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Title: GENE POLYMORPHISMS IN VEGF AND VEGF RECEPTOR 2 AS MARKERS FOR CANCER THERAPY

Response to 5-FU/Oxaliplatin/Bevacizumab by Polymorphisms

FIG. 1

Abstract: The invention provides compositions and methods for determining the likelihood of successful treatment with anti-angiogenic antibodies or equivalent thereof, in combination with a pyrimidine based antimitabolite and a platinum-based alkylating agent based therapy. The methods comprise determining the genomic polymorphism present in a predetermined region of a gene of interest and correlating the polymorphism to the predictive response. Patients identified as responsive are then treated with the appropriate therapy.
GENE POLYMORPHISMS IN VEGF AND VEGF RECEPTOR 2 AS MARKERS FOR CANCER THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of provisional applications Serial Nos.: 60/885,595 filed on January 18, 2007, 60/915,740 filed on May 3, 2007, and 60/941,579 filed on June 1, 2007. The contents of each of these applications are incorporated by reference into the present disclosure in their entirety.

FIELD OF THE INVENTION

This invention relates to the field of pharmacogenomics and specifically to the application of genetic polymorphism(s) to diagnose and treat diseases.

BACKGROUND OF THE INVENTION

In nature, organisms of the same species usually differ from each other in some aspects, e.g., their appearance. The differences are genetically determined and are referred to as polymorphism. Genetic polymorphism is the occurrence in a population of two or more genetically determined alternative phenotypes due to different alleles. Polymorphism can be observed at the level of the whole individual (phenotype), in variant forms of proteins and blood group substances (biochemical polymorphism), morphological features of chromosomes (chromosomal polymorphism) or at the level of DNA in differences of nucleotides (DNA polymorphism).

Polymorphism also plays a role in determining differences in an individual's response to drugs. Pharmacogenetics and pharmacogenomics are multidisciplinary research efforts to study the relationship between genotype, gene expression profiles, and phenotype, as expressed in variability between individuals in response to or toxicity from drugs. Indeed, it is now known that cancer chemotherapy is limited by the predisposition of specific populations to drug toxicity or poor drug response. For a review of the use of germline polymorphisms in clinical oncology, see Lenz (2004) Clin. Oncol. 22(13):2519-

The Food and Drug Administration has approved the use of Cetuximab, an antibody to the epidermal growth factor receptor (EGFR), either alone or in combination with irinotecan (also known as CPT-11 or Camptosar®) to treat patients with EGFR-expressing, metastatic CRC, who are either refractory or intolerant to irinotecan-based chemotherapy. One recent study (Zhang et al. 2006) Pharmacogenetics and Genomics 16:475-483) investigated whether polymorphisms in genes of the EGFR signaling pathway are associated with clinical outcome in CRC patients treated with single-agent Cetuximab. The study also reported that alleles for VEGF and VEGFR2 (receptor 2) as well as the cyclin D1 (CCND1) A870G and the EGF A61G polymorphisms may be useful molecular markers for predicting clinical outcome in CRC patients in stage II or III CRC.

Other polymorphisms have been reported to be associated with clinical outcome. Twenty-one (21) polymorphisms in 18 genes involved in the critical pathways of cancer progression (i.e., drug metabolism, tumor microenvironment, cell cycle regulation, and DNA repair) were investigated to determine if they will predict the risk of tumor recurrence in rectal cancer patients treated with chemoradiation. Gordon et al. (2006) Pharmacogenomics 7(l):67-88. To the best of the Applicant's knowledge, correlation of the genetic polymorphisms identified herein and the responsiveness to combination therapy
of anti-angiogenic antibodies, pyrimidine based anti-metabolites, and platinum-based alkylating agents have not been previously reported.
DESCRIPTION OF THE EMBODIMENTs

This invention provides methods to identify patients likely to respond to a selected therapy and to select the appropriate therapy for patients suffering from a metastatic or non-metastatic gastrointestinal cancer. The method requires detecting the identity of at least one allelic variant of a predetermined gene selected from the group identified in Tables 1, 2, 3 or 4, below.

Table 1 - Study Results for 140 Patients (69 female, 71 male) with Stage II (63) and Stage III (77) Colon Cancer

<table>
<thead>
<tr>
<th>Allele</th>
<th>Predictive Polymorphism Genotype</th>
<th>Measured Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF at nt 936</td>
<td>C/C</td>
<td>High Risk Tumor Recurrence in stage III colon cancer</td>
</tr>
<tr>
<td>VEGFR2 at position 4422</td>
<td>10/10 or 10/11 CA repeats</td>
<td>High Risk Tumor Recurrence in stage III colon cancer</td>
</tr>
<tr>
<td>VEGFR2 at nt 1416</td>
<td>A/A</td>
<td>High Risk Tumor Recurrence in stage III colon cancer</td>
</tr>
<tr>
<td>VEGFR2 at position 4422</td>
<td>11/11 CA repeats</td>
<td>Tumor recurrence in stage II colon cancer</td>
</tr>
</tbody>
</table>

Table 2 - Additional Polymorphism Assayed in Patients with Stage II and Stage III Colon Cancer

<table>
<thead>
<tr>
<th>Allele</th>
<th>Measured Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (G405C)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>VEGFR2 (11T/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>HIF1α (1772C/T)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>Allele</td>
<td>Measured Response</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>ARNT Exon 8 G/C</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IL-8 (-251T/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>AM (3’ end CA repeat)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>NRP (-2548G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>Leptin (-2548G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>TF (-603A/G)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>PLGF (3’UTR G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>PLGF (3’UTR T/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>MMMP7 (-181A/G)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>MMP9 (-1562C/T)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>MMP2 (-1306C/T)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>NFkB (CA repeat in regulatory region)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>p53 (codon 72 Pro-Arg (C/G))</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IGF1 (CA repeat)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IGF2 (3580G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IGFr1 (3174G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>ICAM1 (K469E)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>MDM2 (309T/G)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IL-6 (-174G/C)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>LDH (Exon 5 C/T)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>LDH (Exon 5 G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>GLUT1 (-2841A/T)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>CXCR1 (2607G/C)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>CXCR2 (785C/T)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>COX2 (8437T/C)</td>
<td>No Correlation</td>
</tr>
</tbody>
</table>
Table 3 - Predictive Response for Metastatic Colorectal Cancer Patients treated with combination therapy of 5-FU or Capecitabine in combination with Oxaliplatin and Bevacizumab (FOLFOX/BV or XELOX/BV respectively)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Measured Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF (61A/G)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>EGFR (497G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>TNFα (-308G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IL1b (-511T/C)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IL1b (3954C/T)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IL1Ra (Intron 2 86bp)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>SDF1/CXCL12 (-801G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>FGFR4 (388G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IGFB3 (-202A/C)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IGFB3 (2133G/C)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>Endostatin (G+4349A)</td>
<td>No Correlation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Predictive Polymorphism Genotype</th>
<th>Measured Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin 5'UTR at nt -2548</td>
<td>A/A</td>
<td>Complete or Partial Response; or Stable Disease</td>
</tr>
<tr>
<td>IL-6 at nt 174</td>
<td>G/G or G/C</td>
<td>Complete or Partial Response; or Stable Disease</td>
</tr>
<tr>
<td>IL-8 at nt -251</td>
<td>A/A or A/T</td>
<td>Complete or Partial Response; or Stable Disease</td>
</tr>
<tr>
<td>EGFR at nt 497</td>
<td>G/A or A/A</td>
<td>Complete or Partial Response; or Stable Disease</td>
</tr>
<tr>
<td>ARNT at Exon 8</td>
<td>G/G or G/C</td>
<td>Complete or Partial Response; or Stable Disease</td>
</tr>
</tbody>
</table>
Table 4 - Additional Polymorphisms Assayed for Patients with Metastatic Colorectal Cancer (mCRC) treated with combination therapy of 5-FU or Capecitabine in combination with Oxaliplatin and Bevacizumab (FOLFOX/BV or XELOX/BV respectively)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Predictive Polymorphism Genotype</th>
<th>Measured Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP7 at nt -181</td>
<td>G/G or A/G</td>
<td>Complete or Partial Response; or Stable Disease</td>
</tr>
<tr>
<td>KDR (a.k.a., VEGFR2) at position 4422</td>
<td>Number of CA repeats 10/11 or 10/10</td>
<td>Improved progression free survival</td>
</tr>
<tr>
<td>CXCR2 at nt 785</td>
<td>C/C or C/T</td>
<td>Improved progression free survival</td>
</tr>
<tr>
<td>MMP7 at nt -181</td>
<td>A/G or G/G</td>
<td>Improved progression free survival</td>
</tr>
<tr>
<td>FGFR4 at nt 388</td>
<td>G/G</td>
<td>Improved progression free survival</td>
</tr>
<tr>
<td>NFkB at the 5’ end</td>
<td>≥1 allele with ≥24 CA repeats</td>
<td>Improved progression free survival</td>
</tr>
<tr>
<td>AM at the 3’ end</td>
<td>2 alleles with ≥14 CA repeats</td>
<td>Improved progression free survival</td>
</tr>
<tr>
<td>TF at nt -603</td>
<td>G/G or G/A</td>
<td>Improved progression free survival</td>
</tr>
</tbody>
</table>

Table 4 - Additional Polymorphisms Assayed for Patients with Metastatic Colorectal Cancer (mCRC) treated with combination therapy of 5-FU or Capecitabine in combination with Oxaliplatin and Bevacizumab (FOLFOX/BV or XELOX/BV respectively)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Measured Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (936C/T)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>NRP1 (3’ end C/T)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IL-6 (174 G/C)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>IL-8 (-251 T/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>CXCR1 (2607G/C)</td>
<td>No Correlation</td>
</tr>
</tbody>
</table>
This invention also provides methods for treating metastatic or non-metastatic gastrointestinal cancer patients by administering angiogenesis inhibitors such as anti-angiogenic antibodies, particularly anti-VEGF and anti-VEGFR2. The invention further provides methods for treating metastatic or non-metastatic gastrointestinal cancer patients by administering combination therapy with anti-VEGF antibodies, pyrimidine based antimetabolites and platinum-based alkylating agents.

The various embodiments are set forth herein.

In one embodiment, this invention provides a method for determining if a human stage III colon cancer patient is at risk of developing tumor recurrence by screening a suitable cell or tissue sample isolated from said patient for at least one, or alternatively at least two, or alternatively all three genetic polymorphisms identified in Table 1, above. Patients having a polymorphism genotype selected from (11/1 1 CA repeats) for the VEGFR2 gene at position 4422, indicates that the patient is at high risk for developing tumor recurrence.

In another embodiment, the invention is a method for determining if a human stage II colon cancer patient is at risk of developing tumor recurrence by screening a suitable cell or tissue sample isolated from said patient for the genetic polymorphism identified in Table 1, above. Patients having a polymorphism genotype of (11/1 1 CA repeats) for VEGFR2 at position 4422, indicates that the patient is at high risk for developing tumor recurrence.

After a patient has been identified as positive for one or more of the polymorphisms identified in Table 1, the method may further comprise treating the patient by administering or delivering an effective amount of an anti-VEGF or anti-VEGFR2 antibody or

<table>
<thead>
<tr>
<th>MMP2 (-1306C/T)</th>
<th>No Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP9 (-1562C/T)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>EGFR (497G/A)</td>
<td>No Correlation</td>
</tr>
<tr>
<td>ARNT (Exon 8 G/C)</td>
<td>No Correlation</td>
</tr>
</tbody>
</table>
chemical/biological equivalent thereof. In further aspect, treating the patient may comprise the administration of the anti-VEGF antibody bevacizumab or a chemical/biological equivalent thereof. Methods of administration of pharmaceuticals and biologicals are known in the art and incorporated herein by reference.

In one embodiment, the invention is a method for determining whether a human gastrointestinal cancer patient in need thereof will likely respond to a therapy comprising, or alternatively consisting essentially of, or yet further consisting of the administration of an anti-VEGF antibody, pyrimidine based antimetabolite and platinum-based alkylating agent, or equivalents of each thereof, based therapy comprising screening a suitable cell or tissue sample isolated from said patient for at least one, or alternatively at least two, or alternatively at least three, or alternatively at least four, or alternatively at least five, or alternatively at least six, or alternatively at least seven, or alternatively at least eight, or alternatively at least nine, or alternatively at least ten, or alternatively at least eleven, or alternatively at least twelve, or alternatively all thirteen polymorphisms identified in Table 3, above. Patients having a genotype selected from (A/A) for Leptin 5' UTR at nt -2548; (G/G or A/G) for MMP7 at nt -181; (10/1 1 or 10/10 CA repeats) for VEGFR2 at position 4422; (C/C or C/T) for CXCR2 at nt 785; (A/G or G/G) for MMP7 at nt -181; (G/G) for FGFR4 at nt 388; (≥1 allele with >24 CA repeats) for NFKB at the 5' end; (2 alleles with ≥14 CA repeats) for AM at the 3' end; (G/G or G/A) for TF at nt -603; (G/G or G/C) for IL-6 at nt 174; (A/A or A/T) for IL-8 at nt -251; (G/A or A/A) for EGFR at nt 497 or (G/G or G/C) for ARNT at Exon 8 are likely to show responsiveness to the therapy, wherein responsiveness is any kind of improvement or positive response either clinical or non-clinical selected from, but not limited to, measurable reduction in tumor size or evidence of disease or disease progression, complete response, partial response, stable disease, increase or elongation of progression free survival, increase or elongation of overall survival, or reduction in toxicity.

After a patient has been identified as positive for one or more of the polymorphisms identified in Table 3, the method may further comprise administering or delivering an effective amount of an anti-VEGF antibody, pyrimidine based antimetabolite and platinum-based alkylating agent, or equivalents of each thereof, based therapy. Methods of
administration of pharmaceuticals and biologicals are known in the art and incorporated herein by reference.

In one aspect of the above embodiments, the human patient is suffering from a solid malignant tumor such as a metastatic or non-metastatic gastrointestinal tumor, e.g., from rectal cancer, colorectal cancer, colon cancer, gastric cancer, lung cancer, non-small cell lung cancer and esophageal cancer. In an alternative aspect, the patient is suffering from colorectal cancer. In yet a further aspect, the patient is suffering from metastatic colorectal cancer.

To practice these methods, the patient sample containing the tumor tissue, normal tissue adjacent to said tumor, normal tissue distal to said tumor or peripheral blood lymphocytes. In one aspect, the method also requires isolating a sample containing the genetic material to be tested; however, it is conceivable that one of skill in the art will be able to analyze and identify genetic polymorphisms in situ at some point in the future. Accordingly, the inventions of this application are not to be limited to requiring isolation of the genetic material prior to analysis.

These methods are not limited by the technique that is used to identify the polymorphism of interest. Suitable methods include but are not limited to the use of primers, hybridization probes, antibodies, primers for PCR analysis and gene chips, slides and software for high throughput analysis. Additional polymorphisms can be assayed and used as negative controls.

In each of the above embodiments, an example of anti-VEGF antibody is, but not limited to Bevacizumab (BV) or a biological equivalent thereof. Biological equivalents are described below.

In each of the above aspects, examples of pyrimidine based antimetabolites are include but are not limited to the group, to 5-Fluorouracil (5-FU), ftorafur (1-tetrahydrofuran-5-fluorouracil), S-1(BMS-247616), FdUMP, and Capecitabine (XEL) or chemical equivalents thereof. Additional equivalents are described infra.
In each of the above aspects, an example of a platinum-based alkylating agent is, but not limited to Oxaliplatin (OX) or a chemical equivalent thereof. Additional equivalents are described infra.

In a further aspect of the invention, the therapy comprises a pyrimidine based antimetabolite and a platinum-based alkylating agent in combination with an efficacy enhancing agent for the pyrimidine based antimetabolites. An example of an efficacy enhancing agent is, but not limited to Leucovorin or a chemical equivalent thereof. This combination is known in the art as FOLFOX or XELOX.

This invention also provides a panel, kit, software, support and/or gene chip for patient sampling and performance of the methods of this invention. The kits contain gene chips, probes or primers that can be used to amplify and/or for determining the molecular structure of the polymorphisms identified above. In an alternate embodiment, the kit also contains antibodies or other polypeptide binding agents that are useful to identify a polymorphism of Tables 1, 2, 3 or 4. Instructions for using the materials to carry out the methods are further provided.

The present invention provides methods and kits for identifying patients having solid malignant tumor masses or cancers who are likely to respond to an anti-VEGF antibody, pyrimidine based antimetabolite and platinum-based alkylating agent, or equivalents of each thereof, based therapy. The methods require determining the subject's genotype at the gene of interest. Other aspects of the invention are described below or will be apparent to one of skill in the art in light of the present disclosure.

This invention also provides for a panel of genetic markers selected from, but not limited to the genetic polymorphisms identified in Tables 1, 2, 3 or 4 alone, in combination with each other, or in combination with other genetic polymorphisms or markers. The panel comprises probes or primers that can be used to amplify and/or for determining the molecular structure of the polymorphisms identified above. The probes or primers can be attached or supported by a solid phase support such as, but not limited to a gene chip or microarray. The probes or primers can be detectably labeled. This aspect of the invention is
a means to identify the genotype of a patient sample for the genes of interest identified above.
BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the predictive response to FOLFOX/BV or XELOX/BV therapy associated with MMP7 allele polymorphism at nt -181 or the Leptin allele polymorphism at the 5'UTR at nt -2548 and tumor response. Patients identified as having the genotype G/G or A/G for MMP7 show an increase in response. Patients identified as having the genotype A/A for Leptin also show an increase in response. The letter n equals the number of patients in each group.

Figure 2 shows the predictive response to FOLFOX/BV or XELOX/BV therapy associated with KDR (VEGFR2) polymorphism at position 4422 CA repeats and progression free survival. Patients identified as having the genotype 10/11 or 10/10 show an increase in progression free survival. The letter n equals the number of patients in each group.

Figure 3 shows the predictive response to FOLFOX/BV or XELOX/BV therapy associated with MMP7 allele polymorphism at nt -181 and progression free survival. Patients identified as having the genotype G/G or A/G show an increase in progression free survival. The letter n equals the number of patients in each group.

Figure 4 shows the predictive response to FOLFOX/BV or XELOX/BV therapy associated with NFkB allele polymorphism at the 5' end CA repeat and progression free survival. Patients identified as having the genotype of at least 1 allele with >24 CA repeats show an increase in progression free survival. The letter n equals the number of patients in each group.

