G0, G-1, and G-2 (*see*, *e.g.*, International Patent Publication No. WO 99/22764).

"Glycosylation pattern" is defined as the pattern of carbohydrate units that are covalently attached to a protein (*e.g.*, the glycoform) as well as to the site(s) to which the glycoform(s) are covalently attached to the peptide backbone of a protein, more specifically to an immunoglobulin protein.

It is likely that antibodies expressed by different cell lines or in transgenic animals will have different glycoforms and/or glycosylation patterns compared with each other. However, all antibodies

encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein are part of the instant invention, regardless of the glycosylation of the antibodies.

By the term "effective amount", or "therapeutically effective amount," as used herein, is meant an amount that when administered to a mammal, preferably a human, mediates a detectable therapeutic response compared to the response detected in the absence of the compound. A therapeutic response, such as, but not limited to, inhibition of and/or decreased tumor growth, tumor size, metastasis, and the like, can be readily assessed by a plethora of art-recognized methods, including, *e.g.*, such methods as disclosed herein.

The skilled artisan would understand that the effective amount of the compound or composition administered herein varies and can be readily determined based on a number of factors such as the disease or condition being treated, the stage of the disease, the age and health and physical condition of the mammal being treated, the severity of the disease, the particular compound being administered, and the like.

By the term "compete", as used herein with regard to an antibody, is meant that a first antibody, or an antigen-binding portion thereof, competes for binding with a second antibody, or an antigen-binding portion thereof, where binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s). For instance, cross-competing antibodies can bind to the epitope, or potion of the epitope, to which the antibodies used in the invention bind. Use of both competing and cross-competing antibodies is encompassed by the present invention. Regardless of the mechanism by which such competition or cross-competition occurs (*e.g.*, steric hindrance, conformational change, or binding to a common epitope, or portion thereof, and the like), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

"Instructional material," as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the compound, combination, and/or composition of the invention in the kit for affecting, alleviating or treating the various diseases or disorders recited herein. Optionally, or alternately, the instructional material can describe one

or more methods of alleviating the diseases or disorders in a cell, a tissue, or a mammal, including as disclosed elsewhere herein.

The instructional material of the kit may, for example, be affixed to a container that contains the compound and/or composition of the invention or be shipped together with a container which contains the compound and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively.

Except when noted, the terms "patient" or "subject" are used interchangeably and refer to mammals such as human patients and non-human primates, as well as veterinary subjects such as rabbits, rats, and mice, and other animals. Preferably, patient refers to a human.

Conventional notation is used herein to portray polypeptide sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus.

By the phrase "specifically binds," as used herein, is meant a compound, *e.g.*, a protein, a nucleic acid, an antibody, and the like, which recognizes and binds a specific molecule, but does not substantially recognize or bind other molecules in a sample. For instance, an antibody or a peptide inhibitor which recognizes and binds a cognate ligand (*e.g.*, an anti-CTLA4 antibody that binds with its cognate antigen, CTLA4) in a sample, but does not substantially recognize or bind other molecules in the sample. Thus, under designated assay conditions, the specified binding moiety (*e.g.*, an antibody or an antigen-binding portion thereof) binds preferentially to a particular target molecule and does not bind in a significant amount to other components present in a test sample. A variety of assay formats may be used to select an antibody that specifically binds a molecule of interest. For example, solid-phase ELISA immunoassay, immunoprecipitation, BIAcore and Western blot analysis are used to identify an antibody that specifically reacts with CTLA4. Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 times background, even more specifically, an antibody is said to "specifically bind" an antigen when the equilibrium dissociation constant (K_D) is ≤ 1 μM, preferably ≤ 100 nM and most preferably ≤ 10 nM.

The term "K_D" refers to the equilibrium dissociation constant of a particular antibody-antigen interaction.

As used herein, "substantially pure" means an object species is the predominant species present (*i.e.*, on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species (*e.g.*, an anti-CTLA4 antibody) comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

As used herein, to "treat" means reducing the frequency with which symptoms of a disease (*i.e.*, tumor growth and/or metastasis, or other effect mediated by the numbers and/or activity of immune cells,

and the like) are experienced by a patient. The term includes the administration of the compounds or agents of the present invention to prevent or delay the onset of the symptoms, complications, or biochemical indicia of a disease (*e.g.*, elevation of PSA level), alleviating the symptoms or arresting or inhibiting further development of the disease, condition, or disorder. Treatment may be prophylactic (to prevent or delay the onset of the disease, or to prevent the manifestation of clinical or subclinical symptoms thereof) or therapeutic suppression or alleviation of symptoms after the manifestation of the disease.

I. Antibody-Aromatase Inhibitor Combination Therapy

The invention relates to methods for the administration of an antibody that binds human CTLA4, or an antigen-binding portion of the antibody, in combination with hormonal (*e.g.*, aromatase inhibitor) therapy to treat breast cancer. The combination of CTLA4 blockade with reduction in the level of estrogen in a patient according to a method of the invention may provide a synergistic therapeutic benefit as more fully discussed below.

Although the invention encompasses numerous combination therapies wherein the antibody is administered to the patient in combination with at least one aromatase inhibitor, the present invention is in no way limited to the exemplified agents, which are set forth herein for illustrative purposes only.

In one embodiment, a combination of a CTLA4 antibody, or an antigen-binding portion thereof, and an aromatase inhibitor ("AI") is administered to a breast cancer patient. In one aspect, the aromatase inhibitor is selected from the group consisting of anastrozole, letrozole and exemestane, which are commercially available as ARIMIDEX, FEMARA and AROMASIN, respectively. More preferably, the aromatase inhibitor is exemestane. This is because a synergistic or additive effect is mediated by administration of a combination comprising an anti-CTLA4 antibody and an AI. Without wishing to be bound by any particular theory of the invention, AI therapy may increase, perhaps by apoptotic or other architectural changes, exposure of breast cancer tumor specific antigen(s) to the immune system such that the immune response to the tumor cell is increased. That is, hormonal therapy may create or increase a source of tumor-specific antigen mediated by tumor cell death which, in turn, may feed tumor antigen into host antigen presentation pathways. Anti-CTLA4 antibody mediates an increased immune response to the increased levels of tumor-specific antigen in the antigen presentation pathway thereby providing a potential synergistic therapeutic effect when combined with aromatase inhibitor therapy. Other combination therapies that may result in synergy with anti-CTLA4 enhancement of the immune response through cell death release of tumor-specific antigens are radiation, surgery, and chemotherapy, among others.

Without wishing to be bound by any theory of the invention, the combination of CTLA4 blockade and aromatase inhibition may induce a more robust immunological response to the breast cancer than expected. Therefore, the combination of aromatase blockade using, among others, exemestane, in combination with an anti-CLTA4 antibody, can provide a potential synergistic effect thereby providing an important novel therapeutic treatment for breast cancer.

The methods of the invention may be carried out as a neoadjuvant therapy prior to surgery, radiation therapy, or any other treatment localized treatment, in order to sensitize the tumor cells or to

otherwise confer a therapeutic benefit to the patient. However, the invention is not limited to the neoadjuvant setting. Rather, the methods of the invention may be used along the entire disease and treatment continuum, *e.g.*, but not limited to, adjuvant, first-line, second-line, third-line therapy, and the like, for breast cancer.

In one embodiment, the breast cancer is estrogen receptor positive (ER+), such that aromatase inhibition reduces the level of estrogen thereby affecting the tumor. In one aspect, the ER+ tumor is present in a postmenopausal (post-men) woman patient, such that decreasing the level of estrogen mediates a therapeutic anti-tumor effect in the patient. However, while it may be preferable to administer AI therapy to a post-men ER+ patient, the present invention is not limited to such patient, but rather, comprises administration of anti-CTLA4 antibody-aromatase inhibitor therapy to any patient that may derive a therapeutic benefit from a combination of decreased estrogen level combined with immune enhancement via CTLA4 blockade, as determined by the skilled artisan armed with the teachings provided herein.

II. Dosage Regimens

Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the antibody and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non limiting range for a therapeutically effective amount of an antibody administered according to the invention is at least about 1 mg/kg, at least about 5 mg/kg, at least about 10 mg/kg, more than about 10 mg/kg, or at least about 15 mg/kg, for example about 1-30 mg/kg, or for example about 1-25 mg/kg, or for example about 1-20 mg/kg, or for example about 5-20 mg/kg, or for example about 10-20 mg/kg, or for example about 15-20 mg/kg, or for example, about 15 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. Determining appropriate dosages and regiments for administration of the antibody are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

In one embodiment, the antibody is administered in an intravenous formulation as a sterile aqueous solution containing about 5 to 20 mg/ml of antibody, in an appropriate buffer system.

In one embodiment, for administration of low doses, part of the dose is administered by an intravenous bolus and the rest by infusion of the antibody formulation. For example, a 0.01 mg/kg intravenous injection of the antibody may be given as a bolus, and the rest of a predetermined antibody dose may be administered by intravenous injection. In another embodiment, the entire low dose is administered as a single bolus injection. For higher doses, *e.g.*, 3 mg/kg, the antibody is not administered as a bolus, but the entire amount is administered by infusion. A predetermined dose of the antibody may be administered, for example, over a period of about an hour and a half to about five hours.

The present invention relates to administering a combination of an anti-CTLA4 antibody and an aromatase inhibitor. The skilled artisan would appreciate that the combination can be administered simultaneously or the two agents can be administered at different times. For instance, in one embodiment, the antibody is administered as a single injection and/or infusion and the aromatase inhibitor (*e.g.*, exemestate) is administered once per day commencing before, during, or after administration of the antibody. Typically, exemestane is administered daily, once per day, usually at the same time of the day and accompanied with a meal. However, the present invention is not limited to any particular dosage or administration regimen for an aromatase inhibitor. Rather, the optimal dose, route and regimen for administration of the antibody and the aromatase inhibitor can be readily determined by one of ordinary skill in the relevant art using well-known methods.

For instance, a single dose or multiples doses of the antibody may be administered. Alternatively, at least one dose, or at least three, six or 12 doses may be administered. The doses may be administered, for example, every two weeks, monthly, every twenty days, every 25 days, every 28 days, every 30 days, every 40 days, every 6 weeks, every 50 days, every two months, every 70 days, every 80 days, every three months, every six months or yearly. In addition, the aromatase inhibitor can be administered daily, several times or once per day, weekly, every other week, every third week, every fourth week, monthly, every three months, every six months, once per year, or any other period that provides a therapeutic benefit to the patient as determined by the skilled practitioner.

