Abstract:

This disclosure provides compositions and methods for determining the likely tumor recurrence of gastric cancer patients based on genomic polymorphisms of the SPARC gene. The disclosure also provides compositions and methods for selecting gastric cancer patients for appropriate treatments and methods of treating them.
GERMLINE POLYMORPHISMS IN THE SPARC GENE ASSOCIATED WITH CLINICAL OUTCOME IN GASTRIC CANCER

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Serial No. 61/288,741, filed December 21, 2009, the content of which is incorporated by reference in its entirety into the present disclosure.

FIELD OF THE INVENTION

This invention relates to the filed of pharmacogenomics and specifically to the application of gene expression and genetic polymorphisms to diagnose and treat diseases.

BACKGROUND

The following description is provided to assist the understanding of the reader. None of the information provided or references cited is admitted to be prior art to the present invention. 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, thereby to more fully describe the state of the art to which this invention pertains.

Gastric cancer remains the second most common cause of cancer-related death worldwide, with around 700,000 deaths a year (Parkin et al. (2002) CA Cancer J Clin 55:74-108). Surgical resection is the only treatment that offers cure for this disease. However, because the disease is asymptomatic early on, more than half of gastric carcinomas are diagnosed in the advanced stage, when surgical resection is not possible. The addition of perioperative chemotherapy or adjuvant chemo-radiotherapy has been demonstrated to significantly improve OS and progression-free survival (Cunningham et al. (2006) N Engl J Med 355:1-20 and MacDonald et al. (2001) N Engl J Med 345:725-730). Nevertheless, despite advances in clinical diagnostics, surgical techniques, improvement of chemo- and radiotherapy regimens the prognosis of gastric cancer remains poor.
[0005] Genetically, gastric cancer is a heterogeneous diseases influenced by gene-environment interactions resulting in activation of various molecular pathways. Numerous genetic and epigenetic alterations on oncogenes, tumour-suppressor genes, cell-cycle regulators, as well as cell adhesion molecules have been indentified in the multistep process of gastric carcinogenesis (Tahara (2004) IARC Sci Publ 2004:327-349). However, useful predictive and prognostic biomarkers are still lacking and depend on future studies. The discovery of such biomarkers and the introduction of new therapeutic agents will not only be able to individualize therapeutic strategies but it will also be important to maximize drug efficacy in treatment of gastric cancer patients.

**SUMMARY**

[0006] It has now been discovered that germline variations in the SPARC gene (Secreted Protein Acidic and Rich in Cysteine, also known as osteonectin and BM-40) are associated with clinical outcome in gastric cancer patients. The gastric cancer patients were treated with surgery, or in some instances, with adjuvant 5-fluorouracil therapy and/or adjuvant radiotherapy.

[0007] Accordingly, in one aspect, a method for aiding in selecting or selecting a gastric cancer patient for a therapy comprising surgical resection is provided, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, screening or determining from a tissue or cell sample isolated from the patient for the genotype of at least one polymorphism of SPARC G2120A or SPARC C2217T, wherein the patient is selected for the therapy if at least one genotype of:

- a. (G/G or A/G) for SPARC G2120A; or
- b. (C/C or C/T) for SPARC C2217T

is present, or the patient is not selected if neither of the genotypes is present.

[0008] Also provided, in another aspect, is a method for aiding in determining or determining whether a gastric cancer patient is likely to experience longer or shorter tumor recurrence following a therapy comprising surgical resection, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, screening or determining from a tissue or cell sample isolated from the patient for the genotype of at least one polymorphism of SPARC G2120A or SPARC C2217T, wherein the presence of at least one genotype of:
a. (G/G or A/G) for SPARC G2120A; or
b. (C/C or C/T) for SPARC C2217T

determines that the patient is likely to experience longer tumor recurrence as compared to a patient having neither of the genotypes, or the presence of neither of the genotypes determines that the patient is likely to experience shorter tumor recurrence as compared to a patient having at least one of the genotypes.

[0009] Further, a method for aiding in the treatment of or for treating a gastric cancer patient is provided wherein the treatment is for a patient selected based on the presence of at least one genotype selected from:

a. (G/G or A/G) for SPARC G2120A; or
b. (C/C or C/T) for SPARC C2217T

in a tissue or cell sample from the patient is provided, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient a therapy comprising surgical resection. In one aspect, the patient was identified by a method comprising screening or determining from a tissue or cell sample isolated from the patient for the genotype of at least one polymorphism of SPARC G2120A or SPARC C2217T.

[0010] The gastric cancer patient suffers from gastric adenocarcinoma, or alternatively localized gastric adenocarcinoma. Suitable therapies include surgical resection alone, or in combination with radiotherapy and/or a chemotherapy, such as administration of an effective amount of 5-fluorouracil or a chemical equivalent thereof. In another aspect, the radiotherapy or chemotherapy are adjuvant radiotherapy or chemotherapy.

[0011] The methods are useful in the assistance of an animal, a mammal or yet further a human patient. For the purpose of illustration only, a mammal includes but is not limited to a simian, a murine, a bovine, an equine, a porcine or an ovine.

**DETAILED DESCRIPTION**

[0012] The practice of the present technology employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature for example in the following publications. See, e.g., Sambrook and Russell eds. MOLECULAR CLONING: A

Definitions

[0013] 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" include singular and plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a single cell as well as a plurality of cells, including mixtures thereof.

[0014] 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. "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 disclosure. Accordingly, it is intended that the methods and compositions
can include additional steps and components (comprising) or alternatively including steps and compositions of no significance (consisting essentially of) or alternatively, intending only the stated method steps or compositions (consisting of).

[0015] 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.

[0016] The term "adjuvant" therapy refers to administration of a therapy or chemotherapeutic regimen to a patient after removal of a tumor by surgery. Adjuvant therapy is typically given to minimize or prevent a possible cancer reoccurrence. Alternatively, "neoadjuvant" therapy refers to administration of therapy or chemotherapeutic regimen before surgery, typically in an attempt to shrink the tumor prior to a surgical procedure to minimize the extent of tissue removed during the procedure.

[0017] "5-fluorouracil" or "5-FU" is an antimetabolite agent which inhibits the use of a metabolite, i.e. another chemical that is part of normal metabolism. In cancer treatment, antimetabolites interfere with DNA production, thus cell division and growth of the tumor. 5-FU is transformed into different cytotoxic metabolites that are then incorporated into DNA and RNA thereby inducing cell cycle arrest and apoptosis.

[0018] A "chemical equivalent" of 5-FU is a pyrimidine analog that results in disruption of DNA replication. Chemical equivalents of 5-FU inhibit cell cycle progression at S phase resulting in the disruption of cell cycle and consequently apoptosis. Chemical equivalents of 5-FU include prodrugs, analogs and derivative thereof such as 5'-deoxy-5-fluorouridine (doxifluridine), 1-tetrahydrofuranyl-5-fluorouracil (florafur), Capecitabine (Xeloda®), S-1 (MBMS-247616, consisting of Tegafur and two modulators, a 5-chloro-2,4-dihydroxypyridine and potassium oxonate), ralitrexed (Tomudex®), nolatrexed (Thymitaq, AG337), LY231514 and ZD9331, as described for example in Papamicheal (1999) The Oncologist 4:478-487.
Chemical equivalents of 5-FU also include Capecitabine and Tegafur. Capecitabine is a prodrug of (5-FU) that is converted to its active form by the tumor-specific enzyme PynPase following a pathway 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®. Tegafur is a chemotherapeutic 5-FU prodrug used in the treatment of cancers. It is a component of tegafur-uracil. When metabolized, it becomes 5-FU. Tegafur-uracil, also called UFT or UFUR, is a DPD (DihydroPyrimidine Dehydrogenase) Inhibitory Flouropyrimidine drug. UFT is an oral agent which combines uracil, a competitive inhibitor of DPD, with the 5-FU prodrug tegafur in a 4:1 molar ratio. Excess uracil competes with 5-FU for DPD, thus inhibiting 5-FU catabolism.

