Title: COMpanion DiAgnostic AssAYS For cancer therapy

Abstract: A method for classifying cancer patients as eligible to receive cancer therapy comprising determination of the presence or absence in a patient tissue sample of chromosomal copy number gain at chromosomal locus 18q21-q22. The classification of cancer patients based upon the presence or absence of 18q21-q22 gain allows selection of patients to receive chemotherapy, such as therapy with a Bcl-2 family inhibitor, and for monitoring patient response to therapy.
COMPANION DIAGNOSTIC ASSAYS FOR CANCER THERAPY

FIELD OF THE INVENTION

This invention relates to diagnostic assays useful in classification of patients for selection of cancer therapy, and in particular relates to measurement of certain genomic biomarkers that allow identification of patients eligible to receive Bcl-2-family antagonist therapy and that permit monitoring of patient response to such therapy.

BACKGROUND OF THE INVENTION

Genetic heterogeneity of cancer is a factor complicating the development of efficacious cancer drugs. Cancers that are considered to be a single disease entity according to classical histopathological classification often reveal multiple genomic subtypes when subjected to molecular profiling. In some cases, molecular classification proved to be more accurate than the classical pathology. The efficacy of targeted cancer drugs may correlate with the presence of a genomic feature, such as a gene amplification, Cobleigh, M. A., et al., "Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease", J. Clin. Oncol., 17: 2639-2648, 1999; or a mutation, Lynch, T. J., et al., "Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib", N. Engl. J. Med., 550: 2129-2139, 2004. For Her-2 in breast cancer, it has been demonstrated that detection of gene amplification, provides superior prognostic and treatment selection information as compared with the detection by immunohistochemistry (IHC) of the protein overexpression, Pauletti, G., et al., "Assessment of Methods for Tissue-Based Detection of the HER-2/neu Alteration in Human Breast Cancer: A Direct Comparison of Fluorescence In Situ Hybridization and Immunohistochemistry", J. Clin. Oncol., 18: 3651-3664, 2000. A need therefore exists for genomic classification markers that may improve the response rate of patients to targeted cancer therapy.
Lung cancer is an area of active research for new targeted cancer therapies. Lung malignancies are the leading cause of cancer mortality, which will result in approximately 160,000 deaths in the United States in 2006. Small-cell lung carcinoma (SCLC) is a histopathological subtype of lung cancer, which represents approximately 20% of lung cancer cases. The survival rate for this subtype is low (long-term survival 4-5%) and has not improved significantly in the past decade, despite the introduction of new chemotherapy regimens. The remainder of lung cancer cases are non-small-cell lung carcinomas (NSCLC), a category which is comprised of several common subtypes. In the past several years, there has been substantial progress in the development of targeted therapies for NSCLC, such as erlotinib and gefitinib. Genomic biomarkers have been discovered which enable stratification of NSCLC patients into potential responders and non-responders. In particular, mutations and amplifications in the EGFR kinase domain were shown to correlate with the response to erlotinib and gefitinib. Unfortunately, no such progress has been achieved with SCLC, even though genomic analysis of SCLC cell lines and tumors is reported in Ashman, J. N., et al., Chromosomal alterations in small cell lung cancer revealed by multicolour fluorescence in situ hybridization. *Int. J. Cancer*, 102: 230-236, 2002; 17; Coe, B. P., et al., "Gain of a region on 7p22.3, containing MADII, is the most frequent event in small-cell lung cancer cell lines", *Genes Chromosomes Cancer*, 45: 11-19, 2006; and Kim, Y. H., et al., "Combined microarray analysis of small cell lung cancer reveals altered apoptotic balance and distinct expression signatures of MYC family gene amplification", *Oncogene*, 25: 130-138, 2006.