Figure 5 shows the predictive response to FOLFOX/BV or XELOX/BV therapy associated with CXCR2 allele polymorphism at nt 785 and progression free survival. Patients identified as having the genotype C/C or C/T show an increase in progression free survival. The letter n equals the number of patients in each group.
MODES FOR CARRYING OUT THE INVENTION

Before the compositions and methods are described, it is to be understood that the invention is not limited to the particular methodologies, protocols, cell lines, assays, and reagents described, as these may vary. It is also to be understood that the terminology used herein is intended to describe particular embodiments of the present invention, and is in no way intended to limit the scope of the present invention as set forth in the appended claims.

Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. The disclosures of these publications, patents and published patent specifications are hereby incorporated by reference in their entirety into the present disclosure to more fully describe the state of the art to which this invention pertains.

Definitions

As used herein, certain terms may have the following defined meanings. As used in the specification and claims, the singular form "a," "an" and "the" includes the singular and plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a single cell and a plurality of cells, including mixtures thereof.

As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of when used to define compositions and methods, shall mean excluding other elements of any essential significance to the composition or method for the stated purpose. "Consisting of shall mean excluding more than trace elements of other ingredients for claimed compositions and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention. Accordingly, it is intended that the methods and compositions can include additional steps and components (comprising) or alternatively the steps and compositions of no significance (consisting essentially of) or alternatively, intending only the stated methods steps or compositions (consisting of).

All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term "about". The term "about" also includes the exact value "X" in addition to minor increments of "X" such as "X + 0.1" or "X - 0.1." It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.
The term "antigen" is well understood in the art and includes substances which are immunogenic. The EGFR is an example of an antigen.

A "native" or "natural" or "wild-type" antigen is a polypeptide, protein or a fragment which contains an epitope and which has been isolated from a natural biological source. It also can specifically bind to an antigen receptor.

As used herein, an "antibody" includes whole antibodies and any antigen binding fragment or a single chain thereof. Thus the term "antibody" includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein, any of which can be incorporated into an antibody of the present invention.

The antibodies can be polyclonal or monoclonal and can be isolated from any suitable biological source, e.g., murine, rat, sheep and canine. Additional sources are identified infra.

Bevacizumab is sold under the trade name Avastin by Genentech. It is a humanized monoclonal antibody that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF). Biological equivalent antibodies are identified herein as modified antibodies and those which bind to the same epitope of the antigen, prevent the interaction of VEGF to its receptors (FltOl, KDR a.k.a. VEGFR2) and produce a substantially equivalent response, e.g., the blocking of endothelial cell proliferation and angiogenesis.

In one aspect, the "biological equivalent" means the ability of the antibody to selectively bind its epitope protein or fragment thereof as measured by ELISA or other suitable methods. Biologically equivalent antibodies include, but are not limited to, those antibodies, peptides, antibody fragments, antibody variant, antibody derivative and antibody mimetics that bind to the same epitope as the reference antibody. An example of an
equivalent Bevacizumab antibody is one which binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF).

Fluorouracil (5-FU) belongs to the family of therapy drugs called pyrimidine based anti-metabolites. It is a pyrimidine analog, which is transformed into different cytotoxic metabolites that are then incorporated into DNA and RNA thereby inducing cell cycle arrest and apoptosis. Chemical equivalents are pyrimidine analogs which result in disruption of DNA replication. Chemical equivalents inhibit cell cycle progression at S phase resulting in the disruption of cell cycle and consequently apoptosis. Equivalents to 5-FU include prodrugs, analogs and derivative thereof such as 5'-deoxy-5-fluorouridine (doxifluroidine), 1-tetrahydrofuranyl-5-fluorouracil (ftorafur), Capecitabine (Xeloda), S-I (MBMS-247616, consisting of tegafur and two modulators, a 5-chloro-2,4-dihydroxypyridine and potassium oxonate), ralititrexed (tomudex), nolatrexed (Thymitaq, AG337), LY231514 and ZD9331, as described for example in Papamicheal (1999) The Oncologist 4:478-487.

Capecitabine is a prodrug of (5-FU) that is converted to its active form by the tumor-specific enzyme PynPase following a path of three enzymatic steps and two intermediary metabolites, 5'-deoxy-5-fluorocytidine (5'-DFCR) and 5'-deoxy-5-fluorouridine (5'-DFUR). Capecitabine is marketed by Roche under the trade name Xeloda®.

Leucovorin (Folinic acid) is an adjuvant used in cancer therapy. It is used in synergistic combination with 5-FU to improve efficacy of the chemotherapeutic agent. Without being bound by theory, addition of Leucovorin is believed to enhance efficacy of 5-FU by inhibiting thymidylate synthase. It has been used as an antidote to protect normal cells from high doses of the anticancer drug methotrexate and to increase the antitumor effects of fluorouracil (5-FU) and tegafur-uracil. It is also known as citrovorum factor and Wellcovorin. This compound has the chemical designation of L-Glutamic acid N/[4][(2-amino-5-formyl1,4,5,6,7,8hexahydro4oxo6-pteridinyl]methyl]arnino]benzoyl], calcium salt (1:1).

"Oxaliplatin" (Eloxatin®) is a platinum-based chemotherapy drug in the same family as cisplatin and carboplatin. It is typically administered in combination with
fluorouracil and leucovorin in a combination known as FOLFOX for the treatment of colorectal cancer. Compared to cisplatin the two amine groups are replaced by cyclohexyldiamine for improved antitumour activity. The chlorine ligands are replaced by the oxalato bidentate derived from oxalic acid in order to improve water solubility. Equivalents to Oxaliplatin are known in the art and include without limitation cisplatin, carboplatin, aroplatin, lobaplatin, nedaplatin, and JM-216 (see McKeage et al. (1997) J. Clin. Oncol. 201:1232-1237 and in general, CHEMOTHERAPY FOR GYNECOLOGICAL NEOPLASM, CURRENT THERAPY AND NOVEL APPROACHES, in the Series Basic and Clinical Oncology, Angioli et al. Eds., 2004).

"FOLFOX" is an abbreviation for a type of combination therapy that is used to treat colorectal cancer. In includes 5-FU, oxaliplatin and leucovorin. Information regarding this treatment is available on the National Cancer Institute's web site, cancer.gov, last accessed on January 16, 2008.

In one aspect, the "chemical equivalent" means the ability of the chemical to selectively interact with its target protein, DNA, RNA or fragment thereof as measured by the inactivation of the target protein, incorporation of the chemical into the DNA or RNA or other suitable methods. Chemical equivalents include, but are not limited to, those agents with the same pharmaceutically acceptable salt or mixture thereof that interact with and/or inactivate the same target protein, DNA, or RNA as the reference chemical.

If an antibody is used in combination with the above-noted chemotherapy or for diagnosis or as an alternative to the chemotherapy, the antibodies can be polyclonal or monoclonal and can be isolated from any suitable biological source, e.g., murine, rat, sheep and canine. Additional sources are identified infra.

In one aspect, the "biological activity" means the ability of the antibody to selectively bind its epitope protein or fragment thereof as measured by ELISA or other suitable methods. Biologically equivalent antibodies, include but are not limited to those antibodies, peptides, antibody fragments, antibody variant, antibody derivative and antibody mimetics that bind to the same epitope as the reference antibody.
The term "antibody" is further intended to encompass digestion fragments, specified portions, derivatives and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof, including single chain antibodies and fragments thereof.

Examples of binding fragments encompassed within the term "antigen binding portion" of an antibody include a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH, domains; a F(ab')² fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH, domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, a dAb fragment (Ward et al. (1989) Nature 341:544-546), which consists of a VH domain; and an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, 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 VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv)). Bird et al. (1988) Science 242:423-426 and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883. Single chain antibodies are also intended to be encompassed within the term "fragment of an antibody." Any of the above-noted antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for binding specificity and neutralization activity in the same manner as are intact antibodies.

The term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes 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.

The term "antibody variant" is intended to include antibodies produced in a species other than a mouse. It also includes antibodies containing post-translational modifications to the linear polypeptide sequence of the antibody or fragment. It further encompasses fully human antibodies.
The term "antibody derivative" is intended to encompass molecules that bind an epitope as defined above and which are modifications or derivatives of a native monoclonal antibody of this invention. Derivatives include, but are not limited to, for example, bispecific, multispecific, heterospecific, trispecific, tetraspecific, multispecific antibodies, diabodies, chimeric, recombinant and humanized.

The term "bispecific molecule" is intended to include any agent, e.g., a protein, peptide, or protein or peptide complex, which has two different binding specificities. The term "multispecific molecule" or "heterospecific molecule" is intended to include any agent, e.g. a protein, peptide, or protein or peptide complex, which has more than two different binding specificities.

The term "multispecific molecule" or "heterospecific molecule" is intended to include any agent, e.g. a protein, peptide, or protein or peptide complex, which has more than two different binding specificities.

The term "heteroantibodies" refers to two or more antibodies, antibody binding fragments (e.g., Fab), derivatives thereof, or antigen binding regions linked together, at least two of which have different specificities.

The term "human antibody" as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human 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 antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Thus, as used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C\textsubscript{L}, C\textsubscript{H} domains (e.g., C\textsubscript{H1}, C\textsubscript{H2}, C\textsubscript{H3}), hinge, (VL, VH)) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-
genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

As used herein, a human antibody is "derived from" a particular germline sequence if the antibody is obtained from a system using human immunoglobulin sequences, e.g., by immunizing a transgenic mouse carrying human immunoglobulin genes or by screening a human immunoglobulin gene library. A human antibody that is "derived from" a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequence of human germline immunoglobulins. A selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.
The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

A "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions derived from human germline immunoglobulin sequences.

The term "recombinant human antibody", as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

As used herein, "isotype" refers to the antibody class (e.g., IgM or IgGl) that is encoded by heavy chain constant region genes. The IgGl isotype consist of four subclasses, IgGl, IgG2, IgG3, and IgG4 each of which having specific activities including the ability to cross into the placenta, act as a complement activator, and to bind to Fc receptors on phagocytic cells. In one embodiment, IgGl antibodies can cross into the placenta, is the second highest complement activator and has high affinity to bind to Fc receptors on phagocytic cells.
The term "allele", which is used interchangeably herein with "allelic variant" refers to alternative forms of a gene or portions thereof. Alleles occupy the same locus or position on homologous chromosomes. When a subject has two identical alleles of a gene, the subject is said to be homozygous for the gene or allele. When a subject has two different alleles of a gene, the subject is said to be heterozygous for the gene. Alleles of a specific gene can differ from each other in a single nucleotide, or several nucleotides, and can include substitutions, deletions and insertions of nucleotides. An allele of a gene can also be a form of a gene containing a mutation.

The terms "protein", "polypeptide" and "peptide" are used interchangeably herein when referring to a gene product.

The term "recombinant protein" refers to a polypeptide which is produced by recombinant DNA techniques, wherein generally, DNA encoding the polypeptide is inserted into a suitable expression vector which is in turn used to transform a host cell to produce the heterologous protein.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of preferred vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Preferred vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids" which refer generally to circular double stranded DNA loops which, in their vector form are not bound to the chromosome. In the present specification, "plasmid" and "vector" are used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which become known in the art subsequently hereto.

The term "wild-type allele" refers to an allele of a gene which, when present in two copies in a subject results in a wild-type phenotype. There can be several different wild-
type alleles of a specific gene, since certain nucleotide changes in a gene may not affect the phenotype of a subject having two copies of the gene with the nucleotide changes.

The term "genetic marker" refers to an allelic variant of a polymorphic region of a gene of interest and/or the differentially expressed gene of interest.

The term "allelic variant of a polymorphic region of the gene of interest" refers to a region of the gene of interest having one of a plurality of nucleotide sequences found in that region of the gene in other individuals.

As used herein, the term "gene of interest" intends one or more genes selected from the group consisting of Leptin, MMP7, VEGFR2, CXCR2, FGFR4, NFkB, AM, TF, IL-6, IL-8, EGFR, ARNT, VEGF, HIF1α; NRP, PLGF, MMP9, MMP2, NFKB, p53, IGFI, IGF2, IGFr1, ICAM1, MDM2, LDH, GLUT1, CXCR1, COX2, EGF, TNFα; IL1b, IL1Ra, SDF1/CXCL12, FGFR4, IGFB3 and Endostatin.

"Cells," "host cells" or "recombinant host cells" are terms used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

The expression "amplification of polynucleotides" includes methods such as PCR, ligation amplification (or ligase chain reaction, LCR) and amplification methods. These methods are known and widely practiced in the art. See, e.g., U.S. Pat. Nos. 4,683,195 and 4,683,202 and Innis et al., 1990 (for PCR); and Wu et al. (1989) Genomics 4:560-569 (for LCR). In general, the PCR procedure describes a method of gene amplification which is comprised of (i) sequence-specific hybridization of primers to specific genes within a DNA sample (or library), (ii) subsequent amplification involving multiple rounds of annealing, elongation, and denaturation using a DNA polymerase, and (iii) screening the PCR products for a band of the correct size. The primers used are oligonucleotides of sufficient length and
appropriate sequence to provide initiation of polymerization, i.e. each primer is specifically
designed to be complementary to each strand of the genomic locus to be amplified.

Reagents and hardware for conducting PCR are commercially available. Primers
useful to amplify sequences from a particular gene region are preferably complementary to,
and hybridize specifically to sequences in the target region or in its flanking regions.
Nucleic acid sequences generated by amplification may be sequenced directly.
Alternatively the amplified sequence(s) may be cloned prior to sequence analysis. A
method for the direct cloning and sequence analysis of enzymatically amplified genomic
segments is known in the art.

The term "encode" as it is applied to polynucleotides refers to a polynucleotide
which is said to "encode" a polypeptide if, in its native state or when manipulated by
methods well known to those skilled in the art, it can be transcribed and/or translated to
produce the mRNA for the polypeptide and/or a fragment thereof. The antisense strand is
the complement of such a nucleic acid, and the encoding sequence can be deduced
therefrom.

The term "genotype" refers to the specific allelic composition of an entire cell or a
certain gene, whereas the term 'phenotype' refers to the detectable outward manifestations
of a specific genotype.

As used herein, the term "gene" or "recombinant gene" refers to a nucleic acid
molecule comprising an open reading frame and including at least one exon and (optionally)
an intron sequence. The term "intron" refers to a DNA sequence present in a given gene
which is spliced out during mRNA maturation.

"Homology" or "identity" or "similarity" refers to sequence similarity between two
peptides or between two nucleic acid molecules. Homology can be determined by
comparing a position in each sequence which may be aligned for purposes of comparison.
When a position in the compared sequence is occupied by the same base or amino acid, then
the molecules are homologous at that position. A degree of homology between sequences is
a function of the number of matching or homologous positions shared by the sequences. An
"unrelated" or "non-homologous" sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present invention.

The term "a homolog of a nucleic acid" refers to a nucleic acid having a nucleotide sequence having a certain degree of homology with the nucleotide sequence of the nucleic acid or complement thereof. A homolog of a double stranded nucleic acid is intended to include nucleic acids having a nucleotide sequence which has a certain degree of homology with or with the complement thereof. In one aspect, homologs of nucleic acids are capable of hybridizing to the nucleic acid or complement thereof.

The term "interact" as used herein is meant to include detectable interactions between molecules, such as can be detected using, for example, a hybridization assay. The term interact is also meant to include "binding" interactions between molecules. Interactions may be, for example, protein-protein, protein-nucleic acid, protein-small molecule or small molecule-nucleic acid in nature.

The term "isolated" as used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively, that are present in the natural source of the macromolecule. The term isolated as used herein also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an "isolated nucleic acid" is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term "isolated" is also used herein to refer to polypeptides which are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides.

The term "mismatches" refers to hybridized nucleic acid duplexes which are not 100% homologous. The lack of total homology may be due to deletions, insertions, inversions, substitutions or frameshift mutations.

The term "hybridization" refers to a technique in which single-stranded nucleic acids (DNA or RNA) are allowed to interact so that complexes are formed by molecules with
similar, complementary sequences. Through nucleic acid hybridization, the degree of sequence identity between nucleic acids can be determined and specific sequences detected in them. The hybridization can be carried out in solution or with one component immobilized on, but not limited to a gel, nitrocellulose paper, genechip, or microarray. The conditions for hybridization can be selected for high, moderate, or low stringency. Hybridizations can be done in combinations which include, but are not limited to DNA-DNA, DNA-RNA or RNA-RNA.

Hybridization reactions can be performed under conditions of different "stringency". In general, a low stringency hybridization reaction is carried out at about 40 °C in 10 x SSC or a solution of equivalent ionic strength/temperature. A moderate stringency hybridization is typically performed at about 50 °C in 6 x SSC, and a high stringency hybridization reaction is generally performed at about 60 °C in 1 x SSC.

When hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides, the reaction is called "annealing" and those polynucleotides are described as "complementary". A double-stranded polynucleotide can be "complementary" or "homologous" to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second. "Complementarity" or "homology" (the degree that one polynucleotide is complementary with another) is quantifiable in terms of the proportion of bases in opposing strands that are expected to form hydrogen bonding with each other, according to generally accepted base-pairing rules.

As used herein, the term "nucleic acid" refers to polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, derivatives, variants and analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single (sense or antisense) and double-stranded polynucleotides. Deoxyribonucleotides include deoxyadenosine, deoxycytidine, deoxyguanosine, and deoxythymidine. For purposes of clarity, when referring herein to a nucleotide of a nucleic acid, which can be DNA or an RNA, the terms "adenosine", "cytidine", "guanosine", and
"thymidine" are used. It is understood that if the nucleic acid is RNA, a nucleotide having a uracil base is uridine.

The terms "oligonucleotide" or "polynucleotide", or "portion," or "segment" thereof refer to a stretch of polynucleotide residues which is long enough to use in PCR or various hybridization procedures to identify or amplify identical or related parts of mRNA or DNA molecules. The polynucleotide compositions of this invention include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, car bamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

As used herein, the term "label" intends a directly or indirectly detectable compound or composition that is conjugated directly or indirectly to the composition to be detected, e.g., polynucleotide or protein such as an antibody so as to generate a "labeled" composition. The term also includes sequences conjugated to the polynucleotide that will provide a signal upon expression of the inserted sequences, such as green fluorescent protein (GFP) and the like. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. The labels can be suitable for small scale detection or more suitable for high-throughput screening. As such, suitable labels include, but are not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes. The label may be simply detected or it...
may be quantified. A response that is simply detected generally comprises a response whose existence merely is confirmed, whereas a response that is quantified generally comprises a response having a quantifiable (e.g., numerically reportable) value such as an intensity, polarization, and/or other property. In luminescence or fluorescence assays, the detectable response may be generated directly using a luminophore or fluorophore associated with an assay component actually involved in binding, or indirectly using a luminophore or fluorophore associated with another (e.g., reporter or indicator) component.