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.

As used herein, the term "screening for or determining the genotype of one or more polymorphism a cell or tissue sample" intends to identify the genotypes of polymorphic loci of interest in the cell or tissue sample. In one aspect, a polymorphic locus is a single nucleotide polymorphic (SNP) locus. If the allelic composition of a SNP locus is heterozygous, the genotype of the SNP locus will be identified as "X/Y" wherein X and Y are two different nucleotides, e.g., A/G for the SPARC gene at position 2120. If the allelic composition of a SNP locus is homozygous, the genotype of the SNP locus will be identified as "X/X" wherein X identifies the nucleotide that is present at both alleles, e.g., G/G for the SPARC gene at position 2120.

A polymorphism can be expressed with a GenBank accession number, such as rsl05341, rsl054204, rsl059279, rsl059829 or rs3210714. Alternatively, certainly polymorphisms can be referred to by its relative location and the common genotypes. For
instance, rs1059829 is also known as SPARC G2120A and rs3210714 is also known as SPARC C2217T.

[0023] The term "genetic marker" refers to an allelic variant of a polymorphic region of a gene of interest and/or the expression level of a gene of interest.

[0024] 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.

[0025] 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.

[0026] A "polymorphic gene" refers to a gene having at least one polymorphic region.

[0027] The term "genotype" refers to the specific allelic composition of an entire cell or a certain gene and in some aspects a specific polymorphism associated with that gene, whereas the term "phenotype" refers to the detectable outward manifestations of a specific genotype.

[0028] The phrase "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 "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 refers to molecules or biological or cellular materials being substantially free from other materials associated with it in its natural environment. In one aspect, the term "isolated" refers to nucleic acid, such as DNA or RNA, or protein or polypeptide, or cell or cellular organelle, or tissue or organ, separated or purified from other DNAs or RNAs, or proteins or polypeptides, or cells or cellular organelles, or tissues or organs, respectively, that are present in the natural source. The term "isolated" also refers to a nucleic acid or peptide that is substantially free of (e.g., purified from) 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 "isolated" is also used herein to refer to cells or tissues that are isolated or purified from other cells or tissues and is meant to encompass both cultured and engineered cells or tissues.

[0033] When a genetic marker or polymorphism "is used as a basis" for identifying or 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; or (h) toxicity. 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.

[0034] The term "aiding" intends that the methods can be used in combination with other methods to select a therapy or to determine whether a gastric cancer patient is likely to experience longer or shorter tumor recurrence.

[0035] It is to be understood that information obtained using the diagnostic assays described herein may be used alone or in combination with other information, such as, but not limited to, genotypes or expression levels of other genes, clinical chemical parameters, histopathological parameters, or age, gender and weight of the subject. When used alone, the information obtained using the diagnostic assays described herein is useful in determining or identifying the clinical outcome of a treatment, selecting a patient for a treatment, or treating a patient, etc. When used in combination with other information, on the other hand, the information obtained using the diagnostic assays described herein is useful in aiding in the determination or identification of clinical outcome of a treatment, aiding in the selection of a patient for a treatment, or aiding in the treatment of a patient and etc. In a particular aspect, the genotypes or expression levels of one or more genes as disclosed herein are used in a panel of genes, each of which contributes to the final diagnosis, prognosis or treatment.
[0036] 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, a response 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, time to tumor recurrence, tumor response, complete response, partial response, stable disease, progressive disease, progression free survival, overall survival, each as measured by standards set by the National Cancer Institute and the U.S. Food and Drug Administration for the approval of new drugs. See Johnson et al. (2003) J. Clin. Oncol. 21(7): 1404-1411.

[0037] "An effective amount" intends to indicated the amount of a compound or agent administered or delivered to the patient which is most likely to result in the desired response to treatment. In one aspect, it is understood that the effective amount is a therapeutically effective amount which can be empirically determined by the patient's clinical parameters including, but not limited to the Stage of disease, age, gender, histology, and likelihood for tumor recurrence.

[0038] The term "clinical outcome", "clinical parameter", "clinical response", or "clinical endpoint" refers to any clinical observation or measurement relating to a patient's reaction to a therapy. Non-limiting examples of clinical outcomes include tumor response (TR), overall survival (OS), progression free survival (PFS), disease free survival, time to tumor recurrence (TTR), time to tumor progression (TTP), relative risk (RR), toxicity or side effect.

[0039] The term "suitable for a therapy" or "suitably treated with a therapy" shall mean that the patient is likely to exhibit one or more desirable clinical outcome as compared to patients having the same disease and receiving the same therapy but possessing a different characteristic that is under consideration for the purpose of the comparison. In one aspect, the characteristic under consideration is a genetic polymorphism or a somatic mutation. In another aspect, the characteristic under consideration is expression level of a gene or a polypeptide. In one aspect, a more desirable clinical outcome is relatively higher likelihood of or relatively better tumor response such as tumor load reduction. In another aspect, a more desirable clinical outcome is relatively longer overall survival. In yet another aspect, a more desirable clinical outcome is relatively longer progression free survival or time to...
tumor progression. In yet another aspect, a more desirable clinical outcome is relatively longer disease free survival. In further another aspect, a more desirable clinical outcome is relative reduction or delay in tumor recurrence or a relatively longer tumor recurrence. In another aspect, a more desirable clinical outcome is relatively decreased metastasis. In another aspect, a more desirable clinical outcome is relatively lower relative risk. In yet another aspect, a more desirable clinical outcome is relatively reduced toxicity or side effects. In some embodiments, more than one clinical outcomes are considered simultaneously. In one such aspect, a patient possessing a characteristic, such as a genotype of a genetic polymorphism, may exhibit more than one more desirable clinical outcomes as compared to patients having the same disease and receiving the same therapy but not possessing the characteristic. As defined herein, the patients is considered suitable for the therapy. In another such aspect, a patient possessing a characteristic may exhibit one or more desirable clinical outcome but simultaneously exhibit one or more less desirable clinical outcome. The clinical outcomes will then be considered collectively, and a decision as to whether the patient is suitable for the therapy will be made accordingly, taking into account the patient's specific situation and the relevance of the clinical outcomes. In some embodiments, progression free survival or overall survival is weighted more heavily than tumor response in a collective decision making.

[0040] A "complete response" (CR) to a therapy defines patients with evaluable but non-measurable disease, whose tumor and all evidence of disease had disappeared.

[0041] A "partial response" (PR) to a therapy defines patients with anything less than complete response that were simply categorized as demonstrating partial response.

[0042] "Stable disease" (SD) indicates that the patient is stable.

[0043] "Progressive disease" (PD) indicates that the tumor has grown (i.e. become larger), spread (i.e. metastasized to another tissue or organ) or the overall cancer has gotten worse following treatment. For example, tumor growth of more than 20 percent since the start of treatment typically indicates progressive disease. "Disease free survival" indicates the length of time after treatment of a cancer or tumor during which a patient survives with no signs of the cancer or tumor.
"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.