Targeted cancer therapy research has been reported against members of the Bcl-2 protein family, which are central regulators of programmed cell death. The Bcl-2 family members that inhibit apoptosis are overexpressed in cancers and contribute to tumorigenesis. Bcl-2 expression has been strongly correlated with resistance to cancer therapy and decreased survival. For example, the emergence of androgen independence in prostate cancer is characterized by a high incidence of Bcl-2 expression (> 40% of the cohort examined), see Chaudhary, K. S., et al., "Role of the Bcl-2 gene family in prostate cancer progression and its implications for therapeutic intervention" [Review], *Environmental Health Perspectives* 1999, 107, 49-57, which
also corresponds to an increased resistance to therapy. Furthermore, overexpression of Bcl-2 in both NSCLC and SCLC cell lines, has been demonstrated to induce resistance to cytotoxic agents, Ohmori, T., et al., "Apoptosis of lung cancer cells caused by some anti-cancer agents (MMC, CPT-II, ADM) is inhibited by bcl-2", Biochem. Biophys. Res. Commun. 1993, 192, 30-36. Yasui, K., et al., "Alteration in Copy Numbers of Genes as a Mechanism for Acquired Drug Resistance", Can. Res. 2004, 64, 1403-1410, reports analysis of the etopside resistant ovarian cancer cell line SKOV3/VP for chromosome copy number gain. Yasui et al. describe copy number gain at the Bcl-w (BCL2L2) locus and conclude that Bcl-w expression is "at least partially responsible for the chemoresistance" of SKOV3/VP, Ibid. at p. 1409. Yatsui does not disclose identification of Bcl-2 family copy number change in any other cancer cell line.


A compound called ABT-737 is a small-molecule inhibitor of the Bcl-2 family members Bcl-2, Bcl-XL, and Bcl-w, and has been shown to induce regression of solid tumors, Oltersdorf, T., "An inhibitor of Bcl-2 family proteins induces regression of solid tumours", Nature, 435: 677-681, 2005. ABT-737 has been tested against a
diverse panel of human cancer cell lines and has displayed selective potency against SCLC and lymphoma cell lines. *Ibid.* ABT-737's chemical structure is provided by Oltersdorf et al. at p. 679.

Because of the potential therapeutic use of inhibitors for Bcl-2 family members, companion diagnostic assays that would identify patients eligible to receive Bcl-2 family inhibitor therapy are needed. Additionally, there is a clear need to support this therapy with diagnostic assays using biomarkers that would facilitate monitoring the efficacy of Bcl-2 family inhibition therapy.

**SUMMARY OF THE INVENTION**

The invention provides companion diagnostic assays for classification of patients for cancer treatment which comprise assessment in a patient tissue sample of chromosomal copy number gain at the chromosome 18q21-q22 locus. This chromosome locus includes the Bcl-2 gene at 18q21.3. The inventive assays include assay methods for identifying patients eligible to receive Bcl-2 family antagonist therapy and for monitoring patient response to such therapy. The invention preferably comprises determining by fluorescence in situ hybridization the presence or absence of chromosomal copy number gain at a Bcl-2 family gene chromosomal locus. Patients classified as having copy number gain at a Bcl-2 family gene locus are eligible to receive anti-Bcl-2 family therapy because they are more likely to respond to this therapy. In addition, patients having this copy number gain may be resistant to other cancer therapy. Thus, determination of the presence of 18q21-q22 copy number gain in a cancer tissue is a general therapy stratification marker.

In a preferred embodiment, the invention comprises a method for identifying a patient as eligible to receive Bcl-2 family inhibitor therapy comprising:

(a) providing a tissue sample from a patient; (b) determining chromosomal copy number of chromosome 18q21-q22 and of chromosome 14q1 1.2; and (c) identifying the patient as eligible for Bcl-2 family inhibitor therapy where the patient's sample is classified as having copy number gain of at least one of 18q21-q22 and 14q1 1.2. In
this embodiment, the copy number gain is preferably determined by a multi-color fluorescence in situ hybridization (FISH) assay, for example, performed on a lung cancer tumor biopsy sample.

The invention also comprises a method for monitoring a patient being treated with Bcl-2 family inhibitor therapy comprising: (a) providing a peripheral blood sample from a patient; (b) measuring levels in the peripheral blood sample of circulating tumor cells having increased chromosomal copy number of at least one of 18q21-q22 and 14qll.2; and (c) comparing the level of circulating tumor cells having increased copy number relative to the patient baseline blood level of number of circulating tumor cells having the increased copy number.