The antibody can be administered until disease progression, or intolerable toxicity, or up to 12 consecutive cycles, whichever time is shorter. The antibody can also be administered using a regimen comprising administration of a loading dose followed by a lower dose. Repeat courses of at least one, and more preferably, several cycles of antibody and an aromatase inhibitor can be administered to a patient that experiences a tumor recurrence who had previously derived benefit from administration of the combination of an anti-CTLA4 antibody and an aromatase inhibitor.

In one embodiment, a single injection comprising the anti-CTLA4 antibody is administered to a patient intravenously at a dose of about 3 mg/kg every twenty-eight days. A dose of approximately 25 mg per day of exemestane is also administered to the patient, either before, during and/or after administration of the antibody. In an embodiment of the invention, a single dose comprising the anti-CTLA4 antibody is administered to a patient intravenously at a dose of about 3 mg/kg, preferably, about 6 mg/kg, more preferably, about 10 mg/kg, preferably, about 15 mg/kg, every three months. A dose of approximately 25 mg per day of exemestane is also administered to the patient, either before, during and/or after

administration of the antibody. In another embodiment, a bolus injection comprising an anti-CTLA4 antibody is administered to a patient intravenously at a dose higher than 3 mg/kg (e.g., 6, 10, 15, 20, 25, 30, 35 or 40 mg/kg) every twenty-eight days. The dose can be adjusted as known in the art based on, among other factors, toxicity, if any, and therapeutic effectiveness. A dose of approximately 25 mg per day of exemestane is also administered to the patient, either before, during and/or after administration of the antibody.

The antibody-aromatase inhibitor combination can be administered as a first line systemic adjuvant therapy, or it can be administered to the patient as a neoadjuvant therapy prior to surgery, radiation therapy, or any other treatment, in order to sensitize the tumor cells or to otherwise confer a therapeutic benefit to the patient.

Further, the combination can be administered as a second line therapy, such as, but not limited to, once tamoxifen first line therapy has failed. Alternatively, the combination can be administered concurrently with tamoxifen therapy, and or at any point during tamoxifen therapy, which typically is administered for about five years following initial treatment. In another embodiment, the combination of anti-CTLA4 antibody and exemestane may be administered to a patient following about two or three years of tamoxifen or other adjuvant therapy. This is because it has been demonstrated that exemestane therapy following about two to three years of tamoxifen therapy provides a therapeutic benefit while reducing the side effects of tamoxifen therapy.

Thus, a combination of an anti-CLTA4 antibody and an aromatase inhibitor, preferably, exemestane, can provide a therapeutic benefit once tamoxifen therapy has failed, once systemic adjuvant therapy using another aromatase inhibitor (*e.g.*, anastrozole, letrozole, and the like) has failed, and/or after tamoxifen administration for about two to three years, among others. Therefore, the invention encompasses administration of an anti-CTLA4 antibody and an aromatase inhibitor in combination with or following additional antihormonal therapy, including, but not limited to, tamoxifen, anastrozole, letrozole and fulvestrant as would be appreciated by one skilled in the art based upon the disclosure provided herein.

III. Anti-CTLA4 Antibodies

In one embodiment, the CTLA4 antibody used in the invention comprises a heavy chain wherein the amino acid sequence of the V_H comprises the amino acid sequences set forth in SEQ ID NOs:3, 15 and 27. In yet another embodiment, the V_L of the CTLA4 antibody comprises the amino acid sequences set forth in SEQ ID NOs:9, 21 and 33. More preferably, the V_H and V_L regions of the antibody comprise the amino acid sequences set forth in SEQ ID NO:3 (V_H 4.1.1) and SEQ ID NO:9 (V_L 4.1.1), respectively; the amino acid sequences set forth in SEQ ID NO:15 (V_H 4.13.1) and SEQ ID NO:21 (V_L 4.13.1), respectively; and the amino acid sequences set forth in SEQ ID NO:27 (V_H 11.2.1) and SEQ ID NO:33 (V_L 11.2.1), respectively. Most preferably, the antibody is ticilimumab (also known as CP-675,206), which has the heavy and light chain amino acid sequences of antibody 11.2.1.

In yet another embodiment, the amino acid sequence of the heavy chain comprises the amino acid sequence encoded by a nucleic acid comprising the nucleic acid sequences set forth in SEQ ID NOs:1, 13, and 25. In yet another embodiment, the light chain comprises the amino acid sequence encoded by a nucleic acid comprising the nucleic acid sequences set forth in SEQ ID NOs:7, 19 and 31.

More preferably, the heavy and light chains comprise the amino acid sequences encoded by nucleic acids comprising the nucleic acid sequences set forth in SEQ ID NO:1 (heavy chain 4.1.1) and SEQ ID NO:7 (light chain 4.1.1), respectively; the nucleic acid sequences set forth in SEQ ID NO:13 (heavy chain 4.13.1) and SEQ ID NO:19 (light chain 4.13.1), respectively; and the nucleic acid sequences set forth in SEQ ID NO:25 (heavy chain 11.2.1) and SEQ ID NO:31 (light chain 11.2.1), respectively.

Furthermore, the antibody can comprise a heavy chain amino acid sequence comprising human CDR amino acid sequences derived from the V_H 3-30 or 3-33 gene, or conservative substitutions or somatic mutations therein. It is understood that the V_H 3-30 or V_H 3-33 gene encodes from FR1 through FR3 of the heavy chain variable region of an antibody molecule. Thus, the invention encompasses an antibody that shares at least 85%, more preferably, at least 90%, yet more preferably, at least 91%, even more preferably, at least 94%, yet more preferably, at least 95%, more preferably, at least 97%, even more preferably, at least 98%, yet more preferably, at least 99%, and most preferably, 100% identity, with the sequence from FR1 through FR3 of an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 2.9.1.1, 10D1, and DP-50.

The antibody can further comprise CDR regions in its light chain derived from the A27 or the O12 gene or it may comprise the CDR regions of an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 2.9.1.1, 10D1.

In other embodiments of the invention, the antibody inhibits binding between CTLA4 and B7-1, B7-2, or both. Preferably, the antibody can inhibit binding with B7-1 with an IC_{50} of about 100 nM or lower, more preferably, about 10 nM or lower, for example about 5 nM or lower, yet more preferably, about 2 nM or lower, or even more preferably, for example, about 1 nM or lower. Likewise, the antibody can inhibit binding with B7-2 with an IC_{50} of about 100 nM or lower, more preferably, 10 nM or lower, for example, even more preferably, about 5 nM or lower, yet more preferably, about 2 nM or lower, or even more preferably, about 1 nM or lower.

Further, in another embodiment, the anti-CTLA4 antibody has a binding affinity for CTLA4 of about 10^{-8}, or greater affinity, more preferably, about 10^{-9} or greater affinity, more preferably, about 10^{-10} or greater affinity, and even more preferably, about 10^{-11} or greater affinity.

The anti-CTLA4 antibody includes an antibody that competes for binding with an antibody having heavy and light chain amino acid sequences of an antibody selected from the group consisting of 4.1.1, 6.1.1, 11.2.1, 4.13.1 and 4.14.3. Further, the anti-CTLA4 antibody can compete for binding with antibody 10D1.

In another embodiment, the antibody preferably cross-competes with an antibody having a heavy and light chain sequence, a variable heavy and a variable light chain sequence, and/or the heavy and light CDR sequences of antibody 4.1.1, 4.13.1, 4.14.3, 6.1.1. or 11.2.1. For example, the antibody can bind to the epitope to which an antibody that has heavy and light chain amino acid sequences, variable sequences and/or CDR sequences, of an antibody selected from the group consisting of 4.1.1, 4.13.1, 4.14.3, 6.1.1, or 11.2.1 binds. In another embodiment, the antibody cross-competes with an antibody having heavy and light chain sequences, or antigen-binding sequences, of 10D1 (MDX-D010).

In another embodiment, the invention is practiced using an anti-CTLA4 antibody that comprises a heavy chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3, and a light chain

comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3, of an antibody selected from the group consisting of 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1, or sequences having changes from the CDR sequences selected from the group consisting of conservative changes, wherein the conservative changes are selected from the group consisting of replacement of nonpolar residues by other nonpolar residues, replacement of polar charged residues other polar uncharged residues, replacement of polar charged residues by other polar charged residues, and substitution of structurally similar residues; non-conservative substitutions, wherein the non-conservative substitutions are selected from the group consisting of substitution of polar charged residue for polar uncharged residues and substitution of nonpolar residues for polar residues, additions and deletions.

In a further embodiment of the invention, the antibody contains fewer than 10, 7, 5, or 3 amino acid changes from the germline sequence in the framework or CDR regions. In another embodiment, the antibody contains fewer than 5 amino acid changes in the framework regions and fewer than 10 changes in the CDR regions. In one preferred embodiment, the antibody contains fewer than 3 amino acid changes in the framework regions and fewer than 7 changes in the CDR regions. In a preferred embodiment, the changes in the framework regions are conservative and those in the CDR regions are somatic mutations.

In another embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, preferably, at least 95%, more preferably, at least 99%, sequence(*e.g.*, amino acid, nucleic acid, or both) identity or sequence similarity over the heavy and light chain full-length sequences, or over the heavy or the light chain, separately, with the sequences of antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, 10D1. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy chain and the light chain, or with the heavy chain or the light chain, separately, of an antibody selected from antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, 10D1.

In another embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, more preferably, at least 95%, even more preferably, at least 99%, sequence identity or sequence similarity over the heavy and light chain full-length sequences, or over the heavy or the light chain, separately, with the sequences of germline V_κ A27, germline V_κ O12, and germline DP50 (which is an allele of the V_H 3-33 gene locus). Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy chain sequence of germline DP50 and/or with the light chain sequence of germline A27, or germline O12.

In one embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, preferably, at least 95%, more preferably, at least 99%, sequence(*e.g.*, amino acid, nucleic acid, or both) identity or sequence similarity over the heavy and light chain variable region sequences, or over the heavy or the light chain variable region sequence, separately, with the sequences of antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, 10D1. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy chain and the light chain variable region

sequences, or with the heavy chain or the light chain sequence, separately, of an antibody selected from antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, 10D1.

In another embodiment, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 94%, more preferably, at least 95%, even more preferably, at least 99%, sequence identity or sequence similarity over heavy chain variable region sequence with the heavy chain variable sequence of heavy germline DP50 (which is an allele of the V_H 3-33 gene locus) or with the light chain variable sequence of germline V_κ A27, or germline V_κ O12. Even more preferably, the antibody heavy chain region sequence shares 100% sequence identity or sequence similarity with the sequence of germline DP50 or with the light chain sequence of germline A27, or germline O12.

In one embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, sequence identity or sequence similarity with the heavy chain, the light chain, or both, sequences from FR1 through FR4 with the FR1 through FR4 region sequences of antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, 10D1. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy, light, or both, sequences from FR1 through FR4 with antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, and 10D1.