"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.

"No Correlation" refers to a statistical analysis showing no relationship between the allelic variant of a polymorphic region or gene expression levels and clinical parameters.

"Tumor Recurrence" as used herein and as defined by the National Cancer Institute is cancer that has recurred (come back), usually after a period of time during which the cancer could not be detected. The cancer may come back to the same place as the original (primary) tumor or to another place in the body. It is also called recurrent cancer.

"Time to Tumor Recurrence" (TTR) is defined as the time from the date of diagnosis of the cancer to the date of first recurrence, death, or until last contact if the patient was free of any tumor recurrence at the time of last contact. If a patient had not recurred, then TTR was censored at the time of death or at the last follow-up.

"Relative Risk" (RR), in statistics and mathematical epidemiology, refers to the risk of an event (or of developing a disease) relative to exposure. Relative risk is a ratio of the probability of the event occurring in the exposed group versus a non-exposed group.

A patient's likely clinical outcome following a clinical procedure such as a therapy or surgery can be expressed in relative terms. For example, a patient having a particular genotype or expression level may experience relatively longer overall survival than a patient or patients not having the genotype or expression level. The patient having the particular genotype or expression level, alternatively, can be considered as likely to survive. Similarly, a patient having a particular genotype or expression level may experience relatively longer progression free survival, or time to tumor progression, than a patient or patients not having the genotype or expression level. The patient having the particular
genotype or expression level, alternatively, can be considered as not likely to suffer tumor progression. Further, a patient having a particular genotype or expression level may experience relatively shorter time to tumor recurrence than a patient or patients not having the genotype or expression level. The patient having the particular genotype or expression level, alternatively, can be considered as not likely to suffer tumor recurrence. Yet in another example, a patient having a particular genotype or expression level may experience relatively more complete response or partial response than a patient or patients not having the genotype or expression level. The patient having the particular genotype or expression level, alternatively, can be considered as likely to respond. Accordingly, a patient that is likely to survive, or not likely to suffer tumor progression, or not likely to suffer tumor recurrence, or likely to respond following a clinical procedure is considered suitable for the clinical procedure.

[0052] A "tumor" is an abnormal growth of tissue resulting from uncontrolled, progressive multiplication of cells and serving no physiological function. A "tumor" is also known as a neoplasm.

Descriptive Embodiments

[0053] It has now been discovered that germline variations in the SPARC gene (Secreted Protein Acidic and Rich in Cysteine, also known as osteonectin and BM-40) are associated with clinical outcome in gastric cancer patients. The gastric cancer patients were treated with surgery, or in some instances, in combination with adjuvant 5-fluorouracil therapy and/or adjuvant radiotherapy. These germline variations, therefore, are useful in predicting the likely clinical outcomes of gastric cancer patient receiving these therapies, selecting gastric cancer patients for the therapies on the basis of the germline variations, and treating the gastric cancer patients that are so selected.

[0054] Accordingly, in one aspect, a method for aiding in the selection or selecting a gastric cancer patient for a therapy comprising surgical resection is provided, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, screening or determining from a tissue or cell sample isolated from the patient for the genotype of at least one polymorphism of SPARC G2120A or SPARC C2217T, wherein the patient is selected for the therapy if at least one genotype of:

a. (G/G or A/G) for SPARC G2120A; or
b. (C/C or C/T) for SPARC C2217T

is present, or the patient is not selected if neither of the genotypes is present, e.g. having
A/A for SPARC G2120A or T/T for SPARC C2217T. In one aspect, the patient is selected for the therapy if at least one of the genotypes is present. In another aspect, the patient is not selected if neither of the genotypes is present.

[0055] In one aspect, the patient is selected for the therapy if (G/G or A/G) for SPARC G2120A is present. In another aspect, the patient is not selected if (A/A) for SPARC G2120A is present.

[0056] Also provided, in another aspect, is a method for aiding in the determination or for determining whether a gastric cancer patient is likely to experience longer or shorter tumor recurrence following a therapy comprising surgical resection, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, screening or determining from a tissue or cell sample isolated from the patient for the genotype of at least one polymorphism of SPARC G2120A or SPARC C2217T, wherein the presence of at least one genotype of:

a. (G/G or A/G) for SPARC G2120A; or

b. (C/C or C/T) for SPARC C2217T
determines that the patient is likely to experience longer tumor recurrence as compared to a patient having neither of the genotypes, or the presence of neither of the genotypes determines that the patient is likely to experience shorter tumor recurrence as compared to a patient having at least one of the genotypes. In one aspect, the presence of at least one of the genotypes determines that the patient is likely to experience longer tumor recurrence as compared to a patient having neither of the genotypes. In another aspect, the presence of neither of the genotypes determines that the patient is likely to experience shorter tumor recurrence as compared to a patient having at least one of the genotypes e.g. having A/A for SPARC G2120A or T/T for SPARC C2217T.

[0057] In one aspect, the presence of (G/G or A/G) for SPARC G2120A determines that the patient is likely to experience longer tumor recurrence as compared to a patient having (A/A) for SPARC G2120A. In another aspect, the presence of (A/A) for SPARC G2120A determines that the patient is likely to experience shorter tumor recurrence as compared to a patient having (G/G or A/G) for SPARC G2120A.
[0058] The methods do not require that both of the polymorphisms be tested for every patient. In case only one of the polymorphisms is tested, "neither of the genotype is present" is satisfied when the corresponding genotype is not present for the tested polymorphism.

[0059] Various statistical algorithms are available for a statistical analysis and the presence or absence of a statistical significance may depend on the algorithm or formula used in the analysis. A finding of significance in a statistical testing indicates rejection of the null hypothesis. A finding of lack of significance, however, may only indicate that the null hypothesis is not rejected, and does not indicate that an otherwise confirmed significance is false.

[0060] The terms "longer tumor recurrence" and "shorter recurrence", as used herein, are relative to a control patient having the indicated opposite genotype(s). The control patient may be a patient or a group of patient that is actually tested side by side with the patient to whom the methods are used, or a patient or a group of patients in historical records.

[0061] The gastric cancer patient may suffer from gastric adenocarcinoma, or in some embodiments, suffer from localized gastric adenocarcinoma. Suitable therapies can include surgical resection alone, or in combination with radiotherapy and/or a chemotherapy, such as administration of an effective amount of 5-fluorouracil or a chemical equivalent thereof. In another aspect, the radiotherapy or chemotherapy are adjuvant radiotherapy or chemotherapy. In some embodiments, the patient receives the chemotherapy and/or radiotherapy after surgery.

[0062] The methods are useful in the assistance of an animal, a mammal or yet further a human patient. For the purpose of illustration only, a mammalian patient includes but is not limited to a simian, a murine, a bovine, an equine, a porcine or an ovine.

[0063] In the embodiments of the above noted methods, the sample is any sample having DNA having the gene of interest, e.g. cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, urine or at least one of non-metastatic tumor tissue, a non-metastatic tumor cell, a metastatic tumor tissue, a metastatic tumor cell, a normal tissue, a normal cell, a peripheral blood lymphocyte or a whole blood cell. In some embodiments, the sample is at least one of a fixed tissue, a frozen tissue, a
biopsy tissue, a resection tissue, a microdissected tissue, a formalin fixed paraffin embedded (FFPE) sample, or combinations thereof.