Examples of luminescent labels that produce signals include, but are not limited to bioluminescence and chemiluminescence. Detectable luminescence response generally comprises a change in, or an occurrence of, a luminescence signal. Suitable methods and luminophores for luminescently labeling assay components are known in the art and described for example in Haugland, Richard P. (1996) HANDBOOK OF FLUORESCENT PROBES AND RESEARCH CHEMICALS (6th ed.). Examples of luminescent probes include, but are not limited to, aequorin and luciferases.

Examples of suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue.TM., and Texas Red. Other suitable optical dyes are described in the Haugland, Richard P. (1996) HANDBOOK OF FLUORESCENT PROBES AND RESEARCH CHEMICALS (6th ed.).

In another aspect, the fluorescent label is functionalized to facilitate covalent attachment to a cellular component present in or on the surface of the cell or tissue such as a cell surface marker. Suitable functional groups, including, but not are limited to, isothiocyanate groups, amino groups, haloacetyl groups, maleimides, succi nimidyl esters, and sulfonyl halides, all of which may be used to attach the fluorescent label to a second molecule. The choice of the functional group of the fluorescent label will depend on the site of attachment to either a linker, the agent, the marker, or the second labeling agent.

The term "polymorphism" refers to the coexistence of more than one form of a gene or portion thereof. A portion of a gene of which there are at least two different forms, i.e.,
two different nucleotide sequences, is referred to as a "polymorphic region of a gene". A polymorphic region can be a single nucleotide, the identity of which differs in different alleles.

A "polymorphic gene" refers to a gene having at least one polymorphic region.

When a genetic marker or polymorphism "is used as a basis" for selecting a patient for a treatment described herein, the genetic marker or polymorphism is measured before and/or during treatment, and the values obtained are used by a clinician in assessing any of the following: (a) probable or likely suitability of an individual to initially receive treatment(s); (b) probable or likely unsuitability of an individual to initially receive treatment(s); (c) responsiveness to treatment; (d) probable or likely suitability of an individual to continue to receive treatment(s); (e) probable or likely unsuitability of an individual to continue to receive treatment(s); (f) adjusting dosage; (g) predicting likelihood of clinical benefits. As would be well understood by one in the art, measurement of the genetic marker or polymorphism in a clinical setting is a clear indication that this parameter was used as a basis for initiating, continuing, adjusting and/or ceasing administration of the treatments described herein.

"Administration" of an active agent, e.g., antibody or small molecule, to the patient can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents can be found below.

The term "treating" as used herein is intended to encompass curing as well as ameliorating at least one symptom of the condition or disease. For example, in the case of cancer, likely to respond to treatment includes a reduction in cachexia, increase in survival time, elongation in time to tumor progression, reduction in tumor mass, reduction in tumor burden and/or a prolongation in time to tumor metastasis, each as measured by standards set
by the National Cancer Institute and the U.S. Food and Drag Administration for the approval of new drugs. See Johnson et al. (2003) J. Clin. Oncol. 21(7): 1404-1411.

A "response" implies any kind of improvement or positive response either clinical or non-clinical such as, but not limited to, measurable reduction in tumor size or evidence of disease or disease progression, complete response, partial response, stable disease, increase or elongation of progression free survival, increase or elongation of overall survival, or reduction in toxicity.

The term "likely to respond" shall mean that the patient is more likely than not to exhibit at least one of the described treatment parameters, identified above, as compared to similarly situated patients.

"Progression free survival" (PFS) or "Time to Tumor Progression" (TTP) indicates the length of time during and after treatment that the cancer does not grow. Progression-free survival includes the amount of time patients have experienced a complete response or a partial response, as well as the amount of time patients have experienced stable disease.

A "complete response" (CR) to a therapy defines patients with evaluable but non-measurable disease, whose tumor and all evidence of disease had disappeared.

A "partial response" (PR) to a therapy defines patients with anything less than complete response were simply categorized as demonstrating partial response.

"Stable disease" (SD) indicates that the patient is stable.

"Non-response" (NR) to a therapy defines patients whose tumor or evidence of disease has remained constant or has progressed.

"Overall Survival" (OS) intends a prolongation in life expectancy as compared to naive or untreated individuals or patients.

"No Correlation" refers to a statistical analysis showing no relationship between the allelic variant of a polymorphic region and clinical parameters.
The term "likely to respond" shall mean that the patient is more likely than not to exhibit at least one of the described clinical parameters or treatment responses, identified above, as compared to similarly situated patients.

**Descriptive Embodiments**

This invention provides a method for prognosis of a human cancer patient and for selecting a therapeutic regimen or determining if a certain therapeutic regimen is more likely to treat a malignant condition such as cancer or is the appropriate therapy for that patient than other available chemotherapies. In general, a therapy is considered to "treat" cancer if it provides one or more of the following treatment outcomes: reduce or delay recurrence of the cancer after the initial therapy; time to tumor progression (TTP), decrease in tumor load or size (tumor response or TR), increase median survival time (OS) or decrease metastases. The method is particularly suited to determining which patients will be responsive or experience a positive treatment outcome to an anti-VEGF antibody, pyrimidine based antimetabolite and platinum-based alkylating agent, or equivalents of each thereof, based therapy. These methods are useful to select therapies for highly aggressive cancers such as colon cancer or metastatic colon cancer.

In one embodiment, the therapy further comprises adjuvant radiation therapy or other suitable therapy.

The method comprises screening for a genomic polymorphism or genotype identified in Tables 1-2, 3 or 4, above, determining the identity of the noted position and correlating that result to treatment outcome and treatment selection.

In one embodiment, the invention is a method for determining if a human stage III colon cancer patient is at risk of developing tumor recurrence by screening a suitable sample isolated from the patient for at least one genetic polymorphism selected from VEGF at nt 936; VEGFR2 at position 4422 CA repeats; or VEGFR2 at nt 1416, wherein for the genetic polymorphism screened, the presence of at least one genetic polymorphism genotype selected from (C/C) for VEGF at nt 936; (10/10 or 10/11 CA repeats) for
VEGFR2 at position 4422; or (A/A) for VEGFR2 at nt 1416, indicates the patient is at high risk for developing tumor recurrence.

In another embodiment, the invention is a method for determining if a human stage II colon cancer patient is at risk of developing tumor recurrence by screening a suitable sample isolated from the patient for the genetic polymorphism CA repeats in the VEGFR2 gene at position 4422 CA, wherein the presence of the genetic polymorphism genotype (11/11 CA repeats) for the VEGFR2 gene at position 4422, indicates the patient is at high risk for developing tumor recurrence.

In a further aspect of the above embodiments, the method comprises treating the patient indicated as being at high risk for developing tumor recurrence by administration of an effective amount of the anti-VEGF antibody, for example Bevacizumab or a chemical/biological equivalent thereof. In yet a further aspect, the patient indicated as being at high risk for developing tumor recurrence, is treated by the administration of an effective amount of an anti-VEGFR2 based therapy.

In a further aspect, negative control polymorphisms may be used to screen or identify the patient sample for a polymorphism that is not associated with tumor recurrence selected from VEGF (G405C); VEGFR2 (11IT/A); HIF1α (1772C/T); ARNT Exon 8 G/C; IL-8 (-25 IT/A); AM (3’end CA repeat); NRP (-2548G/A); Leptin (-2548G/A); TF (-603A/G); PLGF (3’UTR G/A); PLGF (3’UTR T/A); MMP7 (-181A/G); MMP9 (-1562C/T); MMP2 (-1306C/T); NFkB (CA repeat in regulatory region); p53 (codon 72 Pro-Arg (C/G)); IGFI (CA repeat); IGF2 (358OG/A); IGFr1 (3174G/A); ICAM1 (K469E); MDM2 (309T/G); IL-6 (-174G/C); LDH (Exon 5 C/T); LDH (Exon 5 G/A); GLUT1 (-2841A/T); CXCR1 (2607G/C); CXCR2 (785C/T); COX2 (8437T/C); EGF (61A/G); EGFR (497G/A); TNFα (-308G/A); IL1b (-511T/C); IL1b (3954C/T); IL1Ra (Intron 2 86bp); SDF1/CXCL12 (-801G/A); FGFR4 (388G/A); IGFB3 (-202A/C); IGFB3 (2133G/C); and Endostatin (G+4349A).

In one embodiment, the invention is a method for determining whether a human gastrointestinal cancer patient is likely responsive to therapy comprising, or alternatively consisting essentially or yet further consisting of, the administration of an anti-VEGF
antibody, pyrimidine based antimetabolite and platinum-based alkylating agent based therapy, by screening a suitable sample isolated from the patient for at least one genetic polymorphism of the group Leptin 5' UTR at nt -2548; MMP7 at nt -181; VEGFR2 at position 4422 CA repeat; CXCR2 at nt 785; MMP7 at nt -181; FGFR4 at nt 388; NFKB at the 5' end CA repeat; AM at the 3' end CA repeat; TF at nt -603; IL-6 at nt 174; IL-8 at nt -251; EGFR at nt 497; or ARNT at Exon 8 G/C, wherein for the genetic polymorphism screened, the presence of at least one genetic polymorphism genotype of the group: (A/A) for Leptin 5' UTR at nt -2548; (G/G or A/G) for MMP7 at nt -181; (10/1 1 or 10/10 CA repeats) for VEGFR2 at position 4422; (C/C or C/T) for CXCR2 at nt 785; (A/G or G/G) for MMP7 at nt -181; (G/G) for FGFR4 at nt 388; (≥1 allele with >24 CA repeats) for NFKB at the 5' end; (2 alleles with >14 CA repeats) for AM at the 3' end; (G/G or G/A) for TF at nt -603; (G/G or G/C) for IL-6 at nt 174; (A/A or AJT) for IL-8 at nt -251; (G/A or A/A) for EGFR at nt 497; or (G/G or G/C) for ARNT at Exon 8, indicates the patient is likely responsive to said therapy.

In one aspect of the above embodiment, the gastrointestinal cancer is a metastatic or non-metastatic gastrointestinal cancer selected from the group consisting of rectal cancer, colorectal cancer, colon cancer, gastric cancer, lung cancer, non-small cell lung cancer and esophageal cancer. In another aspect, the gastrointestinal cancer is colorectal cancer. In yet another aspect, the gastrointestinal cancer is metastatic colorectal cancer.

In a further aspect, negative control polymorphisms may be used to screen or identify the patient sample for a polymorphism that is not associated with response to said therapy, including VEGF (936C/T); NRPI (3' end C/T); CXCR1 (2607G/C); MMP2 (-1306C/T); or MMP9 (-1562C/T).

In one embodiment, the invention is a method for treating a human gastrointestinal cancer patient by administering an effective amount of an anti-VEGF antibody, pyrimidine based antimetabolite and platinum-based alkylating agent based therapy, to a human gastrointestinal cancer patient selected for said therapy based on possession of at least one genetic polymorphism genotype selected from (AJA) for Leptin 5' UTR at nt -2548; (G/G or A/G) for MMP7 at nt -181; (10/1 1 or 10/10 CA repeats) for VEGFR2 at position 4422;
(C/C or C/T) for CXCR2 at nt 785; (A/G or G/G) for MMP7 at nt -181; (G/G) for FGFR4 at nt 388; (>1 allele with >24 CA repeats) for NFKB at the 5' end; (2 alleles with >14 CA repeats) for AM at the 3' end; (G/G or G/A) for TF at nt -603; (G/G or G/C) for IL-6 at nt 174; (A/A or A/T) for IL-8 at nt -25; (G/A or A/A) for EGFR at nt 497 or (G/G or G/C) for ARNT at Exon 8.

In one aspect of the above embodiment, the gastrointestinal cancer is a metastatic or non-metastatic gastrointestinal cancer selected from the group consisting of rectal cancer, colorectal cancer, colon cancer, gastric cancer, lung cancer, non-small cell lung cancer and esophageal cancer. In another aspect, the gastrointestinal cancer is colorectal cancer. In yet another aspect, the gastrointestinal cancer is metastatic colorectal cancer.

In another embodiment, the invention provides for a panel of genetic markers for determining whether a patient is likely responsive to an anti-VEGF antibody, pyrimidine based antimetabolite and platinum-based alkylating agent based therapy, the panel comprising, or alternatively consisting essentially of or yet further consisting of, a group of primers and/or probes that identify a genetic marker selected from Leptin at 5' UTR G-2548A; MMP7 at A-181G; VEGFR2 at position 4422 CA repeat; CXCR2 at nt C785T; MMP7 at nt A-181G; FGFR4 at nt G388A; NFKB at the 5' end CA repeat; AM at the 3' end CA repeat; TF at nt G-603A; IL-6 at nt G174C; IL-8 at nt T-251A; EGFR at nt G497A and ARNT at Exon 8 G/C.

In addition to the above identified genetic markers, the panel may further comprise, consist essentially of or yet further consist of, a primer or probe that identifies a genetic marker selected from VEGF (936C/T); NRPI (3' end C/T); CXCR1 (2607G/C); MMP2 (-1306C/T); or MMP9 (-1562C/T).


Diagnostic Methods

The invention further provides diagnostic methods, which are based, at least in part, on determination of the identity of the polymorphic region of the alleles identified in Tables 1, 2, 3 or 4 above.

For example, information obtained using the diagnostic assays described herein is useful for determining if a subject will respond to cancer treatment of a given type. Based
on the prognostic information, a doctor can recommend a therapeutic protocol, useful for
treating reducing the malignant mass or tumor in the patient or treat cancer in the individual.

In addition, knowledge of the identity of a particular allele in an individual (the gene profile) allows customization of therapy for a particular disease to the individual's genetic profile, the goal of "pharmacogenomics". For example, an individual's genetic profile can enable a doctor: 1) to more effectively prescribe a drug that will address the molecular basis of the disease or condition; 2) to better determine the appropriate dosage of a particular drug and 3) to identify novel targets for drug development. Expression patterns of individual patients can then be compared to the expression profile of the disease to determine the appropriate drug and dose to administer to the patient.

The ability to target populations expected to show the highest clinical benefit, based on the normal or disease genetic profile, can enable: 1) the repositioning of marketed drugs with disappointing market results; 2) the rescue of drug candidates whose clinical development has been discontinued as a result of safety or efficacy limitations, which are patient subgroup-specific; and 3) an accelerated and less costly development for drug candidates and more optimal drug labeling.

Detection of point mutations or additional base pair repeats can be accomplished by molecular cloning of the specified allele and subsequent sequencing of that allele using techniques known in the art. Alternatively, the gene sequences can be amplified directly from a genomic DNA preparation from the tumor tissue using PCR, and the sequence composition is determined from the amplified product. As described more fully below, numerous methods are available for analyzing a subject's DNA for mutations at a given genetic locus such as the gene of interest.

A detection method is allele specific hybridization using probes overlapping the polymorphic site and having about 5, or alternatively 10, or alternatively 20, or alternatively 25, or alternatively 30 nucleotides around the polymorphic region. In another embodiment of the invention, several probes capable of hybridizing specifically to the allelic variant are attached to a solid phase support, e.g., a "chip". Oligonucleotides can be bound to a solid support by a variety of processes, including lithography. For example a chip can hold up to
250,000 oligonucleotides (GeneChip, Affymetrix). Mutation detection analysis using these chips comprising oligonucleotides, also termed "DNA probe arrays" is described e.g., in Cronin et al. (1996) Human Mutation 7:244.

In other detection methods, it is necessary to first amplify at least a portion of the gene of interest prior to identifying the allelic variant. Amplification can be performed, e.g., by PCR and/or LCR, according to methods known in the art. In one embodiment, genomic DNA of a cell is exposed to two PCR primers and amplification for a number of cycles sufficient to produce the required amount of amplified DNA.

Alternative amplification methods include: self sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh et al. (1989) Proc. Natl. Acad. Sci. USA 86:173-1 177), Q-Beta Replicase (Lizardi et al. (1988) Bio/Technology 6:1 197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques known to those of skill in the art. These detection schemes are useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In one embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence at least a portion of the gene of interest and detect allelic variants, e.g., mutations, by comparing the sequence of the sample sequence with the corresponding wild-type (control) sequence. Exemplary sequencing reactions include those based on techniques developed by Maxam and Gilbert (1977) Proc. Natl. Acad. Sci, USA 74:560) or Sanger et al. (1977) Proc. Nat. Acad. Sci, 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the subject assays (Biotechniques (1995) 19:448), including sequencing by mass spectrometry (see, for example, U.S. Patent No. 5,547,835 and International Patent Application Publication Number WO 94/16101, entitled DNA Sequencing by Mass Spectrometry by Koster; U.S. Patent No. 5,547,835 and international patent application Publication Number WO 94/21822 entitled "DNA Sequencing by Mass Spectrometry Via Exonuclease Degradation" by Koster; U.S. Patent No. 5,605,798 and International Patent Application No. PCT/US96/03651 entitled DNA Diagnostics Based on Mass Spectrometry by Koster;
Cohen et al. (1996) Adv. Chromat. 36:127-162; and Griffin et al. (1993) Appl. Biochem. Bio. 38:147-159). It will be evident to one skilled in the art that, for certain embodiments, the occurrence of only one, two or three of the nucleic acid bases need be determined in the sequencing reaction. For instance, A-track or the like, e.g., where only one nucleotide is detected, can be carried out.

Yet other sequencing methods are disclosed, e.g., in U.S. Patent No. 5,580,732 entitled "Method of DNA Sequencing Employing A Mixed DNA-Polymer Chain Probe" and U.S. Patent No. 5,571,676 entitled "Method For Mismatch-Directed In Vitro DNA Sequencing."

In some cases, the presence of the specific allele in DNA from a subject can be shown by restriction enzyme analysis. For example, the specific nucleotide polymorphism can result in a nucleotide sequence comprising a restriction site which is absent from the nucleotide sequence of another allelic variant.

In a further embodiment, protection from cleavage agents (such as a nuclease, hydroxylamine or osmium tetroxide and with piperidine) can be used to detect mismatched bases in RNA/RNA DNA/DNA, or RNA/DNA heteroduplexes (see, e.g., Myers et al. (1985) Science 230:1242). In general, the technique of "mismatch cleavage" starts by providing heteroduplexes formed by hybridizing a control nucleic acid, which is optionally labeled, e.g., RNA or DNA, comprising a nucleotide sequence of the allelic variant of the gene of interest with a sample nucleic acid, e.g., RNA or DNA, obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as duplexes formed based on basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1nuclease to enzymatically digest the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine whether the control and sample nucleic acids have an identical nucleotide sequence or in which nucleotides they
are different. See, for example, U.S. Patent No. 6,455,249; Cotton et al. (1988) Proc. Natl. Acad. Sci. USA 85:4397; Saleeba et al. (1992) Methods Enzy. 217:286-295. In another embodiment, the control or sample nucleic acid is labeled for detection.