In another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the heavy chain sequences from FR1 through FR3 with the FR1 through FR3 region sequences of germline DP50.

In yet another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the light chain sequences from FR1 through FR4 with the FR1 through FR4 region sequences of germline V_κ A27, or germline V_κ O12.

In one embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, sequence identity or sequence similarity with the heavy chain, the light chain, or both, CDR-1, CDR-2 and CDR-3 sequences of antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, 10D1. Even more preferably, the antibody shares 100% sequence identity or sequence similarity over the heavy, light, or both, CDR-1, CDR-2 and CDR-3 sequences with antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, and 10D1.

In another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the heavy chain CDR-1 and CDR-2 sequences with the CDR-1 and CDR-2 sequences of germline DP50.

In yet another embodiment of the present invention, the antibody shares at least 80%, more preferably, at least 85%, even more preferably, at least 90%, yet more preferably, at least 95%, more preferably, at least 99%, and most preferably, about 100%, sequence identity or sequence similarity with the light chain CDR-1, CDR-2 and CDR-3 sequences with the CDR-1, CDR-2 and CDR-3 sequences of germline V_κ A27, or germline V_κ O12.

Examples of antibodies employable in the present invention, and methods of producing them, are described in, among others, U.S. Patent Application No. 09/472,087, now issued as U.S. Patent No. 6,682,736; Int. Appl. No. PCT/US00/23356 (published March 1, 2001, as WO 01/14424) (*e.g.*, antibody 10D1, also known as MDX-010, Medarex, Princeton, NJ); Int. Appl. No. PCT/US99/28739 (published June 8, 2000, as WO 00/32231); U.S. Pat. Nos. 5,811,097, 5,855,887, 6,051,227, and 6,207,156; each of which is incorporated by reference herein. While information on the amino and nucleic acid sequences relating to these antibodies is provided herein, further information can be found in U.S. Patent No. 6,682,736, as well as WO 00/37504; the sequences set forth in those applications are hereby incorporated herein by reference.

Certain uses for these antibodies to treat various cancers were discussed in U.S. Patent Application No. 10/153,382, now published as U.S. Patent Application Publication No. 2003/0086930, which is incorporated by reference as if set forth in its entirety herein.

Characteristics of human anti-CTLA4 antibodies useful in the methods of the invention are extensively discussed in, *e.g.*, U.S. Patent No. 6,682,736, and include antibodies having amino acid sequences of an antibody such as, but not limited to, antibody 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, 12.9.1.1, and 10D1. The invention also relates to methods using antibodies comprising the amino acid sequences of the CDRs of the heavy and light chains of these antibodies, as well as those comprising changes in the CDR regions, as described in the above-cited applications and patent. The invention also concerns antibodies comprising the variable regions of the heavy and light chains of those antibodies. In another embodiment, the antibody is selected from an antibody comprising the full length, variable region, or CDR, amino acid sequences of the heavy and light chains of antibodies 3.1.1, 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1, 12.3.1.1, and 12.9.1.1, and 10D1.

While the anti-CTLA4 antibodies discussed previously herein may be preferred, the skilled artisan, based upon the disclosure provided herein, would appreciate that the invention encompasses a wide variety of anti-CTLA4 antibodies and is not limited to these particular antibodies. More particularly, while human antibodies are preferred, the invention is in no way limited to human antibodies; rather, the invention encompasses useful antibodies regardless of species origin, and includes, among others, chimeric, humanized and/or primatized antibodies. Also, although the antibodies exemplified herein were obtained using a transgenic mammal, *e.g.*, a mouse comprising a human immune repertoire, the skilled artisan, based upon the disclosure provided herein, would understand that the present invention is not limited to an antibody produced by this or by any other particular method. Instead, the invention includes an anti-CTLA4 antibody produced by any method, including, but not limited to, a method known in the art (*e.g.,* screening phage display libraries, and the like) or to be developed in the future for producing an anti-CTLA4 antibody of the invention. Based upon the extensive disclosure provided herein and in, *e.g.*, U.S. Patent No. 6,682,736, to Hanson et al., and U.S. Pat. App. Pub. No. 2002/0088014, one skilled in the

art can readily produce and identify an antibody useful for treatment of breast cancer in combination with a hormonal therapeutic agent using the novel methods disclosed herein.

The present invention encompasses human antibodies produced using a transgenic non-human mammal, *i.e.*, XenoMouse™ (Abgenix, Inc., Fremont, CA) as disclosed in the U.S. 6,682,736, to Hanson et al.

Another transgenic mouse system for production of "human" antibodies is referred to as "HuMAb-Mouse™" (Medarex, Princeton, NJ), which contains human immunoglobulin gene miniloci that encodes unrearranged human heavy (mu and gamma) and kappa light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous mu and kappa chain loci (Lonberg et al. *Nature* **368**:856-859 (1994), and U.S. Pat. No. 5,770,429).

However, the invention uses human anti-CTLA4 antibodies produced using any transgenic mammal such as, but not limited to, the Kirin TC Mouse™ (Kirin Beer Kabushiki Kaisha, Tokyo, Japan) as described in, *e.g.*, Tomizuka et al., *Proc Natl Acad Sci USA* **97**:722 (2000); Kuroiwa et al., *Nature Biotechnol* **18**:1086 (2000); U.S. Patent Application Publication No. 2004/0120948, to Mikayama et al.; and the HuMAb-Mouse™ (Medarex, Princeton, NJ) and XenoMouse™ (Abgenix, Inc., Fremont, CA), supra. Thus, the invention encompasses using an anti-CTLA4 antibody produced using any transgenic or other non-human animal.

In another embodiment, the antibodies employed in methods of the invention are not fully human, but "humanized". In particular, murine antibodies or antibodies from other species can be "humanized" or "primatized" using techniques well known in the art. *See, e.g.,* Winter and Harris *Immunol. Today* **14**:43-46 (1993), Wright et al. *Crit. Reviews in Immunol.* **12**:125-168 (1992), and US Patent No. 4,816,567, to Cabilly et al, and Mage and Lamoyi *in Monoclonal Antibody Production Techniques and Applications* pp. 79-97, Marcel Dekker, Inc., New York, NY (1987). Thus, humanized, chimeric antibodies, anti-CTLA4 antibodies derived from any species (including single chain antibodies obtained from camelids as described in, *e.g.*, U.S. Pat. Nos. 5,759,808 and 6,765,087, to Casterman and Hamers), as well as any human antibody, can be combined with a therapeutic agent to practice the novel methods disclosed herein.

As will be appreciated based upon the disclosure provided herein, antibodies for use in the invention can be obtained from a transgenic non-human mammal, and hybridomas derived therefrom, but can also be expressed in cell lines other than hybridomas.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NS0, Sp2, HEK, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), and human hepatocellular carcinoma cells (e.g., Hep G2). Non-mammalian prokaryotic and eukaryotic cells can also be employed, including bacterial, yeast, insect, and plant cells.

Various expression systems can be used as well known in the art, such as, but not limited to, those described in, *e.g.*, Sambrook and Russell, *Molecular Cloning, A Laboratory Approach*, Cold Spring Harbor Press, Cold Spring Harbor, NY (2001), and Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (2002). These expression systems include dihydrofolate reductase (DHFR)-based systems, among many others. The glutamine synthetase system of expression is discussed in whole or part in connection with European Patents No. 0216846B1, No. 0256055B1, and No. 0323997B1, and European

Patent Application No. EP89303964. In one embodiment, the antibody used is made in NS0 cells using a glutamine synthetase system (GS-NS0). In another embodiment, the antibody is made in CHO cells using a DHFR system. Both systems are well-known in the art and are described in, among others, Barnes et al. *Biotech & Bioengineering* **73**:261-270 (2001), and references cited therein.

Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. Further, the antibody can be deglycosylated by enzymatic (*see, e.g.*, Thotakura et al. *Meth. Enzymol.* 138:350 (1987)) and/or chemical methods (*see, e.g.*, Hakimuddin et al., *Arch. Biochem. Biophys.* **259**:52 (1987)).

Further, the invention encompasses using an anti-CTLA4 antibody comprising an altered glycosylation pattern. The skilled artisan would appreciate, based upon the disclosure provided herein, that an anti-CTLA4 antibody can be modified to comprise additional, fewer, or different glycosylations sites compared with the naturally-occurring antibody. Such modifications are described in, *e.g.*, U.S. Patent Application Publication Nos. 2003/0207336, and 2003/0157108, and International Patent Publication Nos. WO 01/81405 and 00/24893.

Additionally, the invention comprises using an anti-CTLA4 antibody regardless of the glycoform, if any, present on the antibody. Moreover, methods for extensively remodeling the glycoform present on a glycoprotein are well-known in the art and include, *e.g.*, those described in International Patent Publication Nos. WO 03/031464, WO 98/58964, and WO 99/22764, and US Patent Application Publication Nos. 2004/0063911, 2004/0132640, 2004/0142856, 2004/0072290, and US Patent No. 6,602,684 to Umaña et al.

Further, the invention encompasses using an anti-CTLA4 antibody with any art-known covalent and non-covalent modification, including, but not limited to, linking the polypeptide to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in, for example, U.S. Patent Application Publication Nos. 2003/0207346 and 2004/0132640, and U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; 4,179,337.

Additionally, the invention encompasses using an anti-CTLA4 antibody, or antigen-binding portion thereof, chimeric protein comprising, *e.g.,* a human serum albumin polypeptide, or fragment thereof. Whether the chimeric protein is produced using recombinant methods by, *e.g.,* cloning of a chimeric nucleic acid encoding the chimeric protein, or by chemical linkage of the two peptide portions, the skilled artisan would understand once armed with the teachings provided herein that such chimeric proteins are well-known in the art and can confer desirable biological properties such as, but not limited to, increased stability and serum half-life to the antibody of the invention and such molecules are therefore included herein.

Antibodies that are generated for use in the invention need not initially possess a particular desired isotype. Rather, the antibody as generated can possess any isotype and can be isotype switched thereafter using conventional techniques. These include direct recombinant techniques (*see, e.g.*, U.S. Patent 4,816,397), and cell-cell fusion techniques (see e.g., U.S. Patent No. 5,916,771.

The effector function of the antibodies used in the invention may be changed by isotype switching to an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM for various therapeutic uses. Furthermore, dependence on complement for cell killing can be avoided through the use of bispecifics, immunotoxins, or radiolabels, for example.

Although antibody 4.1.1, 4.13.1 and 11.2.1 are IgG2 antibodies and the sequences of the variable regions of the antibodies are provided herein (Figures 1-3), and in the applications and patents referenced and incorporated herein, it is understood that the full-length sequences of these antibodies are encompassed herein, as well as the use of any antibody comprising the sequences set forth in SEQ ID NOs:1-36, and further comprising any constant region, regardless of isotype as more fully discussed elsewhere herein. Likewise, any antibody comprising the full-length sequence of 10D1, or any portion thereof, including a sequence encoding an antigen-binding portion of 10D1, can be used according to the methods of the invention.