[0064] Any suitable method for identifying the genotype in the patient sample can be used and the inventions described herein are not to be limited to these methods. For the purpose of illustration only, the genotype is determined or screened for by a method comprising, or alternatively consisting essentially of, or yet further consisting of, hybridization with a selective probe or primer or amplification of the portion of the SPARC sequence of interest such as using PCR or more specifically, PCR-RFLP or microarray. These methods as well as equivalents are described herein.

Diagnostic Methods

[0065] The disclosure further provides diagnostic, prognostic and therapeutic methods, which are based, at least in part, on determination of the identity of the polymorphic region of the genes identified herein.

Polymorphic Region

[0066] For example, information obtained using the diagnostic assays described herein is useful for determining if a patient will be likely, more likely, or less likely to 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. Thus, the methods are useful in aiding in the selection of a therapy to treat the patient.

[0067] 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. The identity of the genotype or expression patterns of individual patients can then be compared to the genotype or 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, in some aspects, after isolation of a suitable nucleic acid sample using methods 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 isolating and 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 disclosure, 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.

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.

[0073] 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 (1997) 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.

[0074] 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."

[0075] 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.
[0076] 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 SI nuclease 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.

[0077] In other embodiments, alterations in electrophoretic mobility is 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).

[0078] 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).

[0079] 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.

[0080] Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant disclosure. 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).

[0081] 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) Proc. Natl. Acad. Sci. (U.S.A.) 87:8923-8927). In this method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA.

[0082] 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.

[0083] In one embodiment, the single base polymorphism can be detected by using a specialized exonuclease-resistant nucleotide, as disclosed, e.g., in Mundy, C. R. (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.

[0084] In another embodiment of the disclosure, a solution-based method is used for determining the identity of the nucleotide of the polymorphic site. Cohen, D. et al. (French Patent 2,650,840; PCT Appln. No. WO91/02087). As in the Mundy method of U.S. Patent 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.

[0085] 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. W09 1/02087) 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.

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).

[0087] 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.

[0088] Often a solid phase support is used as a support capable of binding of a primer, probe, polynucleotide, an antigen or an antibody. Well-known supports include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the support can be either soluble to some extent or insoluble for the purposes of the present disclosure. 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 supports for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

[0089] 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 is likely to experience tumor recurrence following therapy.

[0090] Sample nucleic acid for use in the above-described diagnostic and prognostic methods can be obtained from any suitable 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). 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, G. J. (1992) PCR in situ hybridization: protocols and applications, Raven Press, NY).

[0091] 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.

[0092] 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 disclosure 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 disclosure. 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 disclosure, 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.

[0093] In one embodiment, it is necessary to first amplify at least a portion of the gene of interest prior to identifying the polymorphic region of the gene of interest in a sample. Amplification can be performed, e.g., by PCR and/or LCR, according to methods known in the art. Various non-limiting examples of PCR include the herein described methods.

[0094] Allele-specific PCR is a diagnostic or cloning technique is used to identify or utilize single-nucleotide polymorphisms (SNPs). It requires prior knowledge of a DNA sequence, including differences between alleles, and uses primers whose 3’ ends encompass the SNP. PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between template and primer, so successful amplification with an SNP-specific primer signals presence of the specific SNP in a sequence (See, Saiki et al. (1986) Nature 324(6093):163-166 and U.S. Patent Nos.: 5,821,062; 7,052,845 or 7,250,258).

[0095] Assembly PCR or Polymerase Cycling Assembly (PCA) is the artificial synthesis of long DNA sequences by performing PCR on a pool of long oligonucleotides with short overlapping segments. The oligonucleotides alternate between sense and antisense directions, and the overlapping segments determine the order of the PCR fragments thereby selectively producing the final long DNA product (See, Stemmer et al. (1995) Gene 164(l):49-53 and U.S. Patent Nos.: 6,335,160; 7,058,504 or 7,323,336).

[0096] Asymmetric PCR is used to preferentially amplify one strand of the original DNA more than the other. It finds use in some types of sequencing and hybridization probing where having only one of the two complementary stands is required. PCR is carried out as usual, but with a great excess of the primers for the chosen strand. Due to the slow amplification later in the reaction after the limiting primer has been used up, extra cycles of PCR are required (See, Innis et al. (1988) Proc. Natl. Acad. Sci. U.S.A. 85(24):9436-9440 and U.S. Patent Nos.: 5,576,180; 6,106,777 or 7,179,600). A recent modification on this process, known as Linear-After-The-Exponential-PCR (LATE-PCR), uses a limiting primer with a higher melting temperature \( T_m \) than the excess primer to maintain reaction efficiency as the limiting primer concentration decreases mid-reaction (Pierce et al. (2007) Methods Mol. Med. 132:65-85).
[0097] Colony PCR uses bacterial colonies, for example E. coli, which can be rapidly screened by PCR for correct DNA vector constructs. Selected bacterial colonies are picked with a sterile toothpick and dabbed into the PCR master mix or sterile water. The PCR is started with an extended time at 95°C when standard polymerase is used or with a shortened denaturation step at 100°C and special chimeric DNA polymerase (Pavlov et al. (2006) "Thermostable DNA Polymerases for a Wide Spectrum of Applications: Comparison of a Robust Hybrid TopoTaq to other enzymes", in Kieleczawa J: DNA Sequencing II: Optimizing Preparation and Cleanup. Jones and Bartlett, pp. 241-257).

[0098] Helicase-dependent amplification is similar to traditional PCR, but uses a constant temperature rather than cycling denaturation and annealing/extension cycles. DNA Helicase, an enzyme that unwinds DNA, is used in place of thermal denaturation (See, Myriam et al. (2004) EMBO reports 5(8):795-800 and U.S. Patent No. 7,282,328).

[0099] Hot-start PCR is a technique that reduces non-specific amplification during the initial set up stages of the PCR. The technique may be performed manually by heating the reaction components to the melting temperature (e.g., 95°C) before adding the polymerase (Chou et al. (1992) Nucleic Acids Research 20:1717-1723 and U.S. Patent Nos.: 5,576,197 and 6,265,169). Specialized enzyme systems have been developed that inhibit the polymerase's activity at ambient temperature, either by the binding of an antibody (Sharkey et al. (1994) Bio/Technology 12:506-509) or by the presence of covalently bound inhibitors that only dissociate after a high-temperature activation step. Hot-start/cold-finish PCR is achieved with new hybrid polymerases that are inactive at ambient temperature and are instantly activated at elongation temperature.

[0100] Intersequence-specific (ISSR) PCR method for DNA fingerprinting that amplifies regions between some simple sequence repeats to produce a unique fingerprint of amplified fragment lengths (Zietkiewicz et al. (1994) Genomics 20(2): 176-83).

[0101] Inverse PCR is a method used to allow PCR when only one internal sequence is known. This is especially useful in identifying flanking sequences to various genomic inserts. This involves a series of DNA digestions and self ligation, resulting in known sequences at either end of the unknown sequence (Ochman et al. (1988) Genetics 120:621-623 and U.S. Patent Nos.: 6,013,486; 6,106,843 or 7,132,587).
Ligation-mediated PCR uses small DNA linkers ligated to the DNA of interest and multiple primers annealing to the DNA linkers; it has been used for DNA sequencing, genome walking, and DNA footprinting (Mueller et al. (1988) Science 246:780-786).