In other embodiments, alterations in electrophoretic mobility are used to identify the particular allelic variant. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al. (1989) Proc. Natl. Acad. Sci USA 86:2766; Cotton (1993) Mutat. Res. 285:125-144 and Hayashi (1992) Genet Anal Tech. Appl. 9:73-79). Single-stranded DNA fragments of sample and control nucleic acids are denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In another preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) Trends Genet. 7:5).

In yet another embodiment, the identity of the allelic variant is obtained by analyzing the movement of a nucleic acid comprising the polymorphic region in polyacrylamide gels containing a gradient of denaturant, which is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers et al. (1985) Nature 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing agent gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) Biophys. Chem. 265:1275).

Examples of techniques for detecting differences of at least one nucleotide between 2 nucleic acids include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide probes
may be prepared in which the known polymorphic nucleotide is placed centrally (allele-specific probes) and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) Nature 324:163); Saiki et al. (1989) Proc. Natl. Acad. Sci. USA 86:6230 and Wallace et al. (1979) Nucl. Acids Res. 6:3543. Such allele specific oligonucleotide hybridization techniques may be used for the detection of the nucleotide changes in the polymorphic region of the gene of interest. For example, oligonucleotides having the nucleotide sequence of the specific allelic variant are attached to a hybridizing membrane and this membrane is then hybridized with labeled sample nucleic acid. Analysis of the hybridization signal will then reveal the identity of the nucleotides of the sample nucleic acid.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the allelic variant of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al. (1989) Nucleic Acids Res. 17:2437-2448) or at the extreme 3’ end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) Tibtech 11:238 and Newton et al. (1989) Nucl. Acids Res. 17:2503). This technique is also termed "PROBE" for Probe Oligo Base Extension. In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al. (1992) Mol. Cell Probes 6:1).

In another embodiment, identification of the allelic variant is carried out using an oligonucleotide ligation assay (OLA), as described, e.g., in U.S. Patent No. 4,998,617 and in Landegren et al. (1988) Science 241:1077-1080. The OLA protocol uses two oligonucleotides which are designed to be capable of hybridizing to abutting sequences of a single strand of a target. One of the oligonucleotides is linked to a separation marker, e.g., biotinylated, and the other is detectably labeled. If the precise complementary sequence is found in a target molecule, the oligonucleotides will hybridize such that their termini abut, and create a ligation substrate. Ligation then permits the labeled oligonucleotide to be recovered using avidin, or another biotin ligand. Nickerson et al. have described a nucleic acid detection assay that combines attributes of PCR and OLA (Nickerson et al. (1990)
In this method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA.

Several techniques based on this OLA method have been developed and can be used to detect the specific allelic variant of the polymorphic region of the gene of interest. For example, U.S. Patent No. 5,593,826 discloses an OLA using an oligonucleotide having 3'-amino group and a 5'-phosphorylated oligonucleotide to form a conjugate having a phosphoramidate linkage. In another variation of OLA described in Tobe et al. (1996) Nucleic Acids Res. 24:3728, OLA combined with PCR permits typing of two alleles in a single microtiter well. By marking each of the allele-specific primers with a unique hapten, i.e. digoxigenin and fluorescein, each OLA reaction can be detected by using hapten specific antibodies that are labeled with different enzyme reporters, alkaline phosphatase or horseradish peroxidase. This system permits the detection of the two alleles using a high throughput format that leads to the production of two different colors.

The invention further provides methods for detecting the single nucleotide polymorphism in the gene of interest. Because single nucleotide polymorphisms constitute sites of variation flanked by regions of invariant sequence, their analysis requires no more than the determination of the identity of the single nucleotide present at the site of variation and it is unnecessary to determine a complete gene sequence for each patient. Several methods have been developed to facilitate the analysis of such single nucleotide polymorphisms.

In one embodiment, the single base polymorphism can be detected by using a specialized exonuclease-resistant nucleotide, as disclosed, e.g., in Mundy (U.S. Patent No. 4,656,127). According to the method, a primer complementary to the allelic sequence immediately 3’ to the polymorphic site is permitted to hybridize to a target molecule obtained from a particular animal or human. If the polymorphic site on the target molecule contains a nucleotide that is complementary to the particular exonuclease-resistant nucleotide derivative present, then that derivative will be incorporated onto the end of the hybridized primer. Such incorporation renders the primer resistant to exonuclease, and thereby permits its detection. Since the identity of the exonuclease-resistant derivative of
the sample is known, a finding that the primer has become resistant to exonucleases reveals
that the nucleotide present in the polymorphic site of the target molecule was
complementary to that of the nucleotide derivative used in the reaction. This method has
the advantage that it does not require the determination of large amounts of extraneous
sequence data.

In another embodiment of the invention, a solution-based method is used for
determining the identity of the nucleotide of the polymorphic site. Cohen et al. (French
No. 4,656,127, a primer is employed that is complementary to allelic sequences
immediately 3’ to a polymorphic site. The method determines the identity of the nucleotide
of that site using labeled dideoxynucleotide derivatives, which, if complementary to the
nucleotide of the polymorphic site will become incorporated onto the terminus of the
primer.

An alternative method, known as Genetic Bit Analysis or GBA™ is described by
Goelet et al. (PCT Appln. No. 92/15712). This method uses mixtures of labeled terminators
and a primer that is complementary to the sequence 3’ to a polymorphic site. The labeled
terminator that is incorporated is thus determined by, and complementary to, the nucleotide
present in the polymorphic site of the target molecule being evaluated. In contrast to the
method of Cohen et al. (French Patent 2,650,840; PCT Appln. No. WO91/02087) the
method of Goelet et al. supra, is preferably a heterogeneous phase assay, in which the
primer or the target molecule is immobilized to a solid phase.

Recently, several primer-guided nucleotide incorporation procedures for assaying
polymorphic sites in DNA have been described (Komher et al. (1989) Nucl. Acids. Res.
17:7779-7784; Sokolov (1990) Nucl. Acids Res. 18:3671; Syvanen et al. (1990) Genomics
Prezant et al. (1992) Hum. Mutat. 1:159-164; Ugozzoli et al. (1992) GATA 9:107-1 12;
Nyren et al. (1993) Anal. Biochem. 208:171-175). These methods differ from GBA™ in
that they all rely on the incorporation of labeled deoxynucleotides to discriminate between
bases at a polymorphic site. In such a format, since the signal is proportional to the number
of deoxynucleotides incorporated, polymorphisms that occur in runs of the same nucleotide can result in signals that are proportional to the length of the run (Syvanen et al. (1993) Amer. J. Hum. Genet. 52:46-59).

If the polymorphic region is located in the coding region of the gene of interest, yet other methods than those described above can be used for determining the identity of the allelic variant. For example, identification of the allelic variant, which encodes a mutated signal peptide, can be performed by using an antibody specifically recognizing the mutant protein in, e.g., immunohistochemistry or immunoprecipitation. Antibodies to the wild-type or signal peptide mutated forms of the signal peptide proteins can be prepared according to methods known in the art.

Antibodies directed against wild type or mutant peptides encoded by the allelic variants of the gene of interest may also be used in disease diagnostics and prognostics. Such diagnostic methods, may be used to detect abnormalities in the level of expression of the peptide, or abnormalities in the structure and/or tissue, cellular, or subcellular location of the peptide. Protein from the tissue or cell type to be analyzed may easily be detected or isolated using techniques which are well known to one of skill in the art, including but not limited to Western blot analysis. For a detailed explanation of methods for carrying out Western blot analysis, see Sambrook and Russell (2001) supra. The protein detection and isolation methods employed herein can also be such as those described in Harlow and Lane, (1999) supra. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody (see below) coupled with light microscopic, flow cytometric, or fluorimetric detection. The antibodies (or fragments thereof) useful in the present invention may, additionally, be employed histologically, as in immunofluorescence or immunoelectron microscopy, for in situ detection of the peptides or their allelic variants. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody of the present invention. The antibody (or fragment) is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the subject polypeptide, but also its distribution in the examined tissue. Using the present invention, one of ordinary skill will readily perceive
that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

In one aspect the invention provided for a panel of genetic markers selected from, but not limited to the genetic polymorphisms above. The panel comprises probes or primers that can be used to amplify and/or for determining the molecular structure of the polymorphisms identified above. The probes or primers can be attached or supported by a solid phase support such as, but not limited to a gene chip or microarray. The probes or primers can be detectably labeled. This aspect of the invention is a means to identify the genotype of a patient sample for the genes of interest identified above. In one aspect, the methods of the invention provided for a means of using the panel to identify or screen patient samples for the presence of the genetic marker identified herein. In one aspect, the various types of panels provided by the invention include, but are not limited to, those described herein. In one aspect, the panel contains the above identified probes or primers as wells as other, probes or primers. In an alternative aspect, the panel includes one or more of the above noted probes or primers and others. In a further aspect, the panel consist only of the above-noted probes or primers.

Often a solid phase support or carrier is used as a support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. or alternatively polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.
Moreover, it will be understood that any of the above methods for detecting alterations in a gene or gene product or polymorphic variants can be used to monitor the course of treatment or therapy.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits, such as those described below, comprising at least one probe or primer nucleic acid described herein, which may be conveniently used, e.g., to determine whether a subject has or is at risk of developing disease such as colorectal cancer.

Sample nucleic acid for use in the above-described diagnostic and prognostic methods can be obtained from any cell type or tissue of a subject. For example, a subject's bodily fluid (e.g. blood) can be obtained by known techniques (e.g., venipuncture). Alternatively, nucleic acid tests can be performed on dry samples (e.g., hair or skin). Fetal nucleic acid samples can be obtained from maternal blood as described in International Patent Application No. WO 91/07660 to Bianchi. Alternatively, amniocytes or chorionic villi can be obtained for performing prenatal testing.

Diagnostic procedures can also be performed in situ directly upon tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections, such that no nucleic acid purification is necessary. Nucleic acid reagents can be used as probes and/or primers for such in situ procedures (see, for example, Nuovo (1992) PCR IN SITU HYBRIDIZATION: PROTOCOLS AND APPLICATIONS, Raven Press, NY).

In addition to methods which focus primarily on the detection of one nucleic acid sequence, profiles can also be assessed in such detection schemes. Fingerprint profiles can be generated, for example, by utilizing a differential display procedure, Northern analysis and/or RT-PCR.

The invention described herein relates to methods and compositions for determining and identifying the allele present at the gene of interest's locus. This information is useful to diagnose and prognose disease progression as well as select the most effective treatment among treatment options. Probes can be used to directly determine the genotype of the sample or can be used simultaneously with or subsequent to amplification. The term
"probes" includes naturally occurring or recombinant single- or double-stranded nucleic acids or chemically synthesized nucleic acids. They may be labeled by nick translation, Klenow fill-in reaction, PCR or other methods known in the art. Probes of the present invention, their preparation and/or labeling are described in Sambrook and Russell (2001) supra. A probe can be a polynucleotide of any length suitable for selective hybridization to a nucleic acid containing a polymorphic region of the invention. Length of the probe used will depend, in part, on the nature of the assay used and the hybridization conditions employed.

In one embodiment of the invention, probes are labeled with two fluorescent dye molecules to form so-called "molecular beacons" (Tyagi and Kramer (1996) Nat. Biotechnol. 14:303-8). Such molecular beacons signal binding to a complementary nucleic acid sequence through relief of intramolecular fluorescence quenching between dyes bound to opposing ends on an oligonucleotide probe. The use of molecular beacons for genotyping has been described (Kostrikis (1998) Science 279:1228-9) as has the use of multiple beacons simultaneously (Marras (1999) Genet. Anal. 14:151-6). A quenching molecule is useful with a particular fluorophore if it has sufficient spectral overlap to substantially inhibit fluorescence of the fluorophore when the two are held proximal to one another, such as in a molecular beacon, or when attached to the ends of an oligonucleotide probe from about 1 to about 25 nucleotides.

Labeled probes also can be used in conjunction with amplification of a polymorphism. (Holland et al. (1991) Proc. Natl. Acad. Sci. 88:7276-7280). U.S. Patent No. 5,210,015 by Gelfand et al. describe fluorescence-based approaches to provide real time measurements of amplification products during PCR. Such approaches have either employed intercalating dyes (such as ethidium bromide) to indicate the amount of double-stranded DNA present, or they have employed probes containing fluorescence-quencher pairs (also referred to as the "Taq-Man" approach) where the probe is cleaved during amplification to release a fluorescent molecule whose concentration is proportional to the amount of double-stranded DNA present. During amplification, the probe is digested by the nuclease activity of a polymerase when hybridized to the target sequence to cause the fluorescent molecule to be separated from the quencher molecule, thereby causing
fluorescence from the reporter molecule to appear. The Taq-Man approach uses a probe containing a reporter molecule—quencher molecule pair that specifically anneals to a region of a target polynucleotide containing the polymorphism.

Primers and/or probes can be affixed to surfaces or supports for use as "gene chips" or "microarray." Such gene chips or microarrays can be used to detect genetic variations by a number of techniques known to one of skill in the art. In one technique, oligonucleotides are arrayed on a gene chip for determining the DNA sequence of a by the sequencing by hybridization approach, such as that outlined in U.S. Patent Nos. 6,025,136 and 6,018,041. The probes of the invention also can be used for fluorescent detection of a genetic sequence. Such techniques have been described, for example, in U.S. Patent Nos. 5,968,740 and 5,858,659. A probe also can be affixed to an electrode surface for the electrochemical detection of nucleic acid sequences such as described by Kayem et al. U.S. Patent No. 5,952,172 and by Kelley et al. (1999) Nucleic Acids Res. 27:4830-4837.

Various "gene chips" or "microarray" and similar technologies are known in the art. Examples of such include, but are not limited to LabCard (ACLARA Bio Sciences Inc.); GeneChip (Affymetric, Inc); LabChip (Caliper Technologies Corp); a low-density array with electrochemical sensing (Clinical Micro Sensors); LabCD System (Gamera Bioscience Corp.); Omni Grid (Gene Machines); Q Array (Genetix Ltd.); a high-throughput, automated mass spectrometry systems with liquid-phase expression technology (Gene Trace Systems, Inc.); a thermal jet spotting system (Hewlett Packard Company); Hyseq HyChip (Hyseq, Inc.); BeadArray (Illumina, Inc.); GEM (Incyte Microarray Systems); a high-throughput microarraying system that can dispense from 12 to 64 spots onto multiple glass slides (Intelligent Bio-Instruments); Molecular Biology Workstation and NanoChip (Nanogen, Inc.); a microfluidic glass chip (Orchid biosciences, Inc.); BioChip Arrayer with four PiezoTip piezoelectric drop-on-demand tips (Packard Instruments, Inc.); FlexJet (Rosetta Inpharmatic, Inc.); MALDI-TOF mass spectrometer (Sequnome); ChipMaker 2 and ChipMaker 3 (TeleChem International, Inc.); and GenoSensor (Vysis, Inc.) as identified and described in Heller (2002) Annu. Rev. Biomed. Eng. 4:129-153. Examples of "Gene chips" or a "microarray" are also described in US Patent Publ. Nos.: 2007-01 11322, 2007-
In one aspect, "gene chips" or "microarrays" containing probes or primers of Table 1 alone or in combination with other genetic polymorphisms or markers are prepared. A suitable sample is obtained from the patient extraction of genomic DNA, RNA, or any combination thereof and amplified if necessary. The DNA or RNA sample is contacted to the gene chip or microarray panel under conditions suitable for hybridization of the gene(s) of interest to the probe(s) or primer(s) contained on the gene chip or microarray. The probes or primers may be detectably labeled thereby identifying the polymorphism in the gene(s) of interest. Alternatively, a chemical or biological reaction may be used to identify the probes or primers which hybridized with the DNA or RNA of the gene(s) of interest. The genotypes of the patient is then determined with the aid of the aforementioned apparatus and methods.

Nucleic Acids

In one aspect, the nucleic acid sequences of the gene's allelic variants, or portions thereof, can be the basis for probes or primers, e.g., in methods for determining the identity of the allelic variant of the IL-8 -251 A/T polymorphic region. Thus, they can be used in the methods of the invention to determine which therapy is most likely to treat an individual's cancer.

The methods of the invention can use nucleic acids isolated from vertebrates. In one aspect, the vertebrate nucleic acids are mammalian nucleic acids. In a further aspect, the nucleic acids used in the methods of the invention are human nucleic acids.