The invention further comprises a reagent kit for an assay for classification of a patient for cancer therapy, such as eligibility for Bcl-2 family inhibitor therapy, comprising a container comprising at least one nucleic acid probe capable of hybridizing under selected stringency conditions to a DNA sequence located within chromosome locus 18q21-q22 or 14qll.2. In a preferred embodiment, the reagent kits of the invention comprise in situ hybridization probes capable of identifying chromosomal copy number change at the chromosomal locus of both Bcl-2 and Bcl-w.

The invention has significant capability to provide improved stratification of patients for cancer therapy, and in particular for Bcl-2 family inhibitor therapy. The assessment of these biomarkers with the invention also allows tracking of individual patient response to the therapy. The inventive assays have particular utility for classification of SCLC and lymphoma patients.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows a plot of experimental quantitative PCR determination of chromosomal copy number on chromosome arm 18q in various SCLC cell lines sensitive and resistant to ABT-737.
Figure 2 depicts the relationship between the Bcl-2 gene copy number of SCLC cell lines and sensitivity of the cell lines to ABT-737.

Figure 3 shows classification of a 62 patient cohort of clinical SCLC samples by chromosome copy number of the Bcl-2 locus.

DETAILED DESCRIPTION OF THE INVENTION

I. General

The invention is based on the discovery by Applicants of chromosome copy number changes in small cell lung cancer cell lines that correlate to therapy sensitivity. In particular, Applicants correlated chromosome copy number gain at 18q21-q22 to sensitivity to a Bcl-2 family inhibitor. The Bcl-2 gene in this locus is a key regulator of cell survival, and other genes in this locus such as NOXA also impact cell survival. Chromosomal gain at 18q21-q22 can thus mark sensitivity to other cancer therapy, such as other chemotherapy or radiation therapy.

As used herein, a "Bcl-2 family inhibitor" refers to a therapeutic compound of any type, including small molecule-, antibody-, antisense-, small interfering RNA-, or microRNA-based compounds, that binds to at least one of Bcl-2, Bcl-XL, and Bcl-w, and antagonizes the activity of the Bcl-2 family related nucleic acid or protein. The inventive methods are useful with any known or hereafter developed Bcl-2 family inhibitor. One Bcl-2 family inhibitor is ABT-737, N-(4-(4-((4'-chloro(l,l'-biphenyl)-2-yl)methyl)piperazin-1-yl)benzoyl)-4-(((1R)-3-(dimethylamino)-1-((phenylsulfanyl)methyl)propyl)amino)-3-nitrobenzenesulfonamide, which binds to each of Bcl-2, Bcl-XL, and Bcl-w. Another Bcl-2 family inhibitor is ABT-263, N-(4-(4-((2-(4-chlorophenyl)-5, 5-dimethyl-1-cyclohex-1-en-1-yl)methyl)piperazin-1-yl)benzoyl)-4-(((IR)-3-(morpholin-4-yl)-l-((phenylsulfanyl)methyl)propyl)amino)-3-((trifluoromethyl)sulfanyl)benzenesulfonamide. The chemical structure of ABT-263 is [related to ABT-737 and its chemical structure is]:

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The assays of the invention have potential use with targeted cancer therapy. In particular, the inventive assays are useful with therapy selection for small cell lung cancer and lymphoma patients, such as therapy with Bcl-2 family inhibitors. The assays can be performed in relation to any cancer type in which copy number gain of Bcl-2, Bcl-XL and Bcl-2 is involved. Other examples of such cancers include solid tissue epithelial cancers, e.g. prostate [cancer], ovarian and esophageal cancer. The inventive assays are performed on a patient tissue sample of any type or on a derivative thereof, including peripheral blood, tumor or suspected tumor tissues (including fresh frozen and fixed or paraffin embedded tissue), cell isolates such as circulating epithelial cells separated or identified in a blood sample, lymph node tissue, bone marrow and fine needle aspirates.