Methylation-specific PCR (MSP) is used to detect methylation of CpG islands in genomic DNA (Herman et al. (1996) Proc Natl Acad Sci U.S.A. 93(13):9821-9826 and U.S. Patent Nos.: 6,811,982; 6,835,541 or 7,125,673). DNA is first treated with sodium bisulfite, which converts unmethylated cytosine bases to uracil, which is recognized by PCR primers as thymine. Two PCRs are then carried out on the modified DNA, using primer sets identical except at any CpG islands within the primer sequences. At these points, one primer set recognizes DNA with cytosines to amplify methylated DNA, and one set recognizes DNA with uracil or thymine to amplify unmethylated DNA. MSP using qPCR can also be performed to obtain quantitative rather than qualitative information about methylation.

Multiplex Ligation-dependent Probe Amplification (MLPA) permits multiple targets to be amplified with only a single primer pair, thus avoiding the resolution limitations of multiplex PCR (see below).

Multiplex-PCR uses of multiple, unique primer sets within a single PCR mixture to produce amplicons of varying sizes specific to different DNA sequences (See, U.S. Patent Nos.: 5,882,856; 6,531,282 or 7,118,867). By targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes, i.e., their base pair length, should be different enough to form distinct bands when visualized by gel electrophoresis.

Nested PCR increases the specificity of DNA amplification, by reducing background due to non-specific amplification of DNA. Two sets of primers are being used in two successive PCRs. In the first reaction, one pair of primers is used to generate DNA products, which besides the intended target, may still consist of non-specifically amplified DNA fragments. The product(s) are then used in a second PCR with a set of primers whose binding sites are completely or partially different from and located 3' of each of the primers used in the first reaction (See, U.S. Patent Nos.: 5,994,006; 7,262,030 or 7,329,493).
Nested PCR is often more successful in specifically amplifying long DNA fragments than conventional PCR, but it requires more detailed knowledge of the target sequences.

[0107] Overlap-extension PCR is a genetic engineering technique allowing the construction of a DNA sequence with an alteration inserted beyond the limit of the longest practical primer length.

[0108] Quantitative PCR (Q-PCR), also known as RQ-PCR, QRT-PCR and RTQ-PCR, is used to measure the quantity of a PCR product following the reaction or in real-time. See, U.S. Patent Nos.: 6,258,540; 7,101,663 or 7,188,030. Q-PCR is the method of choice to quantitatively measure starting amounts of DNA, cDNA or RNA. Q-PCR is commonly used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample. The method with currently the highest level of accuracy is digital PCR as described in U.S. Patent No. 6,440,705; U.S. Publication No. 2007/0202525; Dressman et al. (2003) Proc. Natl. Acad. Sci USA 100(15):8817-8822 and Vogelstein et al. (1999) Proc. Natl. Acad. Sci. USA. 96(16):9236-9241. More commonly, RT-PCR refers to reverse transcription PCR (see below), which is often used in conjunction with Q-PCR. QRT-PCR methods use fluorescent dyes, such as Sybr Green, or fluorophore-containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time.

[0109] Reverse Transcription PCR (RT-PCR) is a method used to amplify, isolate or identify a known sequence from a cellular or tissue RNA (See, U.S. Patent Nos.: 6,759,195; 7,179,600 or 7,317,111). The PCR is preceded by a reaction using reverse transcriptase to convert RNA to cDNA. RT-PCR is widely used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites and, if the genomic DNA sequence of a gene is known, to map the location of exons and introns in the gene. The 5' end of a gene (corresponding to the transcription start site) is typically identified by an RT-PCR method, named Rapid Amplification of cDNA Ends (RACE-PCR).

[0110] Thermal asymmetric interlaced PCR (TAIL-PCR) is used to isolate unknown sequence flanking a known sequence. Within the known sequence TAIL-PCR uses a nested pair of primers with differing annealing temperatures; a degenerate primer is used to amplify in the other direction from the unknown sequence (Liu et al. (1995) Genomics 25(3):674-81).
Touchdown PCR a variant of PCR that aims to reduce nonspecific background by gradually lowering the annealing temperature as PCR cycling progresses. The annealing temperature at the initial cycles is usually a few degrees (3-5°C) above the $T_m$ of the primers used, while at the later cycles, it is a few degrees (3-5°C) below the primer $T_m$. The higher temperatures give greater specificity for primer binding, and the lower temperatures permit more efficient amplification from the specific products formed during the initial cycles (Don et al. (1991) Nucl. Acids Res. 19:4008 and U.S. Patent No. 6,232,063).

In one embodiment of the disclosure, 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 gene of interest. (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.
Probes can be affixed to surfaces for use as "gene chips." Such gene chips 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 disclosure 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.

This disclosure also provides for a prognostic panel of genetic markers selected from, but not limited to the genetic polymorphisms identified herein. The prognostic panel comprises probes or primers that can be used to amplify and/or for determining the molecular structure of the polymorphisms identified herein. 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 disclosure is a means to identify the genotype of a patient sample for the genes of interest identified above.

In one aspect, the panel contains the herein identified probes or primers as well 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.

Primers or probes can be affixed to surfaces 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 disclosure 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.

[0118] Various "gene chips" or "microarrays" 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 U.S. Patent Publ. Nos.: 2007/01 11322, 2007/0099198, 2007/0084997, 2007/0059769 and 2007/0059765 and US Patent 7,138,506, 7,070,740, and 6,989,267.

[0119] In one aspect, "gene chips" or "microarrays" containing probes or primers for the gene of interest are provided alone or in combination with other probes and/or primers. 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 genetic profile of the patient is then determined with the aid of the aforementioned apparatus and methods.
Methods of Treatment

[0120] The disclosure further provides methods of treating a cancer patient after the patient is identified to likely to experience a better clinical outcome, such as longer overall survival, longer progression free survival, better response, longer time to tumor recurrence, or reduced side effects.

[0121] Thus, a method for treating a gastric cancer patient selected for treatment based on the presence of at least one genotype selected from:

a. (G/G or A/G) for SPARC G2120A; or  
b. (C/C or C/T) for SPARC C2217T

in a tissue or cell sample from the patient is provided, comprising, or alternatively consisting essentially of, or yet alternatively consisting of, administering to the patient a therapy comprising surgical resection. In one aspect, the patient is selected for treatment based on the presence of (G/G or A/G) for SPARC G2120A in a tissue or cell sample from the patient.

[0122] Further provided is use of a therapy comprising 5-fluorouracil or a chemical equivalent thereof for the preparation of a medicament to treat a gastric cancer patient selected based on the presence of at least one polymorphism selected from:

a. (G/G or A/G) for SPARC G2120A; or  
b. (C/C or C/T) for SPARC C2217T

in a tissue or cell sample of the patient. In one aspect, the patient is selected based on the presence of (G/G or A/G) for SPARC G2120A. In another aspect, the patient was identified by a method comprising screening a tissue or cell sample isolated from the patient for the genotype of at least one polymorphism selected from SPARC G2120A or SPARC C2217T.

[0123] Also provided is a therapy comprising 5-fluorouracil or a chemical equivalent thereof for use in treating a gastric cancer patient selected based on the presence of at least one polymorphism selected from:

a. (G/G or A/G) for SPARC G2120A; or  
b. (C/C or C/T) for SPARC C2217T

in a tissue or cell sample of the patient. In one aspect, the patient is selected based on the presence of (G/G or A/G) for SPARC G2120A. In another aspect, the patient was identified
by a method comprising screening a tissue or cell sample isolated from the patient for the
genotype of at least one polymorphism selected from SPARC G2120A or SPARC C2217T.