Thus, the skilled artisan, once provided with the teachings provided herein, would readily appreciate that the anti-CTLA4 antibody-therapeutic agent combination of the invention can comprise a wide plethora of anti-CTLA4 antibodies.

Further, one skilled in the art, based upon the disclosure provided herein, would understand that the invention is not limited to administration of only a single antibody; rather, the invention encompasses administering at least one anti-CTLA4 antibody, *e.g.*, one of 4.1.1, 4.13.1, or 11.2.1, in combination with a therapeutic agent. Further, any combination of anti-CLTA4 antibodies can be combined with at least one therapeutic agent and the present invention encompasses any such combination and permutation thereof.

IV. Aromatase Inhibitor

The present invention relates to administering a combination of an anti-CTLA4 antibody and an aromatase inhibitor to a patient for treatment of breast cancer.

Hormone therapy agents for treatment of estrogen receptor expressing breast cancer tumors are well-known in the art. These hormonal therapy agents include compounds that affect the receptor itself, such as, but not limited to, tamoxifen which binds with the receptor thereby inhibiting receptor:ligand interaction, and fulvestrant, which degrades the receptor such that less is available to mediate signaling via receptor:ligand interaction.

Additionally, hormone therapy agents comprise inhibitors of the aromatase enzyme such as, but not limited to, anastrozole, letrozole, and exemestane. These antihormonal agents, unlike tamoxifen and fulvestrant, *supra*, inhibit receptor:ligand interaction by decreasing the level of ligand available to bind with the receptor. More specifically, an aromatase inhibitor inhibits the aromatization of the A ring of an androgen substrate to form an estrogen, which is a necessary step in the biosynthesis of the receptor ligand, *i.e.*, estrogen. Thus, the aromatase inhibitors mediate decreased serum levels of estrogen thereby inhibiting growth of tumor cells requiring the hormone for growth.

Non-steroidal aromatase inhibitors ("AI") such as, but not limited to, anastrozole and letrozole, bind reversibly to the heme portion of the aromatase molecule and are associated with an increase in aromatase activity. This may be associated with development of tumor resistance, particularly with long-term use of the inhibitor. Further, the biosynthesis of estrogen in the presence of these inhibitors can occur without the need for synthesis of new aromatase since the inhibitor is bound reversibly to the enzyme (*see* Miller *Clin Breast Cancer* **1**:S9-S14 (2000)), thereby also providing another opportunity for tumor cells to avoid the inhibition.

Steroidal aromatase inhibitors, such as, exemestane, differ from the non-steroidal inhibitors (*e.g.*, anastrozole and letrozole) in that the steroidal agents bind irreversibly to the substrate-binding site of the enzyme and are associated with a decrease in aromatase activity. For instance, exemestane is structurally related to the natural substrate androstenedione, and is recognized by the enzyme as a false substrate such that it competes with the natural substrate at the active site. Exemestane is then transformed to an intermediate that binds irreversibly to the enzyme causing its inactivation (*i.e.*, "suicide inhibition"). *See, e.g.*, U.S. Patent Application No. 10/611,653, published as U.S. Pat. App. Pub. No. 2004/0082557, U.S. Patent Application No. 10/363,935, published as U.S. Pat. App. Pub. No. 2004/0024044, and U.S. Patent Application No. 10/343,595, published as U.S. Pat. App. Pub. No. 2003/0158168.

Thus, unlike reversible non-steroidal inhibitors, exemestane is a steroidal aromatase inactivator that binds irreversibly to aromatase, causing nearly complete suppression of aromatase activity in peripheral tissues, as well as in the tumor, and mediates substantial lowering of serum estrogen levels without extraneous endocrinologic effects (Kaufmann et al. *J Clin Oncol* **18**:1399-1411 (2000)). Exemestane is currently approved in the US, Canada and Europe for second-line treatment (*e.g.*, where the disease has progressed following tamoxifen first line therapy) of postmenopausal women with metastatic breast cancer. Exemestane produces a response rate of approximately 15% with a median survival of 123.4 weeks and demonstrates a lack of cross-resistance with the non-steroidal agents such that it has been shown to be effective after anastrozole and letrozole treatment (Geisler et al. *Clin Cancer Res* **4**:2089-2093 (1998); Lonning *J Clin Oncol* **18**:2234-2244 (2000)). More recently, exemestane has been approved in Europe for adjuvant treatment of estrogen receptor positive invasive early breast cancer following two-to-three years of initial adjuvant tamoxifen therapy in post-menopausal women.

Because its intermediate is bound irreversibly, exemestane, unlike the non-steroidal aromatase inhibitors, further prevents the biosynthesis of estrogens in that *de novo* synthesis of aromatase enzyme is required. Moreover, the different mechanism of action of the non-steroidal and steroidal aromatase inhibitors may explain the phenomenon that one aromatase inhibitor can be effective following failure of another. More particularly, treatment of postmenopausal breast cancer patients treated with exemestate following failure of both tamoxifen and a non-steroidal aromatase inhibitor provided a clinical benefit (Lonning et al. *J Clin Oncol* **18**:2234-2244 (2000)).

Thus, anti-CTLA4 antibody in combination with any aromatase inhibitor, or multiple inhibitors, can be used to treat a breast cancer patient where the tumor expresses estrogen receptor (ER+). Further, although the present invention is exemplified by a combination comprising exemestane, the invention is in no way limited to this inhibitor or its mechanism of inhibition. Indeed, the mechanism by which the aromatase inhibitor inhibits the enzyme need not be known. Thus, any inhibitor that detectably inhibits aromatization of the A ring of a substrate in the biosynthesis of estrogen using any known assay for assessing such activity can be used in the combination of the invention.

V. Additional Agents

The present invention may be further combined with additional agents and therapies, *e.g.*, chemotherapy, surgery, radiotherapy, transplantation, and the like, to treat a patient. That is, the patient

may be subjected to additional chemotherapy with agents well-known, such as, but not limited to, growth factor inhibitors, biological response modifiers, alkylating agents, intercalating antibiotics, vinca alkaloids, immunomodulators, taxanes, selective estrogen receptor modulators (SERMs), such as, but not limited to, lasofoxifene, and angiogenesis inhibitors.

Therapeutic agents are numerous and have been described in, for instance, U.S. Patent Application Publication No. 2004/0005318, No. 2003/0086930, No. 2002/0086014, and International Publication No. WO 03/086459, all of which are incorporated by reference herein, among many others. Such therapeutic agents include, but are not limited to, topoisomerase I inhibitors; other antibodies (rituximab, bevacizumab, trastuzumab, anti-IGF 1R antibody [*e.g.*, CP-751,871], anti-CD40 antibody [*e.g.*, CP-870,893], and the like); chemotherapeutic agents such as, but not limited to, imatinib (GLEEVEC), SU11248 (SUTENT; sunitinib), SU12662, SU14813; BAY 43-9006, AG-013736 (axitinib), toll-like receptor stimulating agents (*e.g.*, TLR-9 agonist; such as, but not limited to, CPG-7909, also referred to as PF03512676 or PROMUNE), indoleamine-2,3,-dioxygenase (IDO) inhibitors; selective estrogen receptor modulators (SERMs; *e.g.*, lasofoxifene); taxanes; vinca alkaloids; temozolomide; angiogenesis inhibitors; EGFR inhibitors; VEGF inhibitors; erbB2 receptor inhibitors; anti-proliferative agents (*e.g.*, farnesyl protein transferase inhibitors, and αvβ3 inhibitors, αvβ5 inhibitors, p53 inhibitors, and the like); immunomodulators; biological response modifiers; cytokines; tumor vaccines; tumor-specific antigens; heat shock protein-based tumor vaccines; dendritic and stem cell therapies; alkylating agents; folate antagonists; pyrimidine antagonists; anthracycline antibiotics; platinum compounds; immune costimulatory molecules (*e.g.*, CD4, CD25, PD-1, B7-H3, 4-1BB, OX40, ICOS, CD30, HLA-DR, MHCII, and LFA), and agonist antibodies thereto; among many others.

In one embodiment, the methods of the invention may be further combined with transplantation, *e.g.*, stem cell transplantation, to provide a therapeutic benefit to a patient afflicted with breast cancer. Stem cell transplantation may be performed according to the methods known in the art and may be allogeneic or autologous stem cell transplantation. Additionally, one skilled in the art would appreciate, based upon the disclosure provided herein, that transplantation encompasses adoptive transfer of lymphocytes, either autologous or obtained from an HLA-matched donor. Where the method comprises stem cell transplant, the first dose of the antibody-AI therapy agent combination can be administered after the immune system of the mammal has recovered from transplantation, for example, in the period of from one to 12 months post transplantation. In certain embodiments, the first dose is administered in the period of from one to three, or one to four months post transplantation. Transplantation methods are described many treatises, including Appelbaum *in Harrison's Principles of Internal Medicine*, Chapter 14, Braunwald *et al.*, Eds., 15th ed., McGraw-Hill Professional (2001), which is hereby incorporated herein by reference.

As pointed out previously herein, there are many chemotherapeutic agents currently available for the treatment of tumors that are suitable for use in the combination therapy of the present invention. For example, alkylating agents are a class of drugs that alkylate DNA, restricting uncoiling and replication of strands. A preferred alkylating agent for use in the methods of the present invention is cyclophosphamide (CYTOXAN).

Folate antagonists bind to dihydrofolate reductase (DHFR) and interfere with pyrimidine (thymidine) synthesis. Methotrexate and pemetrexed (ALIMTA) are folate antagonists suitable for use in the methods of the present invention. In addition to DHFR, pemetrexed also inhibits thymidylate synthase and glycinamide ribonucleotide formyl transferase, two other folate-dependant enzymes involved in thymidine synthesis.

Pyrimidine antagonists inhibit enzymes involved in pyrimidine synthesis. As pyrimidine analogs, they also interfere with DNA production by competing with normal nucleotides for incorporation into the DNA molecule. Pyrimidine antagonists suitable for use in the methods of the present invention include 5-fluorouracil (5-FU); capecitabine (XELODA), a prodrug of 5'-deoxy-5-fluorouridine (5'-FDUR), which is enzymatically converted to 5-FU *in vivo*; and gemcitabine (GEMZAR).