As used herein, Bcl-2 (official symbol BCL2) means the human B-cell CLL/lymphoma 2 gene; Bcl-xl (official symbol BCL2L1) means the human BCL2-like 1 gene; Bcl-w (official symbol BCL2L2) means the human BCL2-like 2 gene; and NOXA (official symbol PMAIPI) means the human phorbol-12-myristate-13-acetate-induced protein 1 gene; ABL1 (official symbol ABL1) means the human Abelson murine leukemia viral oncogene homolog 1 gene; RAC1 (official symbol RAC1) means the human ras-related C3 botulinum toxin substrate 1 gene; RASSF3 (official symbol RASSF3) means the human Ras association (RalGDS/AF-6) domain family 3 gene; RAB22A (official symbol RAB22A) means the human member RAS oncogene family gene; BI-I or BAX inhibitor 1 (official symbol TEGT) means the human testis enhanced gene transcript gene; FAIM-2 (official symbol FAIM) means the human Fas...
apoptotic inhibitory molecule gene; and RFC2 (official symbol RFC2) means the human replication factor C (activator 1) 2 gene. As used herein, the term "official symbol" refers to EntrezGene database maintained by the United States National Center for Biotechnology Information.

Chromosomal loci cited herein are based on Build 35 of the Human Genome Map, as accessed through the University of California Santa Cruz Genome Browser. As used herein, reference to a chromosome locus or band, such as 18q21, refers to all of the loci or sub bands, for example, such as 18q21.1 or 18q21.3, within the locus or the band.

II. Bcl-2 Family Inhibitor Biomarkers

The invention comprises assessment in a patient tissue sample of chromosome copy number change at chromosome locus 18q21-q22, preferably at either chromosome band 18q21-q22 or band 14q11, and more preferably at both 18q21-q22 and 14q11. Chromosome region 18q21-q22 encompasses the chromosomal DNA sequence of the Bcl-2 gene at 18q21.3 and the NOXA gene at 18q21.32. Chromosome region 14q11 encompasses the chromosomal DNA sequence of the Bcl-w gene at 14q11.2. It is also within the invention to assess the chromosomal locus of the Bcl-XL gene at 20q11.2. Applicants prefer, however, to assess the 18q21-q22 and 14q11 discriminant regions as gains of these loci were correlated to SCLC sensitivity to ABT-737, whereas gain of 20q11.2 showed no correlation to ABT-737 sensitivity.

These genomic biomarkers were identified by Applicants through comparative genomic hybridization (CGH) analysis of 23 SCLC cell lines used to test Bcl-2 inhibitors in vitro and in vivo and investigation of their clinical significance. These genomic biomarkers are of particular interest for use in companion diagnostic assays to the use of ABT-737 Bcl-2 family inhibitor therapy against SCLC and lymphoma. Although Zhao, X., et al., "Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis", Cancer Res., 65: 5561-5570, 2005 (hereafter referred to as Zhao et al.), reports on the
genome-wide analysis of 5 SCLC cell lines and 19 SCLC patient tumors using 100K SNP genotyping microarrays, Zhao et al. do not disclose chromosome copy number gain at 18q21-q22 nor at 14q11.

Applicants' investigation further revealed multiple other novel regions of chromosome copy number change not previously reported in SCLC. These other novel genomic biomarkers are listed in Table 1 below and are also not reported in Zhao et al. A gain of the locus of ABL1 at 9q34 can be potentially used to identify patients for treatment with the ABL1 kinase inhibitor imatinib mesylate, Gleevec® (Gleevec is a registered trademark of Novartis). Copy number gains at three members of the Ras family, RAC1 at 7p22.1 (gains in 69% of lines and 66% of 19 tumors studied), RASSF3 at 12q24 (65% of lines and 70% of 19 tumors studied), and RAB22A at 20q13.3 (42% of lines and 84% of 19 tumors studied), are notable because of the known oncogenic impact of Ras family genes and the high percentage occurrence in the tumor cohort studied. Gains at other anti-apoptotic genes were seen for BI-1 at 12ql2-q4, FAIM-2 (gained in 73% of lines and 58% of 19 tumors studied) at 12ql3.12, and RFC2 (gained in 71% of lines and 60% of 19 tumors studied) at 7q1.1. Diagnostic assays for detecting any of these copy number changes in small cell lung cancer or other cancer is another embodiment of the invention.