[0124]  In one aspect of the above methods, use or therapies, the gastric cancer patient
suffers from gastric adenocarcinoma. In another aspect, the gastric adenocarcinoma is
localized gastric adenocarcinoma.

[0125]  In one aspect, the therapy further comprises, or alternatively consists essentially of,
or yet further consists of radiotherapy. In another aspect, the therapy further comprises
chemotherapy which in turn can comprise, or alternatively consist essentially of, or yet
further consist of administration of an effective amount of 5-fluorouracil or a chemical
equivalent thereof. The tissue or cell sample can comprise or alternatively consist
essentially of or yet further consist any patient sample having DNA having the gene of
interest, e.g. cells, protein or membrane extracts of cells, or biological fluids such as
sputum, blood, serum, plasma, or urine. Further examples of the samples include a non-
metastatic tumor tissue, a non-metastatic tumor cell, a metastatic tumor tissue, a metastatic
tumor cell, a normal tissue, a normal cell, a peripheral blood lymphocyte or a whole blood
cell.

[0126]  The genotype can be determined by any appropriate method non-limiting
examples of such include without limitation hybridization, PCR or PCR-RFLP.

[0127]  The formulation comprising the necessary chemotherapy or chemically 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, 0.4., 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%).
[0128] The chemotherapeutic agents or drugs 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 raffmose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol) and myoinositol.

[0129] 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, carbonic acid, tartaric 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), dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl-quadrate.-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 "pharmaceutically 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., 15th Ed. (Mack Publ. Co., Easton (1975) and Williams & Williams, (1995), and in the "PHYSICIAN'S DESK REFERENCE", 52nd ed., Medical Economics, Montvale, N.J. (1998).

Many combination chemotherapeutic regimens are known to the art, such as combinations of platinum compounds and taxanes, e.g. carboplatin/paclitaxel, capecitabine/docetaxel, the "Cooper regimen", fluorouracil-levamisole, fluorouracil-leucovorin, fluorouracil/oxaliplatin, methotrexate-leucovorin, and the like.

Combinations of chemotherapies and molecular targeted therapies, biologic therapies, and radiation therapies are also well known to the art; including therapies such as trastuzumab plus paclitaxel, alone or in further combination with platinum compounds such as oxaliplatin, for certain breast cancers, and many other such regimens for other cancers; and the "Dublin regimen" 5-fluorouracil IV over 16 hours on days 1-5 and 75 mg/m² cisplatin IV or oxaliplatin over 8 hours on day 7, with repetition at 6 weeks, in combination with 40 Gy radiotherapy in 15 fractions over the first 3 weeks) and the "Michigan regimen" (5-fluorouracil plus cisplatin or oxaliplatin plus vinblastine plus radiotherapy), both for esophageal cancer, and many other such regimens for other cancers, including colorectal cancer.

In another aspect of the invention, the method for treating a patient comprises, or alternatively consists essentially of, or yet further consists of surgical resection of a metastatic or non-metastatic solid malignant tumor and, in some aspects, in combination with radiation. Methods for treating said tumors derived from a gastrointestinal cancer, e.g., metastatic or non-metastatic rectal cancer, metastatic or non-metastatic colon cancer, metastatic or non-metastatic colorectal cancer, gastric cancer, esophageal cancer, stage II colon cancer, stage II rectal cancer or stage III rectal cancer by surgical resection and/or radiation are known to one skilled in the art. Guidelines describing methods for treatment
by surgical resection and/or radiation can be found at the National Comprehensive Cancer Network’s web site, nccn.org, last accessed on May 27, 2008.

[0134] The invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of the chemotherapy as described herein and/or or 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 the chemotherapy and/or 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.

[0135] Chemotherapeutic 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.

[0136] 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 those pen-injector devices for delivery of a solution such as BD Pens, BD Autojectore, Humaject® NovoPen®, B-D®Pen, AutoPen®, and OptiPen®, GenotropinPen®, Genotronorm Pen®, Humatro Pen®, Reco-Pen®, Roferon Pen®, Biojector®, ject®, J-tip Needle-Free Injector®, Intraject®, Medi-Ject®, e.g., as made or developed by Becton Dickensen (Franklin Lakes, N.J. available at bectondickenson.com), Disetronic (Burgdorf, Switzerland, available at disetronic.com; Bioject, Portland, Oregon (available atbioject.com); National Medical Products, Weston Medical (Peterborough, UK, available at weston-medical.com), Medi-Ject Corp (Minneapolis, Minn., available at mediject.com).

[0137] Various delivery systems are known and can be used to administer a chemotherapeutic 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 intra-arterial, intra-muscular, 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.

[0138] 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.

[0139] Also provided is a medicament or a therapy comprising an effective amount of a chemotherapeutic as described herein for treatment of a human cancer patient having high or low gene expression or the polymorphism of the gene of interest as identified in the experimental examples.

[0140] Methods of administering pharmaceutical compositions are well known to those of ordinary skill in the art and include, but are not limited to, oral, microinjection, intravenous or parenteral administration. The compositions are intended for topical, oral, or local
administration as well as intravenously, subcutaneously, or intramuscularly. Administration can be effected continuously or intermittently throughout the course of the treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the cancer being treated and the patient, 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.

Nucleic Acids

[0141] In one aspect, the nucleic acid sequences of the gene of interest, or portions thereof, can be the basis for probes or primers, e.g., in methods for determining expression level of the gene of interest or the allelic variant of a polymorphic region of a gene of interest identified in the experimental section below. Thus, they can be used in the methods of the disclosure to determine which therapy is most likely to treat an individual's cancer.

[0142] The methods of the disclosure 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 disclosure are human nucleic acids.

[0143] Primers for use in the methods of the disclosure 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 disclosure are nucleic acids which hybridize to the gene of interest and which are not further extended. For example, a probe is a nucleic acid which hybridizes to 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 expression levels of the gene of interest. Primers and/or probes for use in the methods can be provided as isolated single stranded oligonucleotides or alternatively, as isolated double stranded oligonucleotides.

[0144] 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.

[0145] 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 disclosure will hybridize selectively to nucleotide sequences located about 100 to about 1000 nucleotides apart.

[0146] 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.

[0147] Yet other preferred primers of the disclosure are nucleic acids which are capable of selectively hybridizing to the gene. 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.

[0148] 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.

[0149] 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).

[0150] The nucleic acids used in the methods of the disclosure 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 Publ. 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 disclosure may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0151] The isolated nucleic acids used in the methods of the disclosure 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 phosphotriester, and a formacetal or analog thereof.

[0152] The nucleic acids, or fragments thereof, to be used in the methods of the disclosure can be prepared according to methods known in the art and described, e.g., in Sambrook et al. (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).

[0153] 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.

Kits

[0154] Also provided is a kit for use in aiding in determining or determining if a gastric cancer patient treated with a therapy comprising surgical resection is likely to experience longer or shorter tumor recurrence, comprising suitable primers or probes for determining the genotype of at least one polymorphism selected from SPARC G2120A or SPARC C2217T, and instructions for use therein.
The gastric cancer patient may suffer from gastric adenocarcinoma, or in some embodiments, suffer from localized gastric adenocarcinoma. Suitable therapies can include surgical resection alone, or in combination with radiotherapy and/or a chemotherapy, such as administration of an effective amount of 5-fluorouracil or a chemical equivalent thereof. In another aspect, the radiotherapy or chemotherapy are adjuvant radiotherapy or chemotherapy.