Anthracycline antibiotics inhibit the uncoiling of DNA by intercalation between DNA strands. Anthracycline antibiotics include doxorubicin hydrochloride (ADRIAMYCIN), epirubicin hydrochloride (ELLENCE, PHARMORUBICIN), daunorubicin (CERUBIDINE, DAUNOXOME), and idarubicin hydrochloride (IDAMYCIN PFS, ZAVEDOS). Preferred anthracyclines for use with the present invention include doxorubicin and epirubicin.

Platinum compounds exert their anti-neoplastic effect by intercalation and intracalation between DNA strands, which inhibits uncoiling of the DNA. Platinum compounds useful in the methods of the present invention include cisplatin (PLATINOL), oxaliplatin (ELOXATIN), and carboplatin (PARAPLATIN).

Taxanes promote assembly of microtubules while inhibiting their disassembly into tubulin, thereby blocking a cell's ability to break down the mitotic spindle during mitosis. They have demonstrated significant activity against many solid tumors as single agent therapy and in combination with other chemotherapy agents. One embodiment of the combination therapy of the present invention includes the use of one or more taxanes in combination with an IGF-1R antibody. Suitable taxanes for use in combination with the IGF-1R antibody include docetaxel (TAXOTERE) and paclitaxel (TAXOL).

Taxane-derivatives, which may be active in cells resistant to doxorubicin, vinblastine, paclitaxel, docetaxel, and the like, include XRP-9981 (Sanofi Aventis), and are encompassed in the invention.

Vinca alkaloids, like taxanes, are "spindle poisons," acting on the microtubules that form the mitotic spindle. They inhibit mitosis by interfering with microtubule assembly, keeping the spindle from being formed. Vinca alkaloids include vindesine (ELDISINE), vinblastine sulfate (VELBAN), vincristine sulfate (ONCOVIN) and vinorelbine tartrate (NAVELBINE). A preferred vinca alkaloid for use in the methods of the present invention is vinorelbine.

BMS-247550 (ixabepilone) promotes tubulin polymerization and microtubule stabilization, thereby arresting cells in the G2-M phase and inducing tumor cell apoptosis. This agent demonstrates activity against taxane-resistant cells.

Analogs of rapamycin, which bind and inhibit the mammalian target of rapamycin (mTOR), are also useful and include, among others, CCI-779 (temsirolimus; Wyeth) and RAD-001 (everolimus, CERTICAN; Novartis).

The camptothecin analogs act through inhibition of topoisomerase I, an enzyme critical for DNA replication and packaging. Levels of topoisomerase I are higher in tumor cells than in normal tissue. A camptothecin analog useful in the methods of the present invention is irinotecan (CAMPTOSAR).

In certain embodiments of the invention, the above described methods are combined with a cancer vaccine. *See, e.g.*, Oh et al., *Cancer Res.* **64**:2610-2618 (2004) (TARP epitopes and breast cancer); Kontani et al., *Int J Molec Med* **12**:493-502 (2003) (dendritic cell vaccine targeting MUC1 mucin); Holmberg & Sandmaier *Expert Rev Vaccines* **3**:269-277 (2004)(vaccination for breast or ovarian cancer). That is, useful vaccines may be, without limitation, those comprised of breast cancer-associated antigens (*e.g.*, HER-2/neu, mammaglobin, prostate and breast tumor-associated protein [TARP], MUC1, CEA, sialyl-Tn and other carbohydrate antigens), other tumor cancer-associated antigens (*e.g.*, p53, telomerase), anti-idiotype antibodies such as 11D10, as well as vaccines comprising GM-CSF, DNA and cell-based vaccines, dendritic cell vaccines, recombinant viral (*e.g.*, vaccinia virus) vaccines, and heat shock protein (HSP) vaccines (*e.g.*, HSPPC-96; Antigenics Inc.). Useful vaccines also include tumor vaccines, such as those formed of breast tumor cells; and may be autologous or allogeneic, and the vaccines may be peptide, DNA or cell-based.

Vaccines may be administered prior to, or subsequent to, administration of the antibody-aromatase inhibitor combination, and when chemotherapy is part of the regimen, a vaccine may be administered prior to chemotherapy. In certain embodiments, the antibody-aromatase inhibitor combination of the invention may also be administered prior to chemotherapy. In yet other embodiments, the treatment can be combined with stem cell transplant. That is, the antibody-aromatase inhibitor combination can be administered before or after stem cell transplant. Vaccine may also be administered before or after stem cell transplantation and, in certain embodiments, concomitantly with the antibody.

The above described treatments can also be used with signal transduction inhibitors, such as agents that can inhibit EGFR (epidermal growth factor receptor) responses, such as EGFR antibodies, EGF antibodies, and molecules that are EGFR inhibitors; VEGF (vascular endothelial growth factor) inhibitors, such as VEGF receptors and molecules that can inhibit VEGF; and erbB2 receptor (HER2) inhibitors, such as organic molecules or antibodies that bind to the erbB2 receptor, for example, trastuzumab (HERCEPTIN, Genentech, Inc., San Francisco, CA), and pertuzumab (2C4, OMNITARG; Genentech), which is a HER dimerization inhibitor (HDI).

EGFR inhibitors are described in, for example in International Patent Publication Nos. WO 95/19970, WO 98/14451, WO 98/02434, and U.S. Patent No. 5,747,498, and such substances can be used in the present invention as described herein. EGFR-inhibiting agents include, but are not limited to, the monoclonal antibodies C225, anti-EGFR 22Mab (ImClone Systems Inc., New York, NY), and ABX-EGF (Abgenix Inc., remont, CA), the compounds ZD-1839 (AstraZeneca), BIBX–1382 (Boehringer Ingelheim), MDX-447 (Medarex,Inc., Annandale, NJ), and OLX-103 (Merck & Co., Whitehouse Station, NJ), VRCTC-310 (Ventech Research) and EGF fusion toxin (Seragen Inc., Hopkinton, MA). These and other EGFR-inhibiting agents can be used in the present invention.

Compounds directed at inhibition of epidermal growth factor receptor (EGFR) tyrosine kinase (TK) represent a relatively new class of antineoplastic drugs that are useful in the method of the present invention. Many human cancers express members of the EGFR family on the cell surface. When a ligand binds to EGFR, it sets off a cascade of cellular reactions that result in increased cell division and influence other aspects of cancer development and progression, including angiogenesis, metastatic spread, and inhibition of apoptosis. EGFR-TK inhibitors may selectively target one of the members of the

EGFR family (EGFR (also known as HER1 or ErbB-1), HER2/neu (also known as ErbB-2), HER3 (also known as ErbB-3), or HER4 (also known as ErbB-4)), or may target two or more of them. EGFR-TK inhibitors suitable for use in the present invention include gefitinib (IRESSA), erlotinib (TARCEVA), CI-1033 (Pfizer), GW2016 (GlaxoSmithKline), EKB-569 (Wyeth), PKI-166 (Novartis), CP-724,714 (Pfizer), and BIBX-1382 (Boeringer-Ingelheim). Additional EGFR-TK inhibitors are described in U.S. Patent No. 6,890,924.

VEGF inhibitors, for example SU-5416, SU-6668, SU-11248, SU-12662, SU-14813 (Sugen Inc., San Francisco, CA), as well as AG-013736 (Pfizer) can also be employed in combination with the antibody. VEGF inhibitors are described for example in International Patent Application No. PCT/IB99/00797 (filed May 3, 1999), International Patent Publication Nos. WO 99/24440; WO 95/21613; WO 99/61422; WO 98/50356; WO 99/10349; WO 97/32856; WO 97/22596; WO 98/54093; WO 98/02438; WO 99/16755; WO 98/02437; U.S. Patent Nos. 5,834,504; 5,883,113; 5,886,020; and 5,792,783. Other examples of some specific VEGF inhibitors useful in the present invention are IM862 (Cytran Inc., Kirkland, WA); IMC-1C11 Imclone antibody, anti-VEGF monoclonal antibody of Genentech, Inc., San Francisco, CA; and angiozyme, a synthetic ribozyme from Ribozyme (Boulder, CO) and Chiron (Emeryville, CA).

ErbB2 receptor inhibitors, such as GW-282974, GW-572016 (lapatinib) (Glaxo Wellcome plc), and the monoclonal antibodies AR-209 (Aronex Pharmaceuticals Inc., Woodlands, TX), trastuzumab (HERCEPTIN; Genentech, Inc., San Francisco, CA), pertuzumab (OMNITARG; 2C4; Genentech, a HER2 dimerization inhibitor HDI)), and 2B-1 (Chiron), can be combined with the antibody-aromatase inhibitor combination therapy. Other erbB2 receptor inhibitors are described in, for example, International Patent Publication Nos. WO 98/02434; WO 99/35146; WO 99/35132; WO 98/02437; WO 97/13760; WO 95/19970; U.S. Patent Nos. 5,587,458, and 5,877,305. ErbB2 receptor inhibitors useful in the present invention are also described in EP1029853 (published August 23, 2000) and in International Patent Publication No. WO 00/44728, (published August 3, 2000). The erbB2 receptor inhibitor compounds and substance described in the aforementioned PCT applications, U.S. patents, and U.S. provisional applications, as well as other compounds and substances that inhibit the erbB2 receptor, can be used with the antibody and aromatase inhibitor combination in accordance with the present invention.

The treatments of the invention also be used with other agents useful in treating abnormal cell growth or cancer, including, but not limited to other agents capable of enhancing antitumor immune responses, such as additional, different, CTLA4 antibodies, and other agents also capable of blocking CTLA4; and anti-proliferative agents such as farnesyl protein transferase inhibitors, and αvβ3 inhibitors, such as the αvβ3 antibody VITAXIN, αvβ5 inhibitors, p53 inhibitors, and the like.

Where the antibody of the invention is administered in combination with another immunomodulatory agent, the immunomodulatory agent can be selected for example from the group consisting of a dendritic cell activator such as CD40 ligand and anti-CD40 agonist antibodies, as well as enhancers of antigen presentation, enhancers of T-cell tropism, inhibitors of tumor-related immunosuppressive factors, such as TGF-β (transforming growth factor beta), and IL-10. Preferred anti-CD40 agonist antibodies encompass antibodies disclosed in International Patent Application No. PCT/US02/36107, filed November 8, 2002, now published as International Patent Publication No. WO

03/040170, and U.S. Patent Application No. 10/292,088, filed November 8, 2002, now published as U.S. Patent Publication No. US2003/0211100, including, but not limited to, an antibody having the heavy and light chain amino acid sequence of antibody 3.1.1, 3.1.1.H-A78T, 3.1.1H-A78T-V88A-V97A, 3.1.1L-L4M-L83V, 3.1.1H-A78T-V88A-V97A/3.1.1L-L4M-L83V, 7.1.2, 10.8.3, 15.1.1, 21.2.1, 21.4.1, 22.1.1, 22.1.1H-C109A, 23.5.1, 23.25.1, 23.28.1, 23.28.1H-D16E, 23.29.1, and 24.2.1.