Applicants used a bioinformatics approach that identified regions of chromosomal aberrations that discriminate between cell line groups that were sensitive and resistant to ABT-737. This approach tested for statistical significance using Fisher's Exact Test to determine if a SNP identified through the CGH analysis shows preferential gain/loss in the sensitive or resistant group. The copy number thresholds for amplifications and deletions used in this analysis were set at 2.8 and 1.5, respectively. Contiguous regions of probesets (SNPs) with low table and two-sided \( p \)-values were then subjected to further analysis. One large region on chromosome 18q was of particular interest because of high copy numbers and low \( p \)-values. This region spans chromosomal bands 18q21.1 through 18q22. Applicants then used real-time qPCR to validate this region as a potential therapy stratification marker. qPCR was used to evaluate six loci starting at 48 Mb (18q21.1) and ending at 62 Mb (18q22).
within chromosome 18. The qPCR results are displayed in Figure 1 and show segregation between the sensitive and resistant lines based on the copy number of the test locus (ANOVA test p-value < 0.0001). The sensitive lines carry an amplification of the region under consideration (3 to 7 copies), whereas the resistant lines display a normal copy number. The target of ABT-737, Bcl-2, is located within this discriminant region and had a low 0.04 p-value for significance in determining sensitivity.

Applicants then analyzed a 62 patient SCLC cohort for copy number gains at 18q21-q22 and found copy number gain in 48% of this cohort, with low-level amplifications of the Bcl-2 gene present in 40% of the patients (25 out of 62) and high-level amplifications in 8% of the tumors (5 out of 62).

Assessment of copy number gain at the 18q21-q22 and 14qll discriminant regions are believed applicable for patient classification for other cancer chemotherapy, such as treatment with cytotoxic drugs, DNA-damaging drugs, tubulin inhibitors, tyrosine kinase inhibitors, and anti-metabolites. The Bcl-2 genes provide significant cell survival benefit, and their chromosome copy number gain driving their expression is expected to mark therapy resistance.

III. Assays

Nucleic acid assay methods useful in the invention comprise detection of chromosomal DNA copy number changes by: (i) in situ hybridization assays to intact tissue or cellular samples, (ii) microarray hybridization assays to chromosomal DNA extracted from a tissue sample, and (iii) polymerase chain reaction (PCR) or other amplification assays to chromosomal DNA extracted from a tissue sample. Assays using synthetic analogs of nucleic acids, such as peptide nucleic acids, in any of these formats can also be used.

The assays of the invention are used to identify the chromosome copy number biomarkers for both predicting therapy response and for monitoring patient response to Bcl-2 family inhibitor therapy. Assays for response prediction are run before start of therapy and patients showing the chromosome copy number gains are eligible to
receive Bcl-2 family inhibitor therapy. The copy number gain can also indicate resistance to other cancer therapy such as chemotherapy or radiation therapy. For monitoring patient response, the assay is run at the initiation of therapy to establish baseline levels of the biomarker in the tissue sample, for example, the percent of total cells or number of cells showing the copy number gain in the sample. The same tissue is then sampled and assayed and the levels of the biomarker compared to the baseline. Where the levels remain the same or decrease, the therapy is likely being effective and can be continued. Where significant increase over baseline level occurs, the patient may not be responding.