Primers for use in the methods of the invention are nucleic acids which hybridize to a nucleic acid sequence which is adjacent to the region of interest or which covers the region of interest and is extended. A primer can be used alone in a detection method, or a primer can be used together with at least one other primer or probe in a detection method. Primers can also be used to amplify at least a portion of a nucleic acid. Probes for use in the methods of the invention are nucleic acids which hybridize to the region of interest and
which are not further extended. For example, a probe is a nucleic acid which hybridizes to the polymorphic region of the gene of interest, and which by hybridization or absence of hybridization to the DNA of a subject will be indicative of the identity of the allelic variant of the polymorphic region of the gene of interest. Primers useful in the methods described herein are found in Table 5.
Table 5 - Primer Sequences, Annealing Temperatures and Enzymes for Determining Polymorphisms.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer (5'-3')</th>
<th>Reverse Primer (5'-3')</th>
<th>Enzyme</th>
<th>Annealing</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>AAGAGGCTGAGTCAGAAGGATGG</td>
<td>GCAACATCATTTAATATCCTGCACAG</td>
<td>$^{32}$P-$\gamma$ATP End-labeled</td>
<td>60°</td>
</tr>
<tr>
<td>3' end CA repeat</td>
<td>ACAGCGAGGGTTGTTGATGTA</td>
<td>CACCTGTCAGGGCATTTGCT</td>
<td>sequencing</td>
<td>60°</td>
</tr>
<tr>
<td>AKN1 Exon 8 G/C</td>
<td>GTTTGAAATTTAAGATGTTTTGAT</td>
<td>TTTCAAAATATTGTTCATTG</td>
<td>BclI</td>
<td>53°</td>
</tr>
<tr>
<td>COX2 8437T/C</td>
<td>CTCATGAGGACCCAGGGATG</td>
<td>GGTTGAGGCACGTATGGGA</td>
<td>AluI</td>
<td>60°</td>
</tr>
<tr>
<td>CXCR1 2607G/C</td>
<td>CATTTTGCTGCTGTCCTCA</td>
<td>CTGTAAGGATGCCCCAGAT</td>
<td>sequencing</td>
<td>60°</td>
</tr>
<tr>
<td>CXCR2 785C/T</td>
<td>TGTCACATAAGGGAAGGA</td>
<td>TGCACAGAAGGGTTAACGCCC</td>
<td>AluI</td>
<td>60°</td>
</tr>
<tr>
<td>EGFR 61A/G</td>
<td>CACCGTTTCTTCTCCAGGAC</td>
<td>CTTGAGAGGATTGCTGCACAG</td>
<td>MseI</td>
<td>60°</td>
</tr>
<tr>
<td>EGFR 497G/A</td>
<td>TGCTGTGACCCACTCCTGTCT</td>
<td>CCAGAGGTTGACACTTGTCC</td>
<td>Bst-NI</td>
<td>59°</td>
</tr>
<tr>
<td>Endostatin G+4349A</td>
<td>CACGTTTCTTCTCCAGGAC</td>
<td>CTC TCAGAGCGTCTACAG</td>
<td>MseI</td>
<td>60°</td>
</tr>
<tr>
<td>FGFR4 388G/A</td>
<td>GACCGCAGCAGCGGCCAGGGCAG</td>
<td>AGAGGAGAGGGAGAGGCTCTG</td>
<td>BstN1</td>
<td>68°</td>
</tr>
<tr>
<td>GLUT1-2841A/T</td>
<td>GCTGAGAATGGGCTTCCCTCAAT</td>
<td>GTCTGCTTTACTCAAGCCATGGTC</td>
<td>HpyCH4V</td>
<td>60°</td>
</tr>
<tr>
<td>HIF1a 1772C/T</td>
<td>CCAATGGATGATGACCTC</td>
<td>AGTGGTGGCATAGCAGTGGG</td>
<td>HphI</td>
<td>60°</td>
</tr>
<tr>
<td>ICAM1 K469E</td>
<td>CCATCGGGGAATCGTG</td>
<td>ACCACTCTGGAGAAGGGTA</td>
<td>$^{32}$P-$\gamma$ATP End-labeled</td>
<td>60°</td>
</tr>
<tr>
<td>IGFB1 5A repeat</td>
<td>GCTGAGCAGCAGCTGGTATT</td>
<td>ACCACTCTGGAGAAGGGTA</td>
<td>MspI</td>
<td>60°</td>
</tr>
<tr>
<td>IGF2 3380G/A</td>
<td>CCACCCCTTCTGGAAGCTAAAAG</td>
<td>CCCCTCGTCTCCAGGAAAGGCA</td>
<td>MspI</td>
<td>60°</td>
</tr>
<tr>
<td>Gene</td>
<td>Forward Primer (5'-3')</td>
<td>Reverse Primer (5'-3')</td>
<td>Enzyme</td>
<td>Annealing</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------</td>
<td>------------------------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td>IGFB3</td>
<td>CCACGAGTGACACACGAATG</td>
<td>AGCCGCAACTGCTGACATCTGG</td>
<td>BsiHKA1</td>
<td>68°</td>
</tr>
<tr>
<td>IGFB3</td>
<td>TGCAGGCGTGCTGACG</td>
<td>CAGCTCGGCACAC</td>
<td>Acil</td>
<td>68°</td>
</tr>
<tr>
<td>IGFr1</td>
<td>CAGGGGTGTTTGGATGTC</td>
<td>CCTGTGCTGATTTTGGCTTTTC</td>
<td>Mn11</td>
<td>60°</td>
</tr>
<tr>
<td>IL1b</td>
<td>TGCCATTGATCTGGTCTAC</td>
<td>GTTTAGGAATCTTCCCACCTT</td>
<td>Aval</td>
<td>60°</td>
</tr>
<tr>
<td>IL1b</td>
<td>GTTGTACAGCGAGCTTTGACC</td>
<td>TTCAGTTATATGGACAGA</td>
<td>Tag1</td>
<td>60°</td>
</tr>
<tr>
<td>IL1Ra</td>
<td>TCAGCAACACTCTCTAT</td>
<td>TCCTGTTCTGAGGTA</td>
<td>n/a</td>
<td>60°</td>
</tr>
<tr>
<td>IL-6</td>
<td>GCCTCAATGACGAC</td>
<td>TCATGGGAAATCC</td>
<td>Nla3</td>
<td>55°</td>
</tr>
<tr>
<td>IL-8</td>
<td>TTGTCTAACACCTGGCATTCT</td>
<td>GGCAAACTCAGTTTCACA</td>
<td>Mfe1</td>
<td>60°</td>
</tr>
<tr>
<td>LDH</td>
<td>GAGATGGGTGATTTTTTATTTTCT</td>
<td>TGGCCCATGACTTTTTATAATCTT</td>
<td>sequencing</td>
<td>60°</td>
</tr>
<tr>
<td>LDH</td>
<td>GAGATGGGTGATTTTTTATTTTCT</td>
<td>TGGCCCATGACTTTTTATAATCTT</td>
<td>sequencing</td>
<td>60°</td>
</tr>
<tr>
<td>Leptin</td>
<td>GCAGGAGTTTGATTGGTTAG</td>
<td>GAGTTCTCAACATGCCCACCTG</td>
<td>MspAI1</td>
<td>60°</td>
</tr>
<tr>
<td>MDM2</td>
<td>CGCAGGGAGTTTCTAGGTAAG</td>
<td>CTGAGTCAACCTGCCCACCTG</td>
<td>MspAI1</td>
<td>60°</td>
</tr>
<tr>
<td>MMP7</td>
<td>CAGGTATGACCATACTGAGAGCAGTC</td>
<td>AGAGTCTCACAGAATCTGAAAGATGTGTTATT</td>
<td>sequencing</td>
<td>60°</td>
</tr>
<tr>
<td>MMP2</td>
<td>CTGGGGCGTCTGATGTTTCT</td>
<td>AGGCTTCTGGAAGAAGTGCATTCT</td>
<td>sequencing</td>
<td>60°</td>
</tr>
<tr>
<td>MMP9</td>
<td>GCCCTGGCACATAGTGGGCC</td>
<td>CTTCTAGCCAGCGGCCATC</td>
<td>Sph1</td>
<td>65°</td>
</tr>
<tr>
<td>Gene</td>
<td>Forward-Primer (5'-3')</td>
<td>Reverse-Primer (5'-3')</td>
<td>Enzyme</td>
<td>Annealing</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------------</td>
<td>---------------------------</td>
<td>-----------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NFKB CA repeat in regulatory region (a/k/a 5' end CA repeats)</td>
<td>CTTCAGTATCTAAGAGTATCCT</td>
<td>CAAGTAAGACTCTACGGAGTC</td>
<td>$^{32}$P-$\gamma$ATP End-labeled</td>
<td>60°</td>
</tr>
<tr>
<td>NRP -2548G/A</td>
<td>AGCTTTGAGTGTTTTTGGTG</td>
<td>CCTGGAACCAAAAGGCATTTC</td>
<td>sequencing</td>
<td>60°</td>
</tr>
<tr>
<td>NRP1 3' end C/T</td>
<td>AGCTTTGAGTGTTTTTGGTG</td>
<td>CCTGGAACCAAAAGGCATTTC</td>
<td>sequencing</td>
<td>60°</td>
</tr>
<tr>
<td>p53 codon 72 Pro-Arg C/G</td>
<td>ATCTACAGTCCCCCTGGCG</td>
<td>GCAACTGACGCTGCAAGTCA</td>
<td>BstUl</td>
<td>60°</td>
</tr>
<tr>
<td>PLGF 3'UTR G/A</td>
<td>CGTGATCTCCCCCTCACACTT</td>
<td>CCACTGGCTGAGCTGTCTTCT</td>
<td>BsiE1</td>
<td>60°</td>
</tr>
<tr>
<td>PLGF 3'UTR T/A</td>
<td>GCATCCCTACTTTTGGACAGG</td>
<td>CGCTTTGAAAGAAGCAAGACA</td>
<td>HpaCH111</td>
<td>60°</td>
</tr>
<tr>
<td>SDF1/CXCL12 -801G/A</td>
<td>CAGTCACACCTG GCAAGGCC</td>
<td>AGCTTTGCTGCTGAGACTCC</td>
<td>MspI</td>
<td>60°</td>
</tr>
<tr>
<td>TF -603A/G</td>
<td>AGTCATATCTCTGTGCCTA</td>
<td>CTTCCCTTTCCATTTGGGTGAT</td>
<td>BstN1</td>
<td>60°</td>
</tr>
<tr>
<td>TNFα -308G/A</td>
<td>TGGAGGCAATAGGTGTGAGGAGGAGA</td>
<td>TAGGAACCTGGAGGCTGAACCCGTACCC</td>
<td>NcoI</td>
<td>60°</td>
</tr>
<tr>
<td>VEGF C+936</td>
<td>AAGGAAGAGGAGACT CTGCGCAAGGC</td>
<td>TAAATGTATGTATGTTGCTGTTGCTACAGG</td>
<td>Nla III</td>
<td>60°</td>
</tr>
<tr>
<td>VEGF G405C</td>
<td>ACTTCCCCAATACCTGTGG</td>
<td>GTCACACTTTGGCCCTGT</td>
<td>sequencing</td>
<td>60°</td>
</tr>
<tr>
<td>VEGFR2 4422 CA repeats</td>
<td>GCTTGTAGTAAATTGTCATAAGTGG</td>
<td>GAG C GT ATG TCT ACT ATA CGC CA</td>
<td>$^{32}$P-$\gamma$ATP End-labeled</td>
<td>60°</td>
</tr>
<tr>
<td>Gene</td>
<td>Forward Primer (5'→3')</td>
<td>Reverse Primer (5'→3')</td>
<td>Enzyme</td>
<td>Annealing</td>
</tr>
<tr>
<td>----------</td>
<td>------------------------</td>
<td>------------------------</td>
<td>--------</td>
<td>-----------</td>
</tr>
<tr>
<td>VEGFR2</td>
<td>GCTTGTAGTAAATTGTCAAGTG</td>
<td>GAG CGT ATG TCT ACT ATA CGC CA</td>
<td>sequencing</td>
<td>60°</td>
</tr>
<tr>
<td>+1416T/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGFR2</td>
<td>TGCTCCCTGGAAGCTCTCC</td>
<td>GGCTGCGTGGGAAATTTATTT</td>
<td>AluI</td>
<td>60°</td>
</tr>
<tr>
<td>117/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In one embodiment, primers comprise a nucleotide sequence which comprises a region having a nucleotide sequence which hybridizes under stringent conditions to about: 6, or alternatively 8, or alternatively 10, or alternatively 12, or alternatively 25, or alternatively 30, or alternatively 40, or alternatively 50, or alternatively 75 consecutive nucleotides of the gene of interest.

Primers can be complementary to nucleotide sequences located close to each other or further apart, depending on the use of the amplified DNA. For example, primers can be chosen such that they amplify DNA fragments of at least about 10 nucleotides or as much as several kilobases. Preferably, the primers of the invention will hybridize selectively to nucleotide sequences located about 150 to about 350 nucleotides apart.

For amplifying at least a portion of a nucleic acid, a forward primer (i.e., 5' primer) and a reverse primer (i.e., 3' primer) will preferably be used. Forward and reverse primers hybridize to complementary strands of a double stranded nucleic acid, such that upon extension from each primer, a double stranded nucleic acid is amplified.

Yet other preferred primers of the invention are nucleic acids which are capable of selectively hybridizing to an allelic variant of a polymorphic region of the gene of interest. The hybridization may be carried out under conditions of moderate or high stringency. Thus, such primers can be specific for the gene of interest sequence, so long as they have a nucleotide sequence which is capable of hybridizing to the gene of interest.

The probe or primer may further comprises a label attached thereto, which, e.g., is capable of being detected, e.g. the label group is selected from amongst radioisotopes, fluorescent compounds, enzymes, and enzyme co-factors.

Additionally, the isolated nucleic acids used as probes or primers may be modified to become more stable. Exemplary nucleic acid molecules which are modified include phosphoramidate, phosphothioate and methylphosphonate analogs of DNA (see also U.S. Patent Nos. 5,176,996; 5,264,564 and 5,256,775).

The nucleic acids used in the methods of the invention can also be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the
molecule. The nucleic acids, e.g., probes or primers, may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane. See, e.g., Letsinger et al. (1989) Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al. (1987) Proc. Natl. Acad. Sci. 84:648-652; and PCT Publication No. WO 88/09810, published Dec. 15, 1988), hybridization-triggered cleavage agents, (see, e.g., Krol et al. (1988) BioTechniques 6:958-976) or intercalating agents (see, e.g., Zon (1988) Pharm. Res. 5:539-549. To this end, the nucleic acid used in the methods of the invention may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The isolated nucleic acids used in the methods of the invention can also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose or, alternatively, comprise at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphothriester, and a formacetal or analog thereof.

The nucleic acids, or fragments thereof, to be used in the methods of the invention can be prepared according to methods known in the art and described, e.g., in Sambrook and Russell (2001) supra. For example, discrete fragments of the DNA can be prepared and cloned using restriction enzymes. Alternatively, discrete fragments can be prepared using the Polymerase Chain Reaction (PCR) using primers having an appropriate sequence under the manufacturer's conditions, (described above).

Oligonucleotides can be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein et al. (1988) Nucl. Acids Res. 16:3209, methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports. Sarin et al. (1988) Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451.
Methods of Treatment

The invention further provides methods of treating subjects having solid malignant or non-malignant tissue mass or tumor selected from rectal cancer, colorectal cancer, (including metastatic CRC), colon cancer, gastric cancer, lung cancer (including non-small cell lung cancer) and esophageal cancer. In one embodiment, the method comprises (a) determining the identity of the allelic variant as identified herein; and (b) administering to the subject an effective amount of a compound or therapy (e.g., anti-angiogenic antibody, mimetic or biological equivalent thereof). This therapy can be combined with other suitable therapies or treatments.

The chemotherapies and antibodies, other anti-angiogenic and/or fab fragment compositions or equivalent are administered or delivered in an amount effective to treat the cancer and by any suitable means and with any suitable formulation as a composition and therefore includes a carrier such as a pharmaceutically acceptable carrier. Accordingly, a formulation comprising an antibody or biological equivalent thereof is further provided herein. The formulation can further comprise one or more preservatives or stabilizers. Any suitable concentration or mixture can be used as known in the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, OA, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no preservative, 0.1-2% m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (e.g., 0.005, 0.01), 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, and 1.0%).

The chemotherapies, antibodies or biological equivalents thereof can be administered as a composition. A "composition" typically intends a combination of the active agent and another carrier, e.g., compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like and include pharmaceutically acceptable carriers. Carriers also include pharmaceutical excipients and additives proteins,
peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. Carbohydrate excipients are also intended within the scope of this invention, examples of which include but are not limited to monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltooligosaccharides, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltotol, lactitol, xylitol sorbitol (glucitol) and myoinositol.

The term carrier further includes a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, acetic acid, lactobionic acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Additional carriers include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrans (e.g., cyclodextrins, such as 2-hydroxypropyl-quadrature-cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as "TWEEN 20" and "TWEEN 80"), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

As used herein, the term "pharmacologically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives and any of the above noted carriers with the additional proviso that they be acceptable for use in vivo. For examples of carriers, stabilizers and adjuvants, see Martin REMINGTON'S PHARM. SCI.

An "effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages.

The invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one antibody or its biological equivalent with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. The invention further comprises an article of manufacture, comprising packaging material, a first vial comprising at least one lyophilized antibody or its biological equivalent and a second vial comprising an aqueous diluent of prescribed buffer or preservative, wherein said packaging material comprises a label that instructs a patient to reconstitute the therapeutic in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

The antibody or equivalent thereof is prepared to a concentration includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0 µg/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

The formulations of the present invention can be prepared by a process which comprises mixing at least one antibody or biological equivalent and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal or mixtures thereof in an aqueous diluent. Mixing of the antibody and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. For example, a measured amount of at least one antibody in buffered solution is combined with the desired
preservative in a buffered solution in quantities sufficient to provide the antibody and preservative at the desired concentrations. Variations of this process would be recognized by one of skill in the art, e.g., the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The compositions and formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized antibody that is reconstituted with a second vial containing the aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available. Recognized devices comprising these single vial systems include pen-injector devices for delivery of a solution such as BD Pens, BD Autojectore, Humaject.RTM; NovoPen.RTM., B-D.RTM.Pen, AutoPen.RTM, and OptiPen.RTM., GenotropinPen.RTM., Genotronorm Pen.RTM, Humatro Pen.RTM, Reco-Pen.RTM., Roferon Pen.RTM., Biojector.RTM., iject.RTM., J-tip Needle-Free Injector.RTM., Intraject.RTM., Medi-Ject.RTM., e.g., as made or developed by Becton Dickensen (Franklin Lakes, NJ. available at bectondickenson.com), Disetronic (Burgdorf, Switzerland, available at disetronic.com; Bioject, Portland, Oregon (available at bioject.com); National Medical Products, Weston Medical (Peterborough, UK, available at weston-medical.com), Medi-Ject Corp (Minneapolis, Minn., available at mediject.com).

Various delivery systems are known and can be used to administer a therapeutic agent of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis. See e.g., Wu and Wu (1987) J. Biol. Chem. 262:4429-4432 for construction of a therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of delivery include but are not limited to intravenous, intranasal, and oral routes. In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, by injection or by means of a catheter.

In certain embodiments, an effective amount of Fluorouracil (5-FU), oxaliplatin, or equivalents thereof are administered to the patient. In general, compositions comprising
these compounds can be prepared in accordance with known formulation techniques to provide a composition suitable for oral, topical, transdermal, rectal, inhalation, or parenteral (intravenous, intramuscular, or intraperitoneal) administration, and the like. Detailed guidance for preparing compositions of the invention are found by reference to the 18th or 19th Edition of REMINGTON’S PHARMACEUTICAL SCIENCES, Published by the Mack Publishing Co., Easton, Pa. 18040.

Fluorouracil (5-FU) or a chemical equivalent is administered in a therapeutically effective amount sufficient to treat cancer in a subject and may contain from about 1.0 to 2000 mg/m²/day of compound, for example about 1, 5, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, to 2000 mg/m².

Fluorouracil (5-FU) or a chemical equivalent may be administered parenterally, e.g., intravenously, intramuscularly, intravenously, subcutaneously, or interperitonically. The carrier or excipient or excipient mixture can be a solvent or a dispersive medium containing, for example, various polar or non-polar solvents, suitable mixtures thereof, or oils. As used herein "carrier" or "excipient" means a pharmaceutically acceptable carrier or excipient and includes any and all solvents, dispersive agents or media, coating(s), antimicrobial agents, iso/hypo/hypertonic agents, absorption-modifying agents, and the like. The use of such substances and the agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use in therapeutic compositions is contemplated. Moreover, other or supplementary active ingredients can also be incorporated into the final composition.

Solutions of Fluorouracil (5-FU) or a chemical equivalent may be prepared in suitable diluents such as water, ethanol, glycerol, liquid polyethylene glycol(s), various oils, and/or mixtures thereof, and others known to those skilled in the art.