The kits are useful in the assistance of an animal, a mammal or yet further a human patient. For the purpose of illustration only, a mammal includes but is not limited to a simian, a murine, a bovine, an equine, a porcine or an ovine.

The kit can comprise at least one probe or primer which is capable of specifically hybridizing to 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 disclosure 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 disclosure.

[0161] The test samples used in the diagnostic kits include any sample having DNA having the gene of interest, e.g. cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test samples can also be a tumor cell, a normal cell adjacent to a tumor, a normal cell corresponding to the tumor tissue type, a blood cell, a peripheral blood lymphocyte, or combinations thereof. 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.

[0162] 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.

[0163] 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 Disclosure**

[0164] The identification of the polymorphic region or the expression level 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 example which is included merely for purposes of illustration of certain aspects and embodiments of the present disclosure, and are not intended to limit the invention.
EXPERIMENTAL EXAMPLES

EXAMPLE 1

Patients and Methods

[0166] One hundred and eight patients (n = 108) with localized (stage lb - IV) gastric adenocarcinoma who were treated with surgery alone or surgery and adjuvant (radio-) chemotherapy, at the University of Southern California/Norris Comprehensive Cancer Center (USC/NCCC), the Los Angeles County/University of Southern California medical center from 1992 to 2008 were eligible for our preliminary study. The polymorphisms tested were selected by the following criteria: 1. that the polymorphism has some degree of likelihood to alter the function of the gene in a biological relevant manner; 2. that the frequency of the polymorphism is abundant enough (with a frequency of the variant allele of more than 20 %) to impact the clinical outcome. DNA was extracted from formalin-fixed paraffin-embedded normal tissue and the samples were tested using a PCR-based restriction fragment length polymorphism using primers with sequences shown in Table 1. All five SNP's tested in the SPARC gene are in the 3'UTR (rs1 05341 1, rs1 054204, rs1059279, rs1059829 (G2120A), rs3210714 (C2217T)).

Table 1. Primer sequences

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primer Sequence 5' to 3' (SEQ ID NO.)</th>
<th>Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1053411</td>
<td>CATTTTTAGCAAGTGAATGTATTC (1)</td>
<td>C/G</td>
</tr>
<tr>
<td>Reverse</td>
<td>AATCCACTCCTCCACAGTACC (2)</td>
<td></td>
</tr>
<tr>
<td>rs1054204</td>
<td>GCTCCCCAAAAGTTGAACCA (3)</td>
<td>C/G</td>
</tr>
<tr>
<td>Reverse</td>
<td>GGTTTGCCTGAGGCTGAAC (4)</td>
<td></td>
</tr>
<tr>
<td>rs1059279</td>
<td>AATGCTTGGAGGTGAACGAG (5)</td>
<td>G/T</td>
</tr>
<tr>
<td>Reverse</td>
<td>GGAGTTGGAATCGGTTGT (6)</td>
<td></td>
</tr>
<tr>
<td>rs1059829</td>
<td>CCCAGGAAGGCAGTTTCTAA (7)</td>
<td>G/A</td>
</tr>
<tr>
<td>Reverse</td>
<td>CGTTCACCTCAGCATTTC (8)</td>
<td></td>
</tr>
<tr>
<td>rs3210714</td>
<td>GCAAAAGTTTGCATTTGCT (9)</td>
<td>C/T</td>
</tr>
<tr>
<td>Reverse</td>
<td>TGGGTGATTGACATGGTCT (10)</td>
<td></td>
</tr>
</tbody>
</table>
Results

[0167] 104 patients (63 men, 41 women) with a median age of 57 years (range 26-85) were successfully genotyped. The median time to recurrence (TTR) was 2.2 years (95% CI: 1.7-4.4 years). The median OS was 4.5 years (95% CI: 3.4-5.7 years) with median follow-up of 3.3 years (range 0.1-14.6) years.

TTR associated with the SPARC rs1059829 polymorphism;

[0168] 28 of 100 (28%) patients were homozygous G/G at the SPARC G2120A gene locus; 44 patients (44%) were heterozygous A/G, and 28 patients (28%) were homozygous for the A/A variation. Patients carrying at least one G allele (G/G; AG/) showed a median TTR of 2.9 years compared to 1.7 years TTR for patients with A A (p = 0.0065, log-rank test).

TTR associated with the SPARC rs3210714 polymorphism;

[0169] 32 of 101 (32%) patients were homozygous C/C at the SPARC C2217T gene locus; 41 patients (41%) were heterozygous C/T, and 28 patients (28%) were homozygous for the T/T variation. Patients carrying at least one C allele (C/C; C/T) showed a median TTR of 2.9 years compared to 1.7 years TTR for patients with T/T (p = 0.0048, log-rank test).

[0170] SPARC is an extracellular matrix glycoprotein with high binding affinity to albumin that mediates cell matrix interactions (Sage (1984) J. Biol. Chem. 259:3993-4007). In addition SPARC acts as a key regulator of critical cellular functions such as proliferation, survival, cell migration and angiogenesis. In normal tissue, SPARC expression is limited to bone and tissues undergoing development, remodeling, and repair (Porter et al. (1995) J. Histochem. Cytochem. 43:791-800).

[0171] These data indicate that the common rs1059829 (G2120A) and rs3210714 (C2217T) polymorphisms in the 3'UTR of the SPARC gene significantly affect clinical outcome in patients with localized gastric carcinoma. This can be explained by post-transcriptional regulation of SPARC mRNA level through control of mRNA stability and/or translational efficiency by the 3'UTR. The data therefore indicate that SPARC may play a role in patients with gastric adenocarcinoma.
EXAMPLE 2

[0172] This example is an extension of Example 1. Based on Example 1, two potentially functional germline variations (rs1059829 (G2120A) and rs3210714 (C2217T)) within the SPARC gene are associated with clinical outcome in patients with surgically resected gastric cancer. Data from this present example further confirm that SPARC rs1059829 (G2120A) polymorphism significantly affects tumor recurrence and may therefore act as independent prognostic marker in patients with surgically resected gastric cancer.

[0173] Methods: Either blood or (formalin fixed paraffin embedded) FFPE tissues specimens of 137 patients (54 females and 83 males; median age = 55 yrs; range = 21-85 yrs) were obtained at University of Southern California (n = 105) and Memorial Sloan-Kettering Cancer Center medical facilities (n = 32). The median follow-up was 3.3 years. Sixty-one of 137 patients (45%) had tumor recurrence, with a probability of 3-year recurrence of 52%. Genomic DNA was isolated from peripheral blood or FFPE tissues and two polymorphisms within the SPARC gene were determined by PCR-RFLP technique.

[0174] Results: Patients carrying at least one G allele of the SPARC rs1059829 (G2120A) polymorphism (GG; AG) showed a median TTR of 3.7 years compared to 2.1 years TTR for patients with AA (p = 0.033, log-rank test). In multivariate analysis SPARC rs1059829 (G2120A) showed to be an independent prognostic factor for TTR after adjusting for race, N-stage, T-stage and type of chemotherapy in (adjusted p value = 0.037). SPARC rs3210714 (C2217T) showed no significant associations for TTR in this analysis.