The invention comprises detection of the genomic biomarkers by hybridization assays using detectably labeled nucleic acid-based probes, such as deoxyribonucleic acid (DNA) probes or protein nucleic acid (PNA) probes, or unlabeled primers which are designed/selected to hybridize to the specific designed chromosomal target. The unlabeled primers are used in amplification assays, such as by polymerase chain reaction (PCR), in which after primer binding, a polymerase amplifies the target nucleic acid sequence for subsequent detection. The detection probes used in PCR or other amplification assays are preferably fluorescent, and still more preferably, detection probes useful in "real-time PCR". Fluorescent labels are also preferred for use in situ hybridization but other detectable labels commonly used in hybridization techniques, e.g., enzymatic, chromogenic and isotopic labels, can also be used. Useful probe labeling techniques are described in Molecular Cytogenetics: Protocols and Applications, Y.-S. Fan, Ed., Chap. 2, "Labeling Fluorescence In Situ Hybridization Probes for Genomic Targets", L. Morrison et.al., p. 21-40, Humana Press, © 2002, incorporated herein by reference. In detection of the genomic biomarkers by microarray analysis, these probe labeling techniques are applied to label a chromosomal DNA extract from a patient sample, which is then hybridized to the microarray.

Preferably, in situ hybridization is used to detect the presence of chromosomal copy number increase or gene amplification at either or both of the 18q21-q22 or 14q11 loci, or at the other novel genomic biomarker regions. Probes for use in the in situ hybridization methods of the invention fall into two broad groups: chromosome
enumeration probes, i.e., probes that hybridize to a chromosomal region, usually a repeat sequence region, and indicate the presence or absence of an entire chromosome, and locus specific probes, i.e., probes that hybridize to a specific locus on a chromosome and detect the presence or absence of a specific locus. Chromosome arm probes, i.e., probes that hybridize to a chromosomal region and indicate the presence or absence of an arm of a specific chromosome, can also be used. It is preferred to use a locus specific probe that can detect changes of the unique chromosomal DNA sequences at the interrogated locus such as 18q21-q22. Methods for use of unique sequence probes for in situ hybridization are described in U.S. Patent 5,447,841, incorporated herein by reference.

A chromosome enumeration probe can hybridize to a repetitive sequence, located either near or removed from a centromere, or can hybridize to a unique sequence located at any position on a chromosome. For example, a chromosome enumeration probe can hybridize with repetitive DNA associated with the centromere of a chromosome. Centromeres of primate chromosomes contain a complex family of long tandem repeats of DNA comprised of a monomer repeat length of about 171 base pairs, that are referred to as alpha- satellite DNA. Centromere fluorescence in situ hybridization probes to each of chromosomes 14 and 18 are commercially available from Abbott Molecular (Des Plaines, IL).

The preferred in situ hybridization probes employ directly labeled fluorescent probes, such as described in U.S. Patent 5,491,224, incorporated herein by reference. U.S. Patent 5,491,224 also describes simultaneous FISH assays using more than one fluorescently labeled probe. Use of a pair of fluorescent probes, for example, one for the 18q21-q22 locus of Bcl-2 and one for the centromere of chromosome 18, or one for the 14q11 locus of Bcl-w and one for the centromere of chromosome 14, allows determination of the ratio of the gene locus copy number to the centromere copy number. This multiplex assay can provide a more precise identification of copy number increase through determination on a cell-by-cell basis of whether gene amplification, i.e. a ratio of the number of the gene locus probe signals to the centromere probe signals in each cell that is greater than 2, exists, or whether gain of the entire chromosome has
occurred, i.e. a ratio of the number of the gene locus probe signals to the centromere probe signals in each cell of 1/1 to less than 2/1, but with more than the normal number of two gene locus probe signals. Samples that are classified as amplified from dual probe analysis with ratios of 2/1 or greater, or those having three or more gene locus probe signals, either in dual probe or single probe analysis, are identified as eligible for Bcl-2 family inhibitor therapy.

Useful locus specific probes can be produced in any manner and will generally contain sequences to hybridize to a chromosomal DNA target sequence of about 10,000 to about 1,000,000 bases long. Preferably the probe will hybridize to a target stretch of chromosomal DNA at the target locus of at least 100,000 bases long to about 500,000 bases long, and will also include unlabeled blocking nucleic acid in the probe mix, as disclosed in U.S. Patent 5,756,696, herein incorporated by reference, to avoid non-specific binding of the probe. It is also possible to use unlabeled, synthesized oligomeric nucleic acid or peptide nucleic acid as the blocking nucleic acid or as the centromeric probe. For targeting the particular gene locus, it is preferred that the probes include nucleic acid sequences that span the gene and thus hybridize to both sides of the entire genomic coding locus of the gene. The probes can be produced starting with human DNA containing clones such as Bacterial Artificial Chromosomes (BACs) or the like. BAC libraries for the human genome are available from Invitrogen and can be investigated for identification of useful clones. It is preferred to use the University of California Santa Cruz Genome Browser to identify DNA sequences in the target locus. These DNA sequences can then be used to synthesize PCR primers for use to screen BAC libraries to identify useful clones. The clones can then be labeled by conventional nick translation methods and tested as in situ hybridization probes.