The pharmaceutical forms of Fluorouracil (5-FU) or a chemical equivalent suitable for injectable use include sterile solutions, dispersions, emulsions, and sterile powders. The final form must be stable under conditions of manufacture and storage. Furthermore, the final pharmaceutical form must be protected against contamination and must, therefore, be
able to inhibit the growth of microorganisms such as bacteria or fungi. A single intravenous or intraperitoneal dose can be administered. Alternatively, a slow long term infusion or multiple short term daily infusions may be utilized, typically lasting from 1 to 8 days. Alternate day or dosing once every several days may also be utilized.

Sterile, injectable solutions are prepared by incorporating a compound in the required amount into one or more appropriate solvents to which other ingredients, listed above or known to those skilled in the art, may be added as required. Sterile injectable solutions are prepared by incorporating the compound in the required amount in the appropriate solvent with various other ingredients as required. Sterilizing procedures, such as filtration, then follow. Typically, dispersions are made by incorporating the compound into a sterile vehicle which also contains the dispersion medium and the required other ingredients as indicated above. In the case of a sterile powder, the preferred methods include vacuum drying or freeze drying to which any required ingredients are added.

In all cases the final form, as noted, must be sterile and must also be able to pass readily through an injection device such as a hollow needle. The proper viscosity may be achieved and maintained by the proper choice of solvents or excipients. Moreover, the use of molecular or particulate coatings such as lecithin, the proper selection of particle size in dispersions, or the use of materials with surfactant properties may be utilized.

Prevention or inhibition of growth of microorganisms may be achieved through the addition of one or more antimicrobial agents such as chlorobutanol, ascorbic acid, parabens, thermerosal, or the like. It may also be preferable to include agents that alter the tonicity such as sugars or salts.

In one aspect of the invention, a chemical equivalent of 5-FU (a pyrimidine based anti-metabolite) selected from the group of, but not limited to Cytarabine and Gemcitabine as described in Maring et al. (2005) Pharmacogenomics J. 5(4):226-243; and Floxuridine as described in Mayer (1992) Cancer. 70(5 Suppl): 1414-1424, can be used in combination therapy with the antibody based therapy described above to treat patients identified as having the appropriate genetic markers.
In certain embodiments, an effective amount of Leucovorin (Folinic acid) or a chemical equivalent is administered to the patient for the purpose of enhancing the cytotoxic effects of 5-FU or a chemical equivalent. In general, compositions comprising these compounds can be prepared in accordance with known formulation techniques to provide a composition suitable for oral, topical, transdermal, rectal, inhalation, or parenteral (intravenous, intramuscular, or intraperitoneal) administration, and the like. Detailed guidance for preparing compositions of the invention are found by reference to the 18TH OR 19TH EDITION OF REMINGTON’S PHARMACEUTICAL SCIENCES, Published by the Mack Publishing Co., Easton, Pa. 18040.

Leucovorin or a chemical equivalent is administered in a therapeutically effective amount sufficient to increase the effectiveness of 5-FU or a chemical equivalent to treat cancer in a subject and may contain from about 1.0 to 1000 mg/m^2/day of compound, for example about 1, 5, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, to 1000 mg/m^2.

Leucovorin or a chemical equivalent may be administered parenterally, e.g., intravenously, intramuscularly, intravenously, subcutaneously, or interperitonically. The carrier or excipient or excipient mixture can be a solvent or a dispersive medium containing, for example, various polar or non-polar solvents, suitable mixtures thereof, or oils. As used herein "carrier" or "excipient" means a pharmaceutically acceptable carrier or excipient and includes any and all solvents, dispersive agents or media, coating(s), antimicrobial agents, iso/hypo/hypertonic agents, absorption-modifying agents, and the like. The use of such substances and the agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use in therapeutic compositions is contemplated. Moreover, other or supplementary active ingredients can also be incorporated into the final composition.

Solutions of Leucovorin or a chemical equivalent may be prepared in suitable diluents such as water, ethanol, glycerol, liquid polyethylene glycol(s), various oils, and/or mixtures thereof, and others known to those skilled in the art.
The pharmaceutical forms of Leucovorin or a chemical equivalent suitable for injectable use include sterile solutions, dispersions, emulsions, and sterile powders. The final form must be stable under conditions of manufacture and storage. Furthermore, the final pharmaceutical form must be protected against contamination and must, therefore, be able to inhibit the growth of microorganisms such as bacteria or fungi. A single intravenous or intraperitoneal dose can be administered. Alternatively, a slow long term infusion or multiple short term daily infusions may be utilized, typically lasting from 1 to 8 days. Alternate day or dosing once every several days may also be utilized.

Sterile, injectable solutions are prepared by incorporating a compound in the required amount into one or more appropriate solvents to which other ingredients, listed above or known to those skilled in the art, may be added as required. Sterile injectable solutions are prepared by incorporating the compound in the required amount in the appropriate solvent with various other ingredients as required. Sterilizing procedures, such as filtration, then follow. Typically, dispersions are made by incorporating the compound into a sterile vehicle which also contains the dispersion medium and the required other ingredients as indicated above. In the case of a sterile powder, the preferred methods include vacuum drying or freeze drying to which any required ingredients are added.

In all cases the final form, as noted, must be sterile and must also be able to pass readily through an injection device such as a hollow needle. The proper viscosity may be achieved and maintained by the proper choice of solvents or excipients. Moreover, the use of molecular or particulate coatings such as lecithin, the proper selection of particle size in dispersions, or the use of materials with surfactant properties may be utilized.

Prevention or inhibition of growth of microorganisms may be achieved through the addition of one or more antimicrobial agents such as chlorobutanol, ascorbic acid, parabens, thermerosal, or the like. It may also be preferable to include agents that alter the tonicity such as sugars or salts.

In certain embodiments, an effective amount of Oxaliplatin or a chemical equivalent is administered to the patient. In general, compositions comprising these compounds can be prepared in accordance with known formulation techniques to provide a composition suitable for oral, topical, transdermal, rectal, inhalation, or parenteral (intravenous,
intramuscular, or intraperitoneal) administration, and the like. Detailed guidance for preparing compositions of the invention are found by reference to the 18™ OR 19™ EDITION OF REMINGTON'S PHARMACEUTICAL SCIENCES, Published by the Mack Publishing Co., Easton, Pa. 18040.

Oxaliplatin or a chemical equivalent is administered in a therapeutically effective amount sufficient to treat cancer in a subject and may contain from about 1.0 to 2000 mg/m²/day of compound, for example about 1, 5, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, to 2000 mg/m².

Oxaliplatin or a chemical equivalent may be administered parenterally, e.g., intravenously, intramuscularly, intravenously, subcutaneously, or interperitoneally. The carrier or excipient or excipient mixture can be a solvent or a dispersive medium containing, for example, various polar or non-polar solvents, suitable mixtures thereof, or oils. As used herein "carrier" or "excipient" means a pharmaceutically acceptable carrier or excipient and includes any and all solvents, dispersive agents or media, coating(s), antimicrobial agents, iso/hypo/hypertonic agents, absorption-modifying agents, and the like. The use of such substances and the agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use in therapeutic compositions is contemplated. Moreover, other or supplementary active ingredients can also be incorporated into the final composition.

Solutions of Oxaliplatin or an equivalent thereof may be prepared in suitable diluents such as water, ethanol, glycerol, liquid polyethylene glycol(s), various oils, and/or mixtures thereof, and others known to those skilled in the art.

The pharmaceutical forms of Oxaliplatin or a chemical equivalent suitable for injectable use include sterile solutions, dispersions, emulsions, and sterile powders. The final form must be stable under conditions of manufacture and storage. Furthermore, the final pharmaceutical form must be protected against contamination and must, therefore, be able to inhibit the growth of microorganisms such as bacteria or fungi. A single intravenous or intraperitoneal dose can be administered. Alternatively, a slow long term infusion or
multiple short term daily infusions may be utilized, typically lasting from 1 to 8 days. Alternate day or dosing once every several days may also be utilized.

Sterile, injectable solutions are prepared by incorporating a compound in the required amount into one or more appropriate solvents to which other ingredients, listed above or known to those skilled in the art, may be added as required. Sterile injectable solutions are prepared by incorporating the compound in the required amount in the appropriate solvent with various other ingredients as required. Sterilizing procedures, such as filtration, then follow. Typically, dispersions are made by incorporating the compound into a sterile vehicle which also contains the dispersion medium and the required other ingredients as indicated above. In the case of a sterile powder, the preferred methods include vacuum drying or freeze drying to which any required ingredients are added.

In all cases the final form, as noted, must be sterile and must also be able to pass readily through an injection device such as a hollow needle. The proper viscosity may be achieved and maintained by the proper choice of solvents or excipients. Moreover, the use of molecular or particulate coatings such as lecithin, the proper selection of particle size in dispersions, or the use of materials with surfactant properties may be utilized.

Prevention or inhibition of growth of microorganisms may be achieved through the addition of one or more antimicrobial agents such as chlorobutanol, ascorbic acid, parabens, thermersosal, or the like. It may also be preferable to include agents that alter the tonicity such as sugars or salts.

In one aspect of the invention, a chemical equivalent of Oxaliplatin (a platinum based alkylating agent) selected from the group of, but not limited to Carboplatin and Cisplatin as described in Galanski and Keppler (2007) Anticancer Agents Med. Chem. 7(1):55-73; and BBR3464 as described in Boulikas and Vaugiouka (2003) Oncol. Rep. 10(6):1663-1 682, can be used in combination therapy with the antibody based therapy described above to treat patients identified as having the appropriate genetic markers.

In certain embodiments, an effective amount of Capecitabine or a chemical equivalent is administered to the patient. In general, compositions comprising these compounds can be prepared in accordance with known formulation techniques to provide a
composition suitable for oral, topical, transdermal, rectal, inhalation, or parenteral (intravenous, intramuscular, or intraperitoneal) administration, and the like. Detailed guidance for preparing compositions of the invention are found by reference to the 18TH OR 19TH EDITION OF REMINGTON'S PHARMACEUTICAL SCIENCES, Published by the Mack Publishing Co., Easton, Pa. 18040.

Capecitabine or a chemical equivalent is administered in a therapeutically effective amount sufficient to treat cancer in a subject and may contain from about 1.0 to 6000 mg/day of compound, for example about 1, 5, 10, 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2300, 2600, 3000, 3300, 3600, 4000, 4300, 4600, 5000, 5300, 5600, 6000 mg/day.

Capecitabine or a chemical equivalent can be administered orally in a suitable formulation as an ingestible tablet, a buccal tablet, capsule, caplet, elixir, suspension, syrup, trouche, wafer, lozenge, and the like. Generally, the most straightforward formulation is a tablet or capsule (individually or collectively designated as an "oral dosage unit"). Suitable formulations are prepared in accordance with a standard formulating techniques available that match the characteristics of the compound to the excipients available for formulating an appropriate composition. A tablet or capsule will contain about 50 to about 500 mg.

Capecitabine or a chemical equivalent may deliver the compound rapidly or may be a sustained-release preparation. The compound may be enclosed in a hard or soft capsule, may be compressed into tablets, or may be incorporated with beverages, food or otherwise into the diet. The percentage of the final composition and the preparations may, of course, be varied and may conveniently range between 1 and 90% of the weight of the final form, e.g., tablet. The amount in such therapeutically useful compositions is such that a suitable dosage will be obtained. An alternative composition according to the current invention are prepared so that an oral dosage unit form contains between about 5 to about 50% by weight (% w) in dosage units weighing between 50 and 1000 mg.

The suitable formulation of an oral dosage unit of Capecitabine or a chemical equivalent may also contain: a binder, such as gum tragacanth, acacia, corn starch, gelatin; sweetening agents such as lactose or sucrose; disintegrating agents such as corn starch,
alginate acid and the like; a lubricant such as magnesium stearate; or flavoring such as peppermint, oil of wintergreen or the like. Various other material may be present as coating or to otherwise modify the physical form of the oral dosage unit. The oral dosage unit may be coated with shellac, a sugar or both. Syrup or elixir may contain the compound, sucrose as a sweetening agent, methyl and propylparabens as a preservative, a dye and flavoring. Any material utilized should be pharmaceutically-acceptable and substantially non-toxic. Details of the types of excipients useful may be found in the nineteenth edition of "REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY," Mack Printing Company, Easton, Pa. See particularly chapters 91-93 for a more complete discussion.

In one aspect of the invention, a chemical equivalent of Capecitabine (a pyrimidine based anti-metabolite) selected from the group of, but not limited to Cytarabine and Gemcitabine as described in Maring et al. (2005) Pharmacogenomics J. 5(4):226-243; and Floxuridine as described in Mayer (1992) Cancer. 70(5 Suppl): 1414-1424, can be used in combination therapy with the antibody based therapy described above to treat patients identified as having the appropriate genetic markers.

The agents identified herein as effective for their intended purpose can be administered to subjects or individuals identified by the methods herein as suitable for the therapy, Therapeutic amounts can be empirically determined and will vary with the pathology being treated, the subject being treated and the efficacy and toxicity of the agent.

**Biological Equivalent Antibodies and Therapies**

In one aspect, after determining that antibody therapy alone or in combination with other suitable therapy is likely to provide a benefit to the patient, the invention further comprises administration of an anti-angiogenic antibody, fragment, variant or derivative thereof. The antibodies of this invention are monoclonal antibodies, although in certain aspects, polyclonal antibodies can be utilized. They also can be functional fragments, antibody derivatives or antibody variants. They can be chimeric, humanized, or totally human. A functional fragment of an antibody includes but is not limited to Fab, Fab', Fab2, Fab'2, and single chain variable regions. Antibodies can be produced in cell culture, in
phage, or in various animals, including but not limited to cows, rabbits, goats, mice, rats, hamsters, guinea pigs, sheep, dogs, cats, monkeys, chimpanzees, apes, etc. So long as the fragment or derivative retains specificity of binding or neutralization ability as the antibodies of this invention it can be used. Antibodies can be tested for specificity of binding by comparing binding to appropriate antigen to binding to irrelevant antigen or antigen mixture under a given set of conditions. If the antibody binds to the appropriate antigen at least 2, 5, 7, and preferably 10 times more than to irrelevant antigen or antigen mixture then it is considered to be specific.

The antibodies also are characterized by their ability to specifically bind to an equivalent epitope. The monoclonal antibodies of the invention can be generated using conventional hybridoma techniques known in the art and well-described in the literature. For example, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-I, L.5, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SSI, Sp2 SA5, U397, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, CHO, PerC.6, YB2/0) or the like, or heteromyelomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art (see, e.g., www.atcc.org, www.lifetech.com., and the like), with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. Antibody producing cells can also be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing-heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture
conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods.

Polyclonal antibodies of the invention can be generated using conventional techniques known in the art and are well-described in the literature. Several methodologies exist for production of polyclonal antibodies. For example, polyclonal antibodies are typically produced by immunization of a suitable mammal such as, but not limited to, chickens, goats, guinea pigs, hamsters, horses, mice, rats, and rabbits. An antigen is injected into the mammal, which induces the B-lymphocytes to produce IgG immunoglobulins specific for the antigen. This IgG is purified from the mammals serum. Variations of this methodology include modification of adjuvants, routes and site of administration, injection volumes per site and the number of sites per animal for optimal production and humane treatment of the animal. For example, adjuvants typically are used to improve or enhance an immune response to antigens. Most adjuvants provide for an injection site antigen depot, which allows for a slow release of antigen into draining lymph nodes. Other adjuvants include surfactants which promote concentration of protein antigen molecules over a large surface area and immunostimulatory molecules. Non-limiting examples of adjuvants for polyclonal antibody generation include Freund's adjuvants, Ribi adjuvant system, and Titermax. Polyclonal antibodies can be generated using methods described in U.S. Patent Nos. 7,279,559; 7,119,179; 7,060,800; 6,709,659; 6,656,746; 6,322,788; 5,686,073; and 5,670,153.

The monoclonal antibodies of the invention can be generated using conventional hybridoma techniques known in the art and well-described in the literature. For example, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/O, Sp2/O-AG14, NSO, NSI, NS2, AE-I, L.5, >243, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SSI, Sp2 SA5, U397, MLA 144, ACT IV, MOLT4, DA-I, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, CHO, PerC.6, YB2/O) or the like, or heteromyelomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art (see, e.g., www.atcc.org, www.lifetech.com., last accessed on November 26, 2007, and the like), with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells,
or any other cells expressing heavy or light chain constant or variable or framework or CDR
sequences, either as endogenous or heterologous nucleic acid, as recombinant or
endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish,
mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA,
rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA,
single, double or triple stranded, hybridized, and the like or any combination thereof.
Antibody producing cells can also be obtained from the peripheral blood or, preferably the
spleen or lymph nodes, of humans or other suitable animals that have been immunized with
the antigen of interest. Any other suitable host cell can also be used for expressing-
heterologous or endogenous nucleic acid encoding an antibody, specified fragment or
variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells
can be isolated using selective culture conditions or other suitable known methods, and
cloned by limiting dilution or cell sorting, or other known methods.

In one embodiment, the antibodies described herein can be generated using a
Multiple Antigenic Peptide (MAP) system. The MAP system utilizes a peptidyl core of
three or seven radially branched lysine residues, on to which the antigen peptides of interest
can be built using standard solid-phase chemistry. The lysine core yields the MAP bearing
about 4 to 8 copies of the peptide epitope depending on the inner core that generally
accounts for less than 10% of total molecular weight. The MAP system does not require a
carrier protein for conjugation. The high molar ratio and dense packing of multiple copies
of the antigenic epitope in a MAP has been shown to produce strong immunogenic
response. This method is described in U.S. Patent No. 5,229,490 and is herein incorporated
by reference in its entirety.

Other suitable methods of producing or isolating antibodies of the requisite
specificity can be used, including, but not limited to, methods that select recombinant
antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage,
ribosome, oligonucleotide, RNA, cDNA, or the like, display library; e.g., as available from
various commercial vendors such as Cambridge Antibody Technologies (Cambridgeshire,
UK), MorphoSys (Martinsreid/Planegg, Del.), Biovation (Aberdeen, Scotland, UK)
BiolInvent (Lund, Sweden), using methods known in the art. See U.S. Pat. Nos. 4,704,692;
5,723,323; 5,763,192; 5,814,476; 5,817,483; 5,824,514; 5,976,862. Alternative methods

Antibody variants of the present invention can also be prepared using delivering a polynucleotide encoding an antibody of this invention to a suitable host such as to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. These methods are known in the art and are described for example in U.S. Pat. Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; and 5,304,489.