[0175] 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 aiding in the selection of or selecting a gastric cancer patient for a therapy comprising surgical resection, comprising screening or determining from a tissue or cell sample isolated from the patient for the genotype of at least one polymorphism of SPARC G2120A or SPARC C2217T, wherein the patient is selected for the therapy if at least one genotype of:
   a. (G/G or A/G) for SPARC G2120A; or
   b. (C/C or C/T) for SPARC C2217T

is present, or the patient is not selected if neither of the genotypes is present.

2. The method of claim 1, wherein the patient is selected for the therapy if at least one of the genotypes is present.

3. The method of claim 1, wherein the patient is not selected if neither of the genotypes are present.

4. A method for aiding in the determination of or determining whether a gastric cancer patient is likely to experience longer or shorter tumor recurrence following a therapy comprising surgical resection, comprising screening or determining from a tissue or cell sample isolated from the patient for the genotype of at least one polymorphism of SPARC G2120A or SPARC C2217T, wherein the presence of at least one genotype of:
   a. (G/G or A/G) for SPARC G2120A; or
   b. (C/C or C/T) for SPARC C2217T
determines that the patient is likely to experience longer tumor recurrence as compared to a patient having neither of the genotypes, or the presence of neither of the genotypes determines that the patient is likely to experience shorter tumor recurrence as compared to a patient having at least one of the genotypes.

5. The method of claim 4, wherein the presence of at least one of the genotypes determines that the patient is likely to experience longer tumor recurrence as compared to a patient having neither of the genotypes.
6. The method of claim 4, wherein the presence of neither of the genotypes determines that the patient is likely to experience shorter tumor recurrence as compared to a patient having at least one of the genotypes.

7. A method for aiding in the treatment or treating a gastric cancer patient selected for treatment based on the presence of at least one genotype selected from:
   a. (G/G or A/G) for SPARC G2120A; or
   b. (C/C or C/T) for SPARC C2217T
comprising administering to the patient a therapy comprising surgical resection, wherein the patient was identified by a method comprising screening or determining from a tissue or cell sample isolated from the patient for the genotype of at least one polymorphism of SPARC G2120A or SPARC C2217T, thereby treating the patient.

8. The method of any of claims 1 to 7, wherein the gastric cancer patient suffers from gastric adenocarcinoma.

9. The method of claim 8, wherein the gastric adenocarcinoma is localized gastric adenocarcinoma.

10. The method of any of claims 1 to 9, wherein the therapy further comprises radiotherapy.

11. The method of any of claims 1 to 10, wherein the therapy further comprises chemotherapy.

12. The method of claim 11, wherein the chemotherapy comprises administration of an effective amount of 5-fluorouracil or a chemical equivalent thereof.

13. The method of any of claims 1 to 12, wherein the tissue or cell sample comprises tissue or cell selected from a non-metastatic tumor tissue, a non-metastatic tumor cell, a metastatic tumor tissue, a metastatic tumor cell, a normal tissue, a normal cell, a peripheral blood lymphocyte or a whole blood cell.
14. The method of any of claims 1 to 13, wherein the genotype is determined by a method comprising hybridization, PCR or PCR-RFLP.

15. The method of any of claims 1 to 14, wherein the patient is a mammalian patient.

16. The method of claim 15, wherein the mammalian patient is a simian, a murine, a bovine, an equine, a porcine, an ovine, or a human.

17. The method of claim 16, wherein the patient is a human patient.

18. Use of a therapy comprising 5-fluorouracil or a chemical equivalent thereof for the preparation of a medicament to treat a gastric cancer patient selected based on the presence of at least one polymorphism selected from:

   a. (G/G or A/G) for SPARC G2120A; or
   b. (C/C or C/T) for SPARC C2217T

in a tissue or cell sample isolated from the patient.

19. The use of claim 18, wherein the gastric cancer patient suffers from gastric adenocarcinoma.

20. The use of claim 18, wherein the gastric adenocarcinoma is localized gastric adenocarcinoma.

21. The use of any of claims 18 to 20, wherein the therapy further comprises radiotherapy.

22. The use of any of claims 18 to 21, wherein the therapy further comprises chemotherapy.

23. The use of claim 18, wherein the chemotherapy comprises administration of an effective amount of 5-fluorouracil or a chemical equivalent thereof.

24. The use of any of claims 18 to 23, wherein the tissue or cell sample comprises tissue or cell selected from a non-metastatic tumor tissue, a non-metastatic tumor cell, a metastatic
tumor tissue, a metastatic tumor cell, a normal tissue, a normal cell, a peripheral blood lymphocyte or a whole blood cell.

25. The use of any of claims 18 to 24, wherein the genotype is determined by a method comprising hybridization, PCR or PCR-RFLP.

26. A kit for use in determining if a gastric cancer patient treated with a therapy comprising surgical resection is likely to experience longer or shorter tumor recurrence, comprising suitable primers or probes for determining the genotype of at least one polymorphism selected from SPARC G2120A or SPARC C2217T, and instructions for use therein.

27. The kit of claim 26, wherein the patient is suffering from gastric adenocarcinoma.

28. The kit of claim 26 or 27, wherein the patient is suffering from localized gastric adenocarcinoma.

29. The kit of any of claims 26 to 28, wherein the therapy further comprises radiotherapy.

30. The kit of any of claims 26 to 29, wherein the therapy further comprises chemotherapy.

31. The kit of claim 30, wherein the chemotherapy comprises administration of an effective amount of 5-fluorouracil or a chemical equivalent thereof.

32. The kit any of claims 26 to 31, wherein the tissue or cell sample comprises tissue or cell selected from a non-metastatic tumor tissue, a non-metastatic tumor cell, a metastatic tumor tissue, a metastatic tumor cell, a normal tissue, a normal cell, a peripheral blood lymphocyte or a whole blood cell.

33. The kit of any of claims 26 to 31, wherein the genotype is determined by a method comprising hybridization, PCR or PCR-RFLP.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

**INV. C12Q1/68**

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C12Q

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

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

EPO-Internal, BIOSIS, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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Further documents are listed in the continuation of Box C. See patent family annex.

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<td>&quot;E&quot; earlier document published on or after the international filing date</td>
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<td>&quot;L&quot; document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td>
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<td>&quot;O&quot; document referring to an oral disclosure, use, exhibition or other means</td>
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<td>&quot;P&quot; document published prior to the international filing date but later than the priority date claimed</td>
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<td>&quot;T&quot; later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td>
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<td>&quot;A&quot; document member of the same patent family</td>
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Date of the actual completion of the international search: 11 April 2011

Date of mailing of the international search report: 27/04/2011

Name and mailing address of the ISA:

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer: Cornelis, Karen
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<td>FRANKE KONRAD ET AL: &quot;Differenti al Expression of SPARC in Intestinal-type Gastric Cancer Correl ates with Tumor Progression on and Nodal Spread&quot;, TRANSLATIONAL ONCOLOGY, vol. 2, no. 4, December 2009 (2009-12), pages 310-320, XP002630778, the whole document</td>
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<td>the whole document</td>
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<td>Wo 2006/058425 AI (UNIV BRITISH COLUMBIA [CA]; TAI ISABELLA T [CA]) 8 June 2006 (2006-06-08) the whole document</td>
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**INTERNATIONAL SEARCH REPORT**

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

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<td>[x] subsequently to this Authority for the purpose of search</td>
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<td>2.</td>
<td>In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.</td>
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