Examples of fluorophores that can be used in the in situ hybridization methods described herein are: 7-amino-4-methylcoumarin-3-acetic acid (AMCA), Texas Red™ (Molecular Probes, Inc., Eugene, OR); 5-(and-6)-carboxy-X-rhodamine, lissamine rhodamine B, 5-(and-6)-carboxyfluorescein; fluorescein-5-isothiocyanate (FITC); 7-diethylaminocoumarin-3-carboxylic acid, tetramethyl-rhodamine-5-(and-6)-
isothiocyanate; 5-(and-6)-carboxytetramethylrhodamine; 7-hydroxy-coumarin-3-carboxylic acid; 6-[fluorescein 5-(and-6)-carboxamido]hexanoic acid; N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a diaza-3-indacene)propionic acid; eosin-5-isothiocyanate; erythrosine-5-isothiocyanate; 5-(and-6)-carboxyrhodamine 6G; and Cascade™ blue acetazide (Molecular Probes).

Probes can be viewed with a fluorescence microscope and an appropriate filter for each fluorophore, or by using dual or triple band-pass filter sets to observe multiple fluorophores. See, e.g., U.S. Patent No. 5,776,688 to Bittner, et al., which is incorporated herein by reference. Any suitable microscopic imaging method can be used to visualize the hybridized probes, including automated digital imaging systems, such as those available from MetaSystems or Applied Imaging. Alternatively, techniques such as flow cytometry can be used to examine the hybridization pattern of the chromosomal probes.

Although the cell-by-cell copy number analysis resulting from in situ hybridization is preferred, the genomic biomarkers can also be determined by quantitative PCR. In this embodiment, chromosomal DNA is extracted from the tissue sample, and is then amplified by PCR using a pair of primers specific to at least one of Bcl-2, Bcl-xl or Bcl-w, or by multiplex PCR, using multiple pairs of primers. Any primer sequence for the biomarkers can be used. The copy number of the tissue is then determined by comparison to a reference amplification standard.

Microarray copy number analysis can also be used. In this embodiment, the chromosomal DNA after extraction is labeled for hybridization to a microarray comprising a substrate having multiple immobilized unlabeled nucleic acid probes arrayed at probe densities up to several million probes per square centimeter of substrate surface. Multiple microarray formats exist and any of these can be used, including microarrays based on BACs and on oligonucleotides, such as those available from Agilent Technologies (Palo Alto, California), and Affymetrix (Santa Clara, California). When using a oligonucleotide microarray to detect chromosomal copy
number change, it is preferred to use a microarray that has probe sequences to more than three separate locations in the targeted region.

IV. Sample Processing and Assay Performance

The tissue sample to be assayed by the inventive methods can comprise any type, including a peripheral blood sample, a tumor tissue or a suspected tumor tissue, a thin layer cytological sample, a fine needle aspirate sample, a bone marrow sample, a lymph node sample, a urine sample, an ascites sample, a lavage sample, an esophageal brushing sample, a bladder or lung wash sample, a spinal fluid sample, a brain fluid sample, a ductal aspirate sample, a nipple discharge sample, a pleural effusion sample, a fresh frozen tissue sample, a paraffin embedded tissue sample or an extract or processed sample produced from any of a peripheral blood sample, a tumor tissue or a suspected tumor tissue, a thin layer cytological sample, a fine needle aspirate sample, a bone marrow sample, a lymph node sample, a urine sample, an ascites sample, a lavage sample, an esophageal brushing sample, a bladder or lung wash sample, a spinal fluid sample