The present treatment regimens may also be combined with antibodies or other ligands that inhibit tumor growth by binding to IGF-1R (insulin-like growth factor 1 receptor). Specific anti-IGF-1R antibodies that can be used in the present invention include those described in International Patent Application No. PCT/US01/51113, filed 12/20/01, and published as International Patent Publication No. WO02/053596, International Patent Application No. PCT/IB2004/002555, filed August 3, 2004, and published as International Patent Publication No. WO 2005/016967. Preferred anti-IGFR-1R antibodies encompass an antibody having the heavy and light chain amino acid sequence of, *e.g.*, antibody 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2 and 4.17.3.

The antibody of the invention may also be administered with cytokines such as IL-2, interferon (*e.g.*, IFN-γ, IFN-α, etc.), GM-CSF, IL-12, IL-18, and FLT-3L.

The treatment regimens described herein may be combined with anti-angiogenesis agents, such as MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 9) inhibitors, and COX-II (cyclooxygenase II) inhibitors, can be used in conjunction with the antibody in the method of the invention. Examples of useful COX-II inhibitors include CELEBREX (celecoxib), valdecoxib, and rofecoxib. Examples of useful matrix metalloproteinase inhibitors are described in International Patent Publication Nos. WO 96/33172; WO 96/27583; WO 98/07697, WO 98/03516, WO 98/34918, WO 98/34915, WO 98/33768, WO 98/30566, WO 90/05719, WO 99/52910, WO 99/52889, WO 99/29667, European Patent Application Nos. 780386 (published June 25, 1997), 97304971 (filed July 8, 1997), 99308617 (filed October 29, 1999), 606046 (published July 13, 1994), 931788 (published July 28, 1999), 99302232 (filed March 25, 1999), International Application PCT/IB98/01113 (filed July 21, 1998), Great Britain patent application number 9912961 (filed June 3, 1999), United States Provisional Patent Application No. 60/148,464 (filed August 12, 1999), and U.S. Patent Nos. 5,863,949, and 5,861,510.

Preferred MMP-2 and MMP-9 inhibitors are those that have little or no activity inhibiting MMP-1. More preferred are those that selectively inhibit MMP-2 and/or MMP-9 relative to the other matrix-metalloproteinases (*i.e.*, MMP-1, MMP-3, MMP-4, MMP-5, MMP-6, MMP-7, MMP-8, MMP-10, MMP-11, MMP-12, and MMP-13).

Some specific examples of MMP inhibitors useful in the present invention are AG-3340, RO 32-3555, RS 13-0830, and the compounds recited in the following list:

3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-cyclopentyl)-amino]-propionic acid;

3-exo-3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide;

(2R, 3R) 1-[4-(2-chloro-4-fluoro-benzyloxy)-benzenesulfonyl]-3-hydroxy-3-methyl-piperidine-2-carboxylic acid hydroxyamide;

4-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-4-carboxylic acid hydroxyamide;

3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-cyclobutyl)-amino]-propionic acid;

4-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-4-carboxylic acid hydroxyamide;

(R) 3-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-tetrahydro-pyran-3-carboxylic acid hydroxyamide;

(2R, 3R) 1-[4-(4-fluoro-2-methyl-benzyloxy)-benzenesulfonyl]-3-hydroxy-3-methyl-piperidine-2-carboxylic acid hydroxyamide;

3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(1-hydroxycarbamoyl-1-methyl-ethyl)-amino]-propionic acid;

3-[[4-(4-fluoro-phenoxy)-benzenesulfonyl]-(4-hydroxycarbamoyl-tetrahydro-pyran-4-yl)-amino]-propionic acid;

3-exo-3-[4-(4-chloro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide;

3-endo-3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide; and

(R) 3-[4-(4-fluoro-phenoxy)-benzenesulfonylamino]-tetrahydro-furan-3-carboxylic acid hydroxyamide;

and pharmaceutically acceptable salts and solvates of said compounds.

Radiation therapy can be administered in accordance to well-known radiotherapy methods for treatment of breast cancer. The dose and regimen for radiotherapy can be readily determined by one skilled in the art and is based on the stage of the disease, and other factors well-known in the art.

Co-administration of the antibody with an aromatase inhibitor (combination therapy) encompasses administering a pharmaceutical composition comprising both the anti-CTLA4 antibody and one or more aromatase inhibitor(s), and administering two or more separate pharmaceutical compositions, one comprising the anti-CTLA4 antibody and the other(s) comprising the aromatase inhibitor(s). Further, although co-administration or combination (conjoint) therapy generally mean that the antibody and additional therapeutic agents are administered at the same time as one another, it also encompasses simultaneous, sequential or separate dosing of the individual components of the treatment. Additionally, where an antibody is administered intravenously and the aromatase inhibitor is administered orally (*e.g.*, exemestane), it is understood that their combination is preferably administered as two separate pharmaceutical compositions.

The present invention also encompasses the administration of other therapeutic agents in addition to the first and second components, either concurrently with one or more of those components, or sequentially before and/or after. Such therapeutic agents include cancer vaccines, anti-vascular agents, anti-proliferative agents, and palliative agents to provide supportive care, such as, but not limited to, analgesics, anti-emetic agents, anti-diarrheal agents, and steroids. Preferred anti-emetic agents include ondansetron hydrochloride, granisetron hydrochloride, and metoclopramide. Preferred anti-diarrheal agents include diphenoxylate and atropine (LOMOTIL), loperamide (IMMODIUM), octreotide (SANDOSTATIN), olsalazine (DIPENTUM), and mesalamine (ASACOL). Preferred steroids include the non-absorbable steroid budesonide (ENTOCORT), and the steroids for systemic administration dexametasone (DECADRAN) and prednisone (METICORTEN).

Each administration may vary in its duration from a rapid administration to a continuous perfusion. As a result, for the purposes of the present invention, the combinations are not exclusively limited to those that are obtained by physical association of the constituents, but also to those that permit a separate administration, which can be simultaneous or spaced out over a period of time. The compositions according to the invention are preferably compositions which can be administered parentally. However, these compositions may be administered orally or intraperitoneally in the case of localized regional therapies.

As will be appreciated by one of skill in the art, the choice of therapeutic agents to be used in combination with anti-CTLA4 antibodies and aromatase inhibitor combination therapy, and the timing of their use, will be determined in part by the type and stage of the cancer that is being treated. For example, in early breast cancer (where the cancer has not spread outside the breast), surgery and radiation are generally followed by adjuvant chemotherapy or adjuvant hormonal therapy, either of which may be combined with the anti-CTLA4 antibody-aromatase inhibitor combination in the methods of the present invention. Typical adjuvant chemotherapy for early breast cancer includes cyclophosphamide, methotrexate and 5-FU ("CMF"); 5-FU, doxorubicin, and cyclophosphamide ("FAC"); docetaxel, doxorubicin, and cyclophosphamide ("TAC"); doxorubicin and cyclophosphamide ("AC"); doxorubicin and cyclophosphamide followed by paclitaxel ("AC and T"); and 5-FU, epirubicin, and cyclophosphamide ("FEC"). As discussed previously elsewhere herein, tamoxifen is an antihormonal treatment administered at this stage. Thus, the antibody-aromatase inhibitor combination can be administered in combination with additional hormonal therapy (*e.g.*, another aromatase inhibitor, tamoxifen, fulvestrant, or any combination thereof), and such combination can be coadministered in combination with an adjuvant chemotherapy therapy (*e.g.*, CMF, FAC, TAC, AC, AC and T, and/or FEC, or the chemotherapeutic agents can be administered individually in combination with the antibody-aromatase inhibitor combination, among others).

In locally advanced breast cancer, wherein the cancer has spread only to nearby tissues or lymph nodes, the patient is often given chemotherapy prior to surgery and radiation, which are then followed by adjuvant hormonal therapy. Alternatively, surgery/radiation is followed by adjuvant chemotherapy, then adjuvant hormonal therapy. The anti-CTLA4 antibody-aromatase inhibitor combination can be administered in conjunction with the chemotherapeutic or additional hormonal therapy agents (*e.g.*, another aromatase inhibitor, tamoxifen, fulvestrant, or any combination thereof) whether they are used either before or after surgery/radiation. Typical chemotherapy regimes for locally advanced breast cancer include FAC, AC, FEC, and doxorubicin plus docetaxel ("AT").

Metastatic breast cancer has spread to other parts of the body from the breast in which it started. Chemotherapy optionally may be preceded by hormonal therapy. First line hormonal therapy currently includes tamoxifen and anastrozole. First line chemotherapy regimens currently include FAC, TAC, docetaxel plus epirubicin, docetaxel, paclitaxel, capecitabine, vinorelbine, and trastuzumab (HERCEPTIN). Second line treatments include docetaxel, alone or in combination with capecitabine. The methods of the present invention are suitable for use both as first line therapy and second line therapy. Further, the methods of the invention can be combined with radiation therapy and stem cell transplant,

and any combination of any of the treatments described herein, known in the art, or to be developed in the future.

In one embodiment, the additional therapeutic agent administered with antibody-aromatase inhibitor therapy is an alkylating agent. In one aspect, the alkylating agent is cyclophosphamide.

In another embodiment, the additional agent is a folate antagonist. In one aspect, the folate antagonist is selected from the group consisting of methotrexate and pemetrexed.

In one embodiment of the invention, the additional agent is a pyrimidine antagonist. The pyrimidine analog may be selected from the group consisting of 5-FU, capecitabine, and gemcitabine.

In another embodiment of the present invention, the additional agent is an anthracycline antibiotic. In one aspect, the anthracycline antibiotic is selected from the group consisting of epirubicin and doxorubicin. In a further aspect, the anthracycline antibiotic is doxorubicin.

In another embodiment of the invention, the additional agent is a platinum compound. The platinum compound may be selected from the group consisting of cisplatin and carboplatin.

In yet another embodiment of the invention, the additional agent is a taxane. In one aspect, the taxane is docetaxel. In another aspect, the taxane is paclitaxel.

In an embodiment of the present invention, the anti-CTLA4 antibody and aromatase inhibitor combination is administered with docetaxel in further combination with at least one of capecitabine, cisplatin, gemcitabine, and epirubicin.

In an embodiment of the invention, the anti-CTLA4 and aromatase inhibitor combination is administered in further combination with paclitaxel and an additional agent selected from the group consisting of carboplatin, cisplatin, and gemcitabine. In one aspect, the additional agent is carboplatin.

In another embodiment, the anti-CTLA4 and aromatase inhibitor combination is administered with an additional agent where the additional agent is a vinca alkaloid. In one aspect, the vinca alkaloid is vinorelbine.