The term "antibody variant" includes post-translational modification to linear polypeptide sequence of the antibody or fragment. For example, U.S. Patent No. 6,602,684 B1 describes a method for the generation of modified glycol-forms of antibodies, including whole antibody molecules, antibody fragments, or fusion proteins that include a region equivalent to the Fc region of an immunoglobulin, having enhanced Fc-mediated cellular toxicity, and glycoproteins so generated.

Antibody variants also can be prepared by delivering a polynucleotide of this invention to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco, maize, and duckweed) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom. For example, Cramer et al. (1999) Curr. Top. Microbiol. Immunol. 240:95-18 and references cited therein, describe the production of transgenic tobacco leaves expressing large amounts of recombinant proteins, e.g., using an inducible promoter. Transgenic maize have been used to express mammalian proteins at
commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al. (1999) Adv. Exp. Med. Biol. 464:127-147 and references cited therein. Antibody variants have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv’s), including tobacco seeds and potato tubers. See, e.g., Conrad et al.(1998) Plant Mol. Biol. 38:101-109 and reference cited therein. Thus, antibodies of the present invention can also be produced using transgenic plants, according to known methods.

Antibody derivatives can be produced, for example, by adding exogenous sequences to modify immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids.

In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Humanization or engineering of antibodies of the present invention can be performed using any known method, such as but not limited to those described in U.S. Pat. Nos. 5,723,323, 5,976,862, 5,824,514, 5,817,483, 5,814,476, 5,763,192, 5,723,323, 5,766,886, 5,714,352, 6,204,023, 6,180,370, 5,693,762, 5,530,101, 5,585,089, 5,225,539; and 4,816,567.

Human monoclonal antibodies can also be produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, e.g., a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

The antibodies of this invention also can be modified to create chimeric antibodies. Chimeric antibodies are those in which the various domains of the antibodies’ heavy and light chains are coded for by DNA from more than one species. See, e.g., U.S. Patent No. 4,816,567.

Alternatively, the antibodies of this invention can also be modified to create vereered antibodies. Vereered antibodies are those in which the exterior amino acid residues of the antibody of one species are judiciously replaced or "veneered" with those of a second species so that the antibodies of the first species will not be immunogenic in the second species thereby reducing the immunogenicity of the antibody. Since the antigenicity of a protein is primarily dependent on the nature of its surface, the immunogenicity of an antibody could be reduced by replacing the exposed residues which differ from those usually found in another mammalian species antibodies. This judicious replacement of exterior residues should have little, or no, effect on the interior domains, or on the interdomain contacts. Thus, ligand binding properties should be unaffected as a consequence of alterations which are limited to the variable region framework residues. The process is referred to as "veneering" since only the outer surface or skin of the antibody is altered, the supporting residues remain undisturbed.

The term "antibody derivative" also includes "diabodies" which are small antibody fragments with two antigen-binding sites, wherein fragments comprise a heavy chain variable domain (V) connected to a light chain variable domain (V) in the same polypeptide chain (VH V). See for example, EP 404,097; WO 93/1 1161 ; and Hollinger et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448. By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. See also, U.S. Patent No. 6,632,926 to Chen et al. which discloses antibody variants that have one or more amino acids inserted into a hypervariable region of the parent antibody and a binding affinity for a target antigen which is at least about two fold stronger than the binding affinity of the parent antibody for the antigen.

The term "antibody derivative" further includes "linear antibodies". The procedure for making this is known in the art and described in Zapata et al. (1995) Protein Eng. 8(10): 1057-1062. Briefly, these antibodies comprise a pair of tandem Fd segments (V-C 1-VH -Cl) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

The antibodies of this invention can be recovered and purified from recombinant cell cultures by known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be used for purification.
Antibodies of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells, or alternatively from a prokaryotic cells as described above.

If a monoclonal antibody being tested binds with protein or polypeptide, then the antibody being tested and the antibodies provided by the hybridomas of this invention are equivalent. It also is possible to determine without undue experimentation, whether an antibody has the same specificity as the monoclonal antibody of this invention by determining whether the antibody being tested prevents a monoclonal antibody of this invention from binding the protein or polypeptide with which the monoclonal antibody is normally reactive. If the antibody being tested competes with the monoclonal antibody of the invention as shown by a decrease in binding by the monoclonal antibody of this invention, then it is likely that the two antibodies bind to the same or a closely related epitope. Alternatively, one can pre-incubate the monoclonal antibody of this invention with a protein with which it is normally reactive, and determine if the monoclonal antibody being tested is inhibited in its ability to bind the antigen. If the monoclonal antibody being tested is inhibited then, in all likelihood, it has the same, or a closely related, epitopic specificity as the monoclonal antibody of this invention.

The term "antibody" also is intended to include antibodies of all isotypes. Particular isotypes of a monoclonal antibody can be prepared either directly by selecting from the initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of different isotype by using the sib selection technique to isolate class switch variants using the procedure described in Steplewski et al. (1985) Proc. Natl. Acad. Sci. USA 82:8653 or Spira et al. (1984; J. Immunol. Methods 74:307.

The isolation of other hybridomas secreting monoclonal antibodies with the specificity of the monoclonal antibodies of the invention can also be accomplished by one of ordinary skill in the art by producing anti-idiotypic antibodies. Herlyn et al. (1986) Science 232:100. An anti-idiotypic antibody is an antibody which recognizes unique determinants present on the monoclonal antibody produced by the hybridoma of interest.
Idiotypic identity between monoclonal antibodies of two hybridomas demonstrates that the two monoclonal antibodies are the same with respect to their recognition of the same epitopic determinant. Thus, by using antibodies to the epitopic determinants on a monoclonal antibody it is possible to identify other hybridomas expressing monoclonal antibodies of the same epitopic specificity.

It is also possible to use the anti-idiotype technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the mirror image of the epitope bound by the first monoclonal antibody. Thus, in this instance, the anti-idiotypic monoclonal antibody could be used for immunization for production of these antibodies.

In some aspects of this invention, it will be useful to detectably or therapeutically label the antibody. Suitable labels are described supra. Methods for conjugating antibodies to these agents are known in the art. For the purpose of illustration only, antibodies can be labeled with a detectable moiety such as a radioactive atom, a chromophore, a fluorophore, or the like. Such labeled antibodies can be used for diagnostic techniques, either in vivo, or in an isolated test sample.

The coupling of antibodies to low molecular weight haptens can increase the sensitivity of the antibody in an assay. The haptens can then be specifically detected by means of a second reaction. For example, it is common to use haptens such as biotin, which reacts avidin, or dinitrophenol, pyridoxal, and fluorescein, which can react with specific anti-hapten antibodies. See, Harlow and Lane (1999) supra.

Antibodies can be labeled with a detectable moiety such as a radioactive atom, a chromophore, a fluorophore, or the like. Such labeled antibodies can be used for diagnostic techniques, either in vivo, or in an isolated test sample. Antibodies can also be conjugated, for example, to a pharmaceutical agent, such as chemotherapeutic drug or a toxin. They can be linked to a cytokine, to a ligand, to another antibody. Suitable agents for coupling to antibodies to achieve an anti-tumor effect include cytokines, such as interleukin 2 (IL-2) and Tumor Necrosis Factor (TNF); photosensitizers, for use in photodynamic therapy, including aluminum (III) phthalocyanine tetrasulfonate, hematoporphyrin, and
phthalocyanine; radionuclides, such as iodine-131 (¹³¹I), yttrium-90 (⁹⁰Y), bismuth-212 (²¹²Bi), bismuth-213 (²¹³Bi), technetium-99m (⁹⁹ᵐTc), rhenium-186 (¹⁸⁶Re), and rhenium-188 (¹⁸⁸Re); antibiotics, such as doxorubicin, adriamycin, daunorubicin, methotrexate, daunomycin, neocarzinostatin, and carboplatin; bacterial, plant, and other toxins, such as diphtheria toxin, pseudomonas exotoxin A, staphylococcal enterotoxin A, abrin-A toxin, ricin A (deglycosylated ricin A and native ricin A), TGF-alpha toxin, cytotoxin from Chinese cobra (naja naja atra), and gelonin (a plant toxin); ribosome inactivating proteins from plants, bacteria and fungi, such as restrictocin (a ribosome inactivating protein produced by Aspergillus restrictus), saporin (a ribosome inactivating protein from Saponaria officinalis), and RNase; tyrosine kinase inhibitors; ly207702 (a difluorinated purine nucleoside); liposomes containing anti cystic agents (e.g., antisense oligonucleotides, plasmids which encode for toxins, methotrexate, etc.); and other antibodies or antibody fragments, such as F(ab).

The antibodies of the invention also can be bound to many different carriers. Thus, this invention also provides compositions containing the antibodies and another substance, active or inert. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

The anti-angiogenic antibody can be further modified. The modified antibodies of the invention can be produced by reacting a human antibody or antigen-binding fragment with a modifying agent. For example, the organic moieties can be bonded to the antibody in a non-site specific manner by employing an amine-reactive modifying agent, for example, an NHS ester of PEG. Modified human antibodies or antigen-binding fragments can also be prepared by reducing disulfide bonds (e.g., intra-chain disulfide bonds) of an antibody or antigen-binding fragment. The reduced antibody or antigen-binding fragment can then be reacted with a thiol-reactive modifying agent to produce the modified antibody of the invention. Modified human antibodies and antigen-binding fragments comprising an organic moiety that is bonded to specific sites of an antibody of the present invention can be
prepared using suitable methods, such as reverse proteolysis. See generally, Hermanson (1996) BIOCONJUGATE TECHNIQUES, Academic Press: San Diego, Calif.

In one aspect of the invention, biological equivalents of Bevacizumab (an anti-angiogenic antibody) selected from the group of, but not limited to, antibody A4.6.1 and derivatives thereof as described in US Patent Publ. Nos.: 2007/0071749, 20070071748, 2007/0071718, and 2007/002599; any one of the series of humanized and variant anti-VEGF antibodies described in US Patent Publ. Nos. 2005/01 12126, 2003/0190317, and 2002/0032315; or antibody 2C3 and derivatives thereof described in US Patent Publ. No. 2002/01 19153, can be used in combination therapy with antimetabolites and platinum-based alkylating agents based therapy described above to treat patients identified as having the appropriate genetic polymorphisms.

Also provided is a medicament comprising an effective amount of a therapy as described herein for treatment of a human cancer patient having one or more predictive polymorphisms or genetic markers as identified in Tables 1, 2, 3, 4 or the experimental examples.

Kits

As set forth herein, the invention provides diagnostic methods for determining the type of allelic variant of a polymorphic region present in the gene of interest or the expression level of a gene of interest. In some embodiments, the methods use probes or primers comprising nucleotide sequences which are complementary to the polymorphic region of the gene of interest. Accordingly, the invention provides kits for performing these methods as well as instructions for carrying out the methods of this invention such as collecting tissue and/or performing the screen, and/or analyzing the results, and/or administration of an effective amount of the therapies described above.

In an embodiment, the invention provides a kit for determining whether a subject responds to cancer treatment or alternatively one of various treatment options. The kits contain one of more of the compositions described above and instructions for use. As an example only, the invention also provides kits for determining response to cancer treatment containing a first and a second oligonucleotide specific for the polymorphic region of the
gene. Oligonucleotides "specific for" a genetic locus bind either to the polymorphic region of the locus or bind adjacent to the polymorphic region of the locus. For oligonucleotides that are to be used as primers for amplification, primers are adjacent if they are sufficiently close to be used to produce a polynucleotide comprising the polymorphic region. In one embodiment, oligonucleotides are adjacent if they bind within about 1-2 kb, and preferably less than 1 kb from the polymorphism. Specific oligonucleotides are capable of hybridizing to a sequence, and under suitable conditions will not bind to a sequence differing by a single nucleotide.

The kit can comprise at least one probe or primer which is capable of specifically hybridizing to the polymorphic region of the gene of interest and instructions for use. The kits preferably comprise at least one of the above described nucleic acids. Preferred kits for amplifying at least a portion of the gene of interest comprise two primers, at least one of which is capable of hybridizing to the allelic variant sequence. Such kits are suitable for detection of genotype by, for example, fluorescence detection, by electrochemical detection, or by other detection.

Oligonucleotides, whether used as probes or primers, contained in a kit can be detectably labeled. Labels can be detected either directly, for example for fluorescent labels, or indirectly. Indirect detection can include any detection method known to one of skill in the art, including biotin-avidin interactions, antibody binding and the like. Fluorescently labeled oligonucleotides also can contain a quenching molecule. Oligonucleotides can be bound to a surface. In one embodiment, the preferred surface is silica or glass. In another embodiment, the surface is a metal electrode.

Yet other kits of the invention comprise at least one reagent necessary to perform the assay. For example, the kit can comprise an enzyme. Alternatively the kit can comprise a buffer or any other necessary reagent.

Conditions for incubating a nucleic acid probe with a test sample depend on the format employed in the assay, the detection methods used, and the type and nature of the nucleic acid probe used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the nucleic acid probes for use in the present invention.
Examples of such assays can be found in Chard, T. (1986) "An Introduction to
Radioimmunoassay and Related Techniques" Elsevier Science Publishers, Amsterdam, The
Netherlands; Bullock et al., "Techniques in Immunocytochemistry" Academic Press,
THEORY OF IMMUNOASSAYS: LABORATORY TECHNIQUES IN BIOCHEMISTRY
AND MOLECULAR BIOLOGY", Elsevier Science Publishers, Amsterdam, The
Netherlands.

The test samples used in the diagnostic kits include cells, protein or membrane
extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The
test sample used in the above-described method will vary based on the assay format, nature
of the detection method and the tissues, cells or extracts used as the sample to be assayed.
Methods for preparing protein extracts or membrane extracts of cells are known in the art
and can be readily adapted in order to obtain a sample which is compatible with the system
utilized.

The kits can include all or some of the positive controls, negative controls, reagents,
primers, sequencing markers, probes and antibodies described herein for determining the
subject's genotype in the polymorphic region of the gene of interest.

As amenable, these suggested kit components may be packaged in a manner
customary for use by those of skill in the art. For example, these suggested kit components
may be provided in solution or as a liquid dispersion or the like.

Other Uses for the Nucleic Acids of the Invention

The identification of the allele of the gene of interest can also be useful for
identifying an individual among other individuals from the same species. For example,
DNA sequences can be used as a fingerprint for detection of different individuals within the
same species. Thompson, J. S. and Thompson, eds., (1991) "GENETICS IN MEDICINE",
W B Saunders Co., Philadelphia, Pa. This is useful, e.g., in forensic studies.

The invention now being generally described, it will be more readily understood by
reference to the following examples which are included merely for purposes of illustration
of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

**EXPERIMENTAL EXAMPLE**

For the purpose of illustration only, peripheral blood sample can be collected from each patient, and genomic DNA can be extracted from white blood cells using the QiaAmp kit (Qiagen, Valencia, CA).

**EXAMPLE 1**

**Background:** Despite recent advances in the treatment of metastatic colorectal cancer, tailoring adjuvant treatment of stage II and HI colon cancer patients remains controversial. Identifying a reliable panel of prognostic and predictive markers for tumor recurrence is critical in selecting an individualized and tailored chemotherapy. Tumor angiogenesis plays an important role in tumor development, progression and metastasis. In this retrospective study, tests were conducted to determine whether a specific pattern of 40 functionally significant polymorphisms in 37 genes involved in angiogenesis and tumor microenvironment will predict the risk of tumor recurrence in stage II and III colon cancer patients treated with adjuvant chemotherapy.

**Methods:** Between 1999 and 2006 blood specimens from 140 patients (69 females and 71 males with a median age of 59 years; range=28-86) were obtained at the University of Southern California/Norris Comprehensive Cancer Center (USC/NCCC). Sixty-three patients had stage II and 77 had stage III colon cancer. The median follow-up was 5.4 years (range=2.0-16.8). 51 of 140 patients (36.4%) developed tumor recurrence with a 5-year probability of 0.28 ± 0.06 for stage II and 0.40 ± 0.06 for stage III colon cancer patients. Genomic DNA was extracted from peripheral blood and genotypes were determined using PCR based RFLP. Probes and primers for this analysis are known in the art as described herein, examples of which are provided in Table 5.

**Results:** Polymorphisms in VEGF (C936T; p=0.009, log-rank) and VEGFR2 (+4422 AC- repeat; p=0.04, log-rank and +1416 T/A; p=0.0009, log-rank) were associated with risk of tumor recurrence in stage III colon cancer patients (n=77). VEGFR2 AC-repeat polymorphisms were additionally associated with risk of recurrence in Stage II colon cancer
patients (n=63, \( p=0.02 \), log-rank). The associated predictive polymorphisms for these alleles are show in Table 1. Angiogenesis seem to play a crucial role in tumor recurrence, thus targeting VEGF and VEGFR2 are predicted to be of clinical benefit for stage II and stage III colon cancer patients.

EXAMPLE 2

**Background:** The inhibition of angiogenesis is thought to be central to the mechanism of action of BV, a monoclonal antibody to vascular endothelial growth factor (VEGF). We evaluated polymorphisms of genes involved in the angiogenesis/VEGF pathway as potential molecular predictors of clinical outcome in pts with mCRC who received BV as part of their frontline therapy. These genes included: VEGF, VEGF receptor 2 (KDR or VEGFR2), neuropilin 1 (NRP 1), Interleukin (IL) 6 and 8, IL receptor 1 and 2 (CXCR 1, CXCR 2) adrenomedullin (AM), leptin, fibroblast growth factor receptor 4 (FGFR4), tissue factor (TF), matrix metalloproteinases (MMP 2,7,9), epidermal growth factor receptor (EGFR), aryl hydrocarbon receptor nuclear translocator (ARNT), and nuclear factor kappa b (NFkb).

**Methods:** PCR-RFLP assays were performed on genomic DNA extracted from the blood of 30 pts with mCRC treated with first-line FOLFOX/BV or XELOX/BV at USC. Probes and primers for this analysis are known in the art as described herein, examples of which are provided in Table 5.

**Results:** The cohort consisted of 21 males and 9 females with a median age of 56 years (range: 29-81). 20 pts received XELOX/BV as part of an on-going phase II study, 10 pts received FOLFOX/BV. Radiologic response was evaluable in 27/30 pts: 2/27 (7%) complete response (CR), 14/27 (52%) partial response (PR), 10/27 (37%) stable disease (SD) and 1/27 (4%) progressive disease. At a median follow-up of 19.4 months, 16/30 pts progressed with a median progression free survival (PFS) of 11.8 months. Pts homozygous A/A at the leptin 5'UTR region had a higher probability of response than pts with the G/A or G/G genotypes (\( p=0.03 \), Fisher's exact test). Pts with one or more G allele (G/G or A/G) at locus -181 in the promoter region of MMP7 had a higher probability of response than pts with the AA genotype (\( p=0.014 \)). There were statistically significant associations between genomic polymorphisms of KDR, CXCR2, MMP7 and PFS (\( p<0.05 \), Log-rank test),
whereas FGFR4, NFKB, AM, and TF related polymorphisms demonstrated a trend towards improved PFS.