In an embodiment of the invention, the additional agent administered with the antibody-aromatase inhibitor combination is a camptothecin analog. In one aspect, the camptothecin analog is irinotecan.

In another embodiment, the additional agent is an EGFR inhibitor.

In an embodiment of the invention, the additional agent is an erbB2 inhibitor. In one aspect, the erbB2 inhibitor is selected from trastuzumab and pertuzumab. In yet another aspect, the erbB2 inhibitor is trastuzumab (HERCEPTIN).

In an embodiment of the invention, the anti-CTLA4 and aromatase inhibitor combination is administered in further combination with a combination comprising 5-FU, doxorubicin, and cyclophosphamide.

In another embodiment of the present invention, the anti-CTLA4 and aromatase inhibitor combination is administered in further combination with a combination comprising docetaxel, doxorubicin, and cyclophosphamide.

The methods of the present invention also relate to the treatment of cancer in a mammal, preferably a human, who has undergone stem cell transplantation, which methods comprise administering to the mammal an amount of a human anti-CTLA4 antibody that is effective in treating the cancer in combination with an aromatase inhibitor in further combination with stem cell transplantation. Stem cell

transplantation may be allogeneic or autologous stem cell transplantation, and more preferably, the aromatase inhibitor is exemestane. Where the method comprises stem cell transplant, the first dose of the antibody-aromatase inhibitor combination can be administered after the immune system of the mammal has recovered from transplantation, for example, in the period of from one to 12 months post transplantation. In certain embodiments, the first dose is administered in the period of from one to three, or one to four months post transplantation. The patient may undergo stem cell transplantation preparatory treatment(s).

VI. Pharmaceutical Compositions

The invention encompasses the preparation and use of pharmaceutical compositions comprising a human anti-CTLA4 antibody of the invention as an active ingredient in combination with an aromatase inhibitor, preferably, a steroidal aromatase inhibitor, and even more preferably, the aromatase inhibitor is exemestane. Such a pharmaceutical composition may consist of each active ingredient alone, as a combination of at least one active ingredient (*e.g.*, an effective dose of an anti-CTLA4, an effective does of an aromatase inhibitor) in a form suitable for administration to a subject, or the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carriers, one or more additional (active and/or inactive) ingredients, or some combination of these.

In one embodiment, the antibody is administered parenterally (*e.g.*, intravenously) in an aqueous solution while the aromatase inhibitor (*e.g.*, exemestane) is administered orally in pill/capsule form. However, the skilled artisan would understand, based upon the disclosure provided herein, that the invention is not limited to these, or any other, formulations, doses, routes of administration, and the like. Rather, the invention encompasses any formulation or method of administering an antibody in combination with an aromatase inhibitor, including, but not limited to, administering each agent separately in a different formulation via a different route of administration, and administering the antibody and aromatase inhibitor in a single composition (*e.g.,* in an aqueous composition administered, *inter alia*, i.v.), among many others. Thus, the following discussion describes various formulations for practicing the methods of the invention comprising administration of any anti-CTLA4 antibody in combination with any aromatase inhibitor, but the invention is not limited to these formulations, but comprises any formulation as can be readily determined by one skilled in the art once armed with the teachings provided herein for use in the methods of the invention.

The antibodies employed in the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises the antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable substances such as wetting or minor amounts of auxiliary substances such as

wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

The antibodies may be in a variety of forms. These include, for example, liquid, semi solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The antibodies can be administered by a variety of methods known in the art, including, without limitation, oral, parenteral, mucosal, by-inhalation, topical, buccal, nasal, and rectal. For many therapeutic applications, the preferred route/mode of administration is subcutaneous, intramuscular, intravenous or infusion. Non-needle injection may be employed, if desired. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

In certain embodiments, the antibody may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J. R. Robinson, ed., Marcel Dekker, Inc., New York (1978).

Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is

especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the antibody and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

In one embodiment, the antibody is administered in an intravenous formulation as a sterile aqueous solution containing 5 mg/m, or more preferably, about 10 mg/ml, or yet more preferably, about 15 mg/ml, or even more preferably, about 20 mg/ml of antibody, with sodium acetate, polysorbate 80, and sodium chloride at a pH ranging from about 5 to 6. Preferably, the intravenous formulation is a sterile aqueous solution containing 5 or 10 mg/ml of antibody, with 20 mM sodium acetate, 0.2 mg/ml polysorbate 80, and 140 mM sodium chloride at pH 5.5. Further, a solution comprising an anti-CTLA4 antibody can comprise, among many other compounds, histidine, mannitol, sucrose, trehalose, glycine, poly(ethylene) glycol, EDTA, methionine, and any combination thereof, and many other compounds known in the relevant art.

In one embodiment, part of the dose is administered by an intraveneous bolus and the rest by infusion of the antibody formulation. For example, a 0.01 mg/kg intravenous injection of the antibody may be given as a bolus, and the rest of a predetermined antibody dose may be administered by intravenous injection. A predetermined dose of the antibody may be administered, for example, over a period of an hour and a half to two hours to five hours.

With regard to an aromatase inhibitor, the inhibitor can be present in the pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents. Particularly contemplated additional agents include anti-emetics, anti-diarrheals, chemotherapeutic agents, cytokines, and the like.

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

A formulation of a pharmaceutical composition of the invention suitable for oral administration may be prepared, packaged, or sold in the form of a discrete solid dose unit including, but not limited to, a tablet, a hard or soft capsule, a cachet, a troche, or a lozenge, each containing a predetermined amount of the active ingredient. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, or an emulsion.

As used herein, an "oily" liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water.

A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture.

Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycolate. Known surface active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and

hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of example, tablets may be coated using methods described in U.S. Patents numbers 4,256,108; 4,160,452; and 4,265,874 to form osmotically-controlled release tablets. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide pharmaceutically elegant and palatable preparation.

Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (*e.g.*, polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para-hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for rectal administration. Such a composition may be in the form of, for example, a suppository, a retention enema preparation, and a solution for rectal or colonic irrigation.

Suppository formulations may be made by combining the active ingredient with a non-irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (*i.e.*, about 20°C) and which is liquid at the rectal temperature of the subject (*i.e.*, about 37°C in a healthy human). Suitable pharmaceutically acceptable excipients include, but are not limited to, cocoa butter, polyethylene glycols, and various glycerides. Suppository formulations may further comprise various additional ingredients including, but not limited to, antioxidants and preservatives.

Retention enema preparations or solutions for rectal or colonic irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, enema preparations may be administered using, and may be packaged within, a delivery device adapted to the rectal anatomy of the subject. Enema preparations may further comprise various additional ingredients including, but not limited to, antioxidants and preservatives.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for vaginal administration. Such a composition may be in the form of, for example, a

suppository, an impregnated or coated vaginally-insertable material such as a tampon, a douche preparation, or gel or cream or a solution for vaginal irrigation.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e. such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

Douche preparations or solutions for vaginal irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, douche preparations may be administered using, and may be packaged within, a delivery device adapted to the vaginal anatomy of the subject. Douche preparations may further comprise various additional ingredients including, but not limited to, antioxidants, antibiotics, antifungal agents, and preservatives.

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations as discussed below. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

A composition of the present invention can be administered by a variety of methods known in the art. The route and/or mode of administration vary depending upon the desired results. The active compounds can be prepared with carriers that protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are described by *e.g.*, *Sustained and Controlled Release Drug Delivery*

Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, (1978). Pharmaceutical compositions are preferably manufactured under GMP conditions.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers, and preferably from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder or using a self-propelling solvent/powder-dispensing container such as a device comprising the active ingredient dissolved or suspended in a low-boiling propellant in a sealed container. Preferably, such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter less than 7 nanometers. More preferably, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. Dry powder compositions preferably include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Low boiling propellants generally include liquid propellants having a boiling point of below 65°F at atmospheric pressure. Generally the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic or solid anionic surfactant or a solid diluent (preferably having a particle size of the same order as particles comprising the active ingredient).

Pharmaceutical compositions of the invention formulated for pulmonary delivery may also provide the active ingredient in the form of droplets of a solution or suspension. Such formulations may be

prepared, packaged, or sold as aqueous or dilute alcoholic solutions or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, or a preservative such as methylhydroxybenzoate. The droplets provided by this route of administration preferably have an average diameter in the range from about 0.1 to about 200 nanometers.

The formulations described herein as being useful for pulmonary delivery are also useful for intranasal delivery of a pharmaceutical composition of the invention.

Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 to 500 micrometers. Such a formulation is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close to the nares.

Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1-1.0% (w/w) solution or suspension of the active ingredient in an aqueous or oily liquid carrier. Such drops may further comprise buffering agents, salts, or one or more other of the additional ingredients described herein. Other ophthalmalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form or in a liposomal preparation.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the

invention are known in the art and described, for example in *Remington's Pharmaceutical Sciences*, Genaro, ed., Mack Publishing Co., Easton, PA (1985), which is incorporated herein by reference.

The anti-CTLA4/aromatase inhibitor active ingredient combination of the invention, can be administered to an animal, preferably a human. While the precise dosage administered of each active ingredient will vary depending upon any number of factors, including but not limited to, the type of animal and type of disease state being treated, the age of the animal and the route of administration.

The anti-CTLA4 antibody may be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

The aromatase inhibitor, preferably, exemestane, may be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

The antibody and aromatase can be co-administered in that they can be administered separately, on different dates or at different times of the day, as well as simultaneously or on the same date. Co-administration thus encompasses any temporal combination of administration of the antibody and the aromatase inhibitor such that administration of the two agents mediates a therapeutic benefit to the patient that is detectably greater than administration of either agent in the absence of the other.

An antibody-aromatase inhibitor combination of the invention may be co-administered with numerous other compounds (other antihormonal therapy agents, cytokines, chemotherapeutic and/or antiviral drugs, among many others). Alternatively, the compound(s) may be administered an hour, a day, a week, a month, or even more, in advance of the antibody-aromatase inhibitor combination, or any permutation thereof. Further, the compound(s) may be administered an hour, a day, a week , or even more, after administration of radiation, stem cell transplant, or administration of any therapeutic agent (*e.g.*, cytokine, chemotherapeutic compound, and the like), or any permutation thereof. The frequency and administration regimen will be readily apparent to the skilled artisan and will depend upon any number of factors such as, but not limited to, the type and severity of the disease being treated, the age and health status of the animal, the identity of the compound or compounds being administered, the route of administration of the various compounds, and the like.

VII. Kits

The invention includes various kits which comprise a therapeutically effective amount of a human anti-CTLA4 antibody of the invention and a therapeutically effective amount of an aromatase inhibitor, along with an applicator and instructional materials which describe use of the combination to perform the methods of the invention. Although exemplary kits are described below, the contents of other useful kits

will be apparent to the skilled artisan in light of the present disclosure. Each of these kits is included within the invention.