**EXAMPLE 3**

In an extension of the experiment described in Example 2, Applicant provides the following Example 3.

**Background:** The inhibition of angiogenesis is central to the mechanism of action of BV, a monoclonal antibody to vascular endothelial growth factor (VEGF). We evaluated functionally significant polymorphisms of genes involved in the angiogenesis/VEGF pathway as potential molecular predictors of clinical outcome in pts with mCRC who received BV as part of their frontline therapy. These genes included: VEGF, VEGF receptor 2 (KDR, a.k.a. VEGFR2), neuropilin 1 (NRP 1), Interleukin (IL) 6 and 8, IL receptor 1 and 2 (CXCR 1, CXCR 2) adrenomedullin (AM), leptin, fibroblast growth factor receptor 4 (FGFR4), tissue factor (TF), matrix metalloproteinases (MMP 2,7,9), epidermal growth factor receptor (EGFR), aryl hydrocarbon receptor nuclear translocator (ARNT), and nuclear factor kappa b (NFkb).

**Methods:** A total of 31 patients with metastatic colon cancer treated at the University of Southern California/Norris Comprehensive Cancer Center (USC/NCCC) or the Los Angeles County/University of Southern California Medical Center (LACAJSCMC) who received first line treatment with 5FU/LV or capecitabine in combination with oxaliplatin and bevacizumab are included in this study. XELOX/BV patients are part of an on-going phase II clinical trial and all patients gave informed consent. Patient information was collected through prospective database review and retrospective chart review. The end point of this study was to identify molecular predictors of clinical outcome including overall response and progression free survival (PFS). The progression free survival was determined by calculating the difference between the date of first treatment at USC/NCCC or LACAJSC and the date of last follow-up appointment or date of progression of disease. Peripheral blood samples were collected from each patient and genomic DNA was extracted from white blood cells using the QiaAmp kit (Qiagen, Valencia, CA). PCR-RFLP assays were performed on genomic DNA extracted from the blood of 31 patients. Probes and
primers for this analysis are known in the art as described herein, examples of which are provided in Table 5.

**Results:** The cohort consisted of 22 males and 9 females with a median age of 56 years (range: 29-81). 20 pts received XELOX/BV as part of an on-going phase II study, 10 pts received FOLFOX/BV, and one patient first received XELOX/BV then FOLFOX/BV. Radiologic response was evaluable in 28/31 pts: 2/28 (7%) complete response (CR), 14/28 (50%) partial response (PR), 11/28 (39%) stable disease (SD) and 1/28 (4%) progressive disease. Patients had a median follow-up of 19.4 months and a median progression free survival (PFS) of 11.8 months. Patients homozygous A/A at the Leptin 5’UTR region, with one or more G allele (G/G or A/G) at locus -181 in the promoter region of MMP7 or with one or more G allele (G/G or G/C) for the ARNT Exon 8 had a higher probability of response (Table 5 and Figure 1). There was also a trend in response to treatment for patients having genomic polymorphisms in IL-6, IL-8, and EGFR (Table 6). There were statistically significant associations between genomic polymorphisms of KDR (a.k.a. VEGFR2), MMP7, NFkB, CXCR2 and progression free survival (Figures 2-5, p<0.05, Log-rank test), whereas FGFR4, AM, and TF related polymorphisms demonstrated a trend towards improved PFS (Table 7).
<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>N</th>
<th>Response</th>
<th>Non-Response</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP7, locus -181</td>
<td>0.033</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>16</td>
<td>6 (38%)</td>
<td>10 (62%)</td>
<td></td>
</tr>
<tr>
<td>A/G</td>
<td>11</td>
<td>9 (82%)</td>
<td>2 (18%)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>1</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Leptin 5'UTR</td>
<td>0.032</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>12</td>
<td>5 (42%)</td>
<td>7 (58%)</td>
<td></td>
</tr>
<tr>
<td>G/A</td>
<td>8</td>
<td>4 (50%)</td>
<td>4 (50%)</td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>7</td>
<td>7 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>IL6</td>
<td>0.090</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>18</td>
<td>9 (50%)</td>
<td>9 (50%)</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>8</td>
<td>7 (88%)</td>
<td>1 (13%)</td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>1</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td></td>
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<tr>
<td>IL8 T-251A</td>
<td>0.086</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>2</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td></td>
</tr>
<tr>
<td>A/T</td>
<td>16</td>
<td>12 (75%)</td>
<td>4 (25%)</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>9</td>
<td>3 (33%)</td>
<td>6 (67%)</td>
<td></td>
</tr>
<tr>
<td>EGFR G497A</td>
<td>0.060</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>15</td>
<td>6 (40%)</td>
<td>9 (60%)</td>
<td></td>
</tr>
<tr>
<td>G/A</td>
<td>8</td>
<td>6 (75%)</td>
<td>2 (25%)</td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>4</td>
<td>4 (100%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>ARNT</td>
<td>0.038</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>11</td>
<td>5 (45%)</td>
<td>6 (55%)</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>14</td>
<td>11 (79%)</td>
<td>3 (21%)</td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>2</td>
<td>0 (0%)</td>
<td>2 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Based on Fisher’s exact test.
### Table 7. Polymorphisms and Progression-Free Survival (PFS)

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>N</th>
<th>Median PFS, Mo (95% CI)</th>
<th>Relative Risk (95% CI)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>KDR</td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>10/10</td>
<td>15</td>
<td>9.8 (7.0, 12.9)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10/1</td>
<td>15</td>
<td>28.7+ (7.8, 28.7+)</td>
<td>0.37 (0.12, 1.16)</td>
<td></td>
</tr>
<tr>
<td>11/1</td>
<td>1</td>
<td>5.2</td>
<td>6.34 (0.46, 86.51)</td>
<td></td>
</tr>
<tr>
<td>MMP7</td>
<td></td>
<td></td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>A/A</td>
<td>18</td>
<td>7.2 (6.5, 11.8)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A/G</td>
<td>12</td>
<td>16.7 (12.9, 22.7+)</td>
<td>0.27 (0.08, 0.85)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>1</td>
<td>26.0+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFkb</td>
<td></td>
<td></td>
<td></td>
<td>0.047</td>
</tr>
<tr>
<td>&lt; 24/24</td>
<td>12</td>
<td>7.2 (6.5, 14.7)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>≥ 24</td>
<td>19</td>
<td>16.7 (9.8, 28.7+)</td>
<td>0.38 (0.14, 1.04)</td>
<td></td>
</tr>
<tr>
<td>CXCR2</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>C/C</td>
<td>6</td>
<td>16.7+ (7.0, 16.7+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>17</td>
<td>28.7+ (9.8, 28.7+)</td>
<td>0.84 (0.17, 4.10)</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>8</td>
<td>7.0 (5.1, 7.8)</td>
<td>4.39 (0.90, 21.34)</td>
<td></td>
</tr>
<tr>
<td>FGFR4</td>
<td></td>
<td></td>
<td></td>
<td>0.092</td>
</tr>
<tr>
<td>G/G</td>
<td>7</td>
<td>28.7+ (11.8, 28.7+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>G/A</td>
<td>19</td>
<td>9.8 (7.1, 22.7+)</td>
<td>2.87 (0.64, 12.91)</td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>5</td>
<td>7.0 (5.3, 14.7+)</td>
<td>5.60 (1.00, 31.45)</td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td></td>
<td></td>
<td></td>
<td>0.057</td>
</tr>
<tr>
<td>&lt; 14</td>
<td>15</td>
<td>7.2 (7.0, 16.7+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt;14/&gt;14</td>
<td>16</td>
<td>14.7 (11.8, 28.7+)</td>
<td>0.42 (0.14, 1.19)</td>
<td></td>
</tr>
<tr>
<td>TF</td>
<td></td>
<td></td>
<td></td>
<td>0.087</td>
</tr>
<tr>
<td>A/A</td>
<td>11</td>
<td>7.2 (6.1, 16.7+)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A/G</td>
<td>11</td>
<td>12.9 (7.1, 26.0+)</td>
<td>0.41 (0.13, 1.27)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>9</td>
<td>28.7+ (11.8, 28.7+)</td>
<td>0.31 (0.08, 1.19)</td>
<td></td>
</tr>
</tbody>
</table>

* Based on the log-rank test
+ Estimates were not reached
† The patient did not progress by the last follow-up.

It is to be understood that while the invention has been described in conjunction with the above embodiments, that the foregoing description and examples are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.
WHAT IS CLAIMED IS:

1. A method for determining if a human stage III colon cancer patient is at risk for developing tumor recurrence, comprising screening a suitable cell or tissue sample isolated from said patient for at least one genetic polymorphism of the group:
   
   (i) VEGF at nt 936;
   
   (ii) VEGFR2 at position 4422 CA repeats; or
   
   (iii) VEGFR2 at nt 1416,

   wherein for the genetic polymorphism screened, the presence of at least one genetic polymorphism of the group:
   
   (i) (C/C) for VEGF at nt 936;
   
   (ii) (10/10 or 10/1 1 CA repeats) for VEGFR2 at position 4422; or
   
   (iii) (A/A) for VEGFR2 at nt 1416,

   indicates the patient is at risk for developing tumor recurrence.

2. A method for determining if a human stage II colon cancer patient is at risk of developing tumor recurrence, comprising screening a suitable cell or tissue sample isolated from said patient for a genetic polymorphism comprising CA repeats in the VEGFR2 gene at position 4422 CA, wherein the presence of (11/1 1 CA repeats) for the VEGFR2 gene at position 4422, indicates the patient is at risk for developing tumor recurrence.

3. The method of claims 1 or 2, further comprising treating the patient indicated as being at risk for developing tumor recurrence, comprising administration of an effective amount of an anti-VEGF antibody.

4. The method of claim 3, wherein the anti-VEGF antibody is Bevacizumab (BV) or a biological equivalent thereof.
5. The method of claims 1 or 2, further comprising treating the patient indicated as being at risk for developing tumor recurrence, comprising administration of an effective amount of a chemical or biological anti-VEGFR2 inhibitor.

6. The method of claim 1 or 2, further comprising screening the patient sample for a polymorphism selected from the group consisting of:

(i) VEGF (G405C);
(ii) VEGFR2 (11T/A);
(iii) HIF1α (1772C/T);
(iv) ARNT Exon 8 G/C;
(v) IL-8 (-251T/A);
(vi) AM (3’ end CA repeat);
(vii) NRP (-2548G/A);
(viii) Leptin (-2548G/A);
(ix) TF (-603A/G);
(x) PLGF (3’UTR G/A);
(xi) PLGF (3’UTR T/A);
(xii) MMMP7 (-181A/G);
(xiii) MMP9 (-1562C/T);
(xiv) MMP2 (-1306C/T);
(xv) NFkB (CA repeat in regulatory region);
(xvi) p53 (codon 72 Pro-Arg (C/G));
(xvii) IGFl (CA repeat);
(xviii) IGF2 (3580G/A);
(xix) IGFrI (3174G/A);
(xx) ICAM1 (K469E);
(xxii) IL-6 (-174G/C);
(xxiii) LDH (Exon 5 C/T);
(xxiv) LDH (Exon 5 G/A);
(xxv) GLUT1 (-2841VT);
(xxvi) CXCR1 (2607G/C);
(xxvii) CXCR2 (785C/T);
(xxviii) COX2 (8437T/C);
(xxix) EGF (61A/G);
(29870) EGFR (497G/A);
(29911) TNFα (-308G/A);
(30303) IL1b (-511T/C);
(30304) IL1b (3954C/T);
(30305) IL1Ra (Intron 2 86bp);
(30306) SDF1/CXCL12 (-801G/A);
(30307) FGFR4 (388G/A);
(30308) IGFB3 (-202A/C);
(30309) IGFB3 (2133G/C); and
7. A method for determining whether a human gastrointestinal cancer patient is likely responsive to therapy comprising the administration of an anti-VEGF antibody, pyrimidine based antimetabolite and platinum-based alkylating agent based therapy, comprising screening a suitable cell or tissue sample isolated from said patient for at least one genetic polymorphism of the group:

(i) Leptin 5' UTR at nt -2548;

(ii) MMP7 at nt -181;

(iii) VEGFR2 at position 4422 CA repeat;

(iv) CXCR2 at nt 785;

(v) MMP7 at nt -181;

(vi) FGFR4 at nt 388;

(vii) NFKB at the 5' end CA repeat;

(viii) AM at the 3' end CA repeat;

(ix) TF at nt -603;

(x) IL-6 at nt 174;

(xi) IL-8 at nt -251;

(xii) EGFR at nt 497; or

(xiii) ARNT at Exon 8 G/C,

wherein for the genetic polymorphism screened, the presence of at least one genetic polymorphism of the group:

(xiv) (A/A) for Leptin 5' UTR at nt -2548;
(xv) \((G/G\) or \(AJG)\) for MMP7 at nt -181;
(xvi) \(10/1\) 1 or \(10/10\) CA repeats) for VEGFR2 at position 4422;
(xvii) \((C/C\) or \(C/T)\) for CXCR2 at nt 785;
(xviii) \((A/G\) or \(G/G)\) for MMP7 at nt -181 ;
(xix) \((G/G)\) for FGFR4 at nt 388;
(xx) \((\geq 1\) allele with \(>24\) CA repeats) for NFKB at the 5’ end;
(xxi) \((2\) alleles with \(\geq 14\) CA repeats) for AM at the 3’ end;
(xxii) \((G/G\) or \(G/A)\) for TF at nt -603;
(xxiii) \((G/G\) or \(G/C)\) for IL-6 at nt 174;
(xxiv) \((A/A\) or \(A/T)\) for IL-8 at nt -251 ;
(xxv) \((G/A\) or \(A/A)\) for EGFR at nt 497; or
(xxvi) \((G/G\) or \(G/C)\) for ARNT at Exon 8,
indicates the patient is likely responsive to said therapy.

8. The method of claim 7, wherein the gastrointestinal cancer is a metastatic or non-metastatic gastrointestinal cancer selected from the group consisting of rectal cancer, colorectal cancer, colon cancer, gastric cancer, lung cancer, non-small cell lung cancer and esophageal cancer.

9. The method of claim 7, wherein the gastrointestinal cancer is colorectal cancer.

10. The method of claim 7, wherein the gastrointestinal cancer is metastatic colorectal cancer.

11. The method of claim 7, wherein the suitable cell or tissue sample is a tumor cell or tissue sample.
12. The method of claim 7, wherein the suitable cell or tissue sample is a metastatic colorectal tumor cell or tissue sample.

13. The method of claim 7, wherein the suitable cell or tissue sample is a normal cell or tissue sample.

14. The method of claim 7, wherein the suitable cell or tissue sample is peripheral blood lymphocytes.

15. The method of claim 7, further comprising screening the patient sample for a polymorphism selected from the group:

   (i) VEGF (936C/T);
   (ii) NRPI (3' end C/T);
   (iii) CXCR1 (2607G/C);
   (iv) MMP2 (-1306C/T); or
   (v) MMP9 (-1562C/T).

16. A method for treating a human gastrointestinal cancer patient comprising administering an effective amount of an anti-VEGF antibody, pyrimidine based antimetabolite and platinum-based alkylating agent based therapy, to a human gastrointestinal cancer patient selected for said therapy based on possession of at least one genetic polymorphism of the group:

   (i) (A/A) for Leptin 5' UTR at nt -2548;
   (ii) (G/G or A/G) for MMP7 at nt -181;
   (iii) (10/11 or 10/10 CA repeats) for VEGFR2 at position 4422;
   (iv) (C/C or C/T) for CXCR2 at nt 785;
   (v) (A/G or G/G) for MMP7 at nt -181;
   (vi) (G/G) for FGFR4 at nt 388;
(vii) (≥1 allele with >24 CA repeats) for NFKB at the 5' end;
(viii) (2 alleles with ≥14 CA repeats) for AM at the 3' end;
(ix) (G/G or G/A) for TF at nt -603;
(x) (G/G or G/C) for IL-6 at nt 174;
(xi) (A/A or AJT) for IL-8 at nt -251;
(xii) (G/A or A/A) for EGFR at nt 497; or
(xiii) (G/G or G/C) for ARNT at Exon 8.

17. The method of claim 16, wherein the gastrointestinal cancer is a metastatic or non-metastatic cancer selected from the group consisting of rectal cancer, colorectal cancer, colon cancer, gastric cancer, lung cancer, non-small cell lung cancer and esophageal cancer.

18. The method of claim 16, wherein the gastrointestinal cancer is metastatic colorectal cancer.

19. The method of claim 16, wherein the gastrointestinal cancer is colorectal cancer.

20. The method of claim 16, wherein the suitable cell or tissue sample is a tumor cell or tissue sample.

21. The method of claim 16, wherein the suitable cell or tissue sample is a metastatic colorectal tumor cell or tissue sample.

22. The method of claim 16, wherein the suitable cell or tissue sample is a normal cell or tissue sample.

23. The method of claim 16, wherein the suitable cell or tissue sample is peripheral blood lymphocytes.

24. A panel of genetic markers for determining whether a patient is likely responsive to an anti-VEGF antibody, pyrimidine based antimetabolite and platinum-based...
alkylating agent based therapy, the panel comprising a group of primers and/or probes that identify a genetic marker of the group:

(i) Leptin at 5' UTR G-2548A;
(ii) MMP7 at A-181G;
(iii) VEGFR2 at position 4422 CA repeat;
(iv) CXCR2 at nt C785T;
(v) MMP7 at nt A-181G;
(vi) FGFR4 at nt G388A;
(vii) NFKB at the 5' end CA repeat;
(viii) AM at the 3' end CA repeat;
(ix) TF at nt G-603A;
(x) IL-6 at nt G174C;
(xi) IL-8 at nt T-251A;
(xii) EGFR at nt G497A; and
(xiii) ARNT at Exon 8 G/C.
25. The panel of claim 24, further comprising a primer or probe that identifies a genetic marker of the group:

(i) VEGF (936C/T);

(ii) NRPI (3' end C/T);

(iii) CXCL1 (2607G/C);

(iv) MMP2 (-1306C/T); or

(v) MMP9 (-1562C/T).
Response to 5-FU/Oxaliplatin/Bevacizumab by Polymorphisms

FIG. 1

Fisher's exact $P = 0.033$

Fisher's exact $P = 0.032$
MMP7 polymorphism and progression-free survival

Log-rank P value = 0.015

MMP7 G/G (n=1)

MMP7 A/G (n=12)

MMP7 A/A (n=18)

Estimated Probability of Progression-Free Survival

Months Since Start of Treatment

FIG. 3