The invention includes a kit for treatment of breast cancer in a patient in need thereof. The kit includes a human anti-CTLA4 antibody of the invention and at least one aromatase inhibitor. The inhibitor encompasses, but is not limited to, fulvestrant, anastrozole, letrozole and exemestane. The kit further comprises an applicator, including, but not limited to, a syringe, for administration of the components of the kit to a patient. Further, the kit comprises an instructional material setting forth the pertinent information for the use of the kit to treat breast cancer in the patient.

More preferably, the kit comprises at least one anti-CTLA4 antibody selected from an antibody having the heavy and light chain amino acid sequence of antibody 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and 10D1 (MDX-010), even more preferably, the antibody is an antibody having the heavy and light chain amino acid sequence of antibody 4.13.1, 11.2.1, and 10D1 (MDX-010).

In one embodiment, the aromatase inhibitor is at least one inhibitor selected from anastrozole, letrozole, and exemestane. More preferably, the aromatase inhibitor is exemestane.

The kit can comprise any number of additional agents for treatment of breast cancer. Such agents are set forth previously and include chemotherapeutic compounds, cancer vaccines, signal transduction inhibitors, agents useful in treating abnormal cell growth or cancer, antibodies or other ligands that inhibit tumor growth by binding to IGF-1R, and cytokines, among many others.

The invention also relates to an article of manufacture (*e.g.*, dosage form adapted for i.v. administration) comprising a human anti-CTLA4 antibody in the amount effective to treat cancer (*e.g.*, more than 10 mg/kg, at least 15 mg/kg, or 15 mg/kg) and a therapeutically effective amount of an aromatase inhibitor. In certain embodiments, the article of manufacture comprises a container or containers comprising a human anti-CTLA4 antibody, the aromatase inhibitor, and a label and/or instructions for use to treat cancer.

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

EXAMPLE

Anti-CLTA4 antibody in combination with exemestane in metastatic or locally advanced breast cancer in postmenopausal patient

Patients having metastatic or locally advanced breast cancer with at least one lesion that can be accurately measured in two dimensions and whose size is ≥ 2 cm x 1 cm by conventional CT scan or ≥ 1 cm x 1 cm by spiral CT scan were administered an IV infusion (100 mL/hr) of anti-CTLA4 antibodies as described herein. Prophylactic anti-emetics and anti-diarrheals are given as appropriate.

The treatment is repeated after 28 days. Successive cohorts receive escalating doses of the anti-CTLA4 antibody dose, starting at 3 mg/kg and increasing to 6 mg/kg, 10 mg/kg and 15 mg/kg, every 28 days thereafter, respectively, for maximum of 12 cycles in the absence of intolerable toxicity or disease progression.

Preferably, the patients are premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

All patients receive exemestane, as continuous oral dose of 25 mg per day of every 28-day cycle. Preferably, exemestane is taken at the same time each day.

Doses are escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there is no required waiting period between subjects. Subsequent cohorts may not be opened until the first subject at the current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

The antibody is provided in 10 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 5 mg/ml (with a nominal fill of 50 mg/vial) of anti-CTLA4 antibody, in a sterile aqueous solution comprising 20 mM sodium acetate, 0.2 mg/ml polysorbate 80, and 140 mM sodium chloride at pH 5.5.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

Preferably, the anti-CTLA4 antibody has the heavy and light chain amino acid sequences of at least one antibody selected from 4.1.1, 4.13.1, 11.2.1, and 10D1. More preferably, the antibody has the heavy and light chain amino acid sequences of 11.2.1.

Three patients were administered anti-CTLA4 antibody (ticilimumab) at 3 mg/kg. All three patients were administered exemestane at 25 mg daily. One patient received two doses of anti-CTLA4 every twenty-eight days and exemestane daily for 56 days. Cycle 2 staging CT revealed a lung lesion not present at baseline. Also, the patient demonstrated new, mild circumferential wall thickening involving the terminal ileum, distal descending colon, and sigmoid colon of uncertain etiology, possible infections or inflammatory. The patient did not have a clinical history to explain these changes and the patient felt well and was clinically asymptomatic. No infection was detected. This patient went off-study for progression of disease (PD).

A second patient was administered two cycles of anti-CTLA4 antibody, and had approximately fourteen (14) days of exemestane therapy (25 mg daily). Following initial ticilimumab dose, the patient reported some baseline lymphedema of the right arm that became somewhat worse then subsided. Also, the patient reported transient, mild non-pruritic rash.

A third patient received one cycle of ticilimumab and approximately twenty-eight days of daily 25 mg exemestane thus far. On day twenty-eight, routine laboratory studies detected Grade 1 elevated ALP and AST as well as Grade 2 elevated ALT. The patient was asymptomatic. Therapy was placed on-hold pending improvement of these laboratory values.

These data suggest that patients treated with the combination of ticilimumab and exemestane demonstrated biological activity.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While the invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

1. A method for the treatment of breast cancer in a patient in need of such treatment, said method comprising administering to said patient a therapeutically effective amount of an anti-CTLA4 antibody, or antigen-binding portion thereof, in combination with a therapeutically effective amount of an aromatase inhibitor.

2. The method of claim 1, wherein said aromatase inhibitor is at least one inhibitor selected from the group consisting of anastrozole, letrozole, and exemestane.

3. The method of claim 2, wherein said aromatase inhibitor is exemestane.

4. The method of claim 3, wherein said therapeutically effective amount of a human anti-CTLA4 antibody amount ranges from about 1 mg/kg to 40 mg/kg.

5. The method of claim 4, wherein said therapeutically effective amount of a human anti-CTLA4 antibody amount ranges from about 3 mg/kg to 15 mg/kg.

6. The method of claim 3, wherein said therapeutically effective amount of exemestane ranges from about 25 mg per day to 200 mg per day.

7. The method of claim 6, wherein said therapeutically effective amount of exemestane is about 25 mg per day.

8. The method of claim 1, wherein said treatment is selected from the group consisting of a neoadjuvant therapy, an adjuvant therapy, a first line treatment, a second line treatment, and a third line treatment.

9. The method of claim 1, wherein said antibody is selected from a group consisting of a non-human mammalian antibody, a chimeric antibody, and a human antibody.

10. The method of claim 9, wherein said antibody is a human antibody.

11. The method of claim 1, wherein said anti-CTLA4 antibody, or antigen-binding portion thereof, is at least one antibody selected from the group consisting of:

(a) a human antibody having a binding affinity for CTLA4 of about 10^{-8} or greater, and which inhibits binding between CTLA4 and B7-1, and binding between CTLA4 and B7-2;

(b) a human antibody having an amino acid sequence comprising at least one human CDR sequence that corresponds to a CDR sequence from an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and 10D1;

(c) a human antibody having the heavy and light chain amino acid sequences of an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, and 12.9.1.1;

(d) a human antibody having the amino acid sequences of a heavy chain variable region and a light chain variable region of an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and 10D1;

(e) an antibody, or antigen-binding portion thereof, that competes for binding with CTLA4 with at least one antibody having the heavy and light chain amino acid sequences of an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and 10D1; and

(f) an antibody, or antigen-binding portion thereof, that cross-competes for binding with CTLA4 with at least one antibody having the heavy and light chain amino acid sequences of an antibody selected from the group consisting of 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1, 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and 10D1.

12. The method of claim 1, wherein said antibody is a human antibody having the heavy and light chain amino acid sequences of antibody 11.2.1.

13. The method of claim 1, wherein said antibody comprises a heavy chain and a light chain wherein the amino acid sequences of the heavy chain variable region of said heavy chain and the light chain variable region of said light chain are selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:3 and the amino acid sequence of SEQ ID NO:9;

(b) the amino acid sequence of SEQ ID NO:15 and the amino acid sequence of SEQ ID NO:21 ;

(c) the amino acid sequence of SEQ ID NO:27 and the amino acid sequence of SEQ ID NO:33;

(d) the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:1 and the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:7;

(e) the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:13 and the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:19;

(f) the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:25 and the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:31;

(g) the amino acid sequence of a heavy chain variable region and a light chain variable region of antibody 10D1.

14. The method of claim 1, wherein said antibody, or antigen-binding portion thereof, is an antibody selected from the group consisting of:

(a) an antibody having a heavy chain variable region comprising the amino acid sequences set forth in SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, and further having a light chain variable region comprising the amino acid sequences set forth in SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12;

(b) an antibody having a heavy chain variable region comprising the amino acid sequences set forth in SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18, and further having a light chain variable region comprising the amino acid sequences set forth in SEQ ID NO:22, SEQ ID NO:23 and SEQ ID NO:24;

(c) an antibody having a heavy chain variable region comprising the amino acid sequences set forth in SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, and further having a light chain variable region comprising the amino acid sequences set forth in SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36; and

(d) an antibody having a heavy chain variable region comprising the amino acid sequences of the heavy chain CDR1, CDR2, and CDR3 of antibody 10D1, further having a light chain variable region comprising the amino acid sequences of the light chain CDR1, CDR2, and CDR3 of antibody 10D1.

15. The method of claim 1, said method further comprising administering to said patient at least one agent selected from the group consisting of an alkylating agent, a folate antagonist, a pyrimidine antagonist, an anthracycline antibiotic, a platinum compound, a taxane, a vinca alkaloid, a camptothecin analog, a toll-like receptor stimulating agent, a heat shock protein-based tumor vaccine, an antigen presenting cell-based therapy, a mammalian target of rapamycin inhibitor, an erbB2 inhibitor, an EGFR inhibitor, a VEGF inhibitor, a VEGFR inhibitor, an angiogenesis inhibitor, an antibody, an immunomodulator, a selective estrogen receptor modulator, a cytokine, a tumor vaccine, an antiproliferative agent, an immune costimulatory molecule, and a cytokine.

16. A pharmaceutical composition for the treatment of breast cancer comprising:
a therapeutically effective amount of an anti-CTLA4 antibody;
a therapeutically effective amount of an aromatase inhibitor; and
a pharmaceutically acceptable carrier.

17. The pharmaceutical composition of claim 16, wherein said aromatase inhibitor is at least one aromatase inhibitor selected from the group consisting of anastrozole, letrozole, and exemestane.

18. The pharmaceutical composition of claim 17, wherein said aromatase inhibitor is exemestane.

19. A use of an amount of an anti-CTLA4 antibody in the preparation of a composition for the treatment of breast cancer in a patient wherein said treatment further comprises administering to said patient an amount of an aromatase inhibitor.

20. The use of claim 19, wherein said aromatase inhibitor is exemestane and further wherein said antibody is a human antibody having the heavy and light chain variable region amino acid sequences of antibody 11.2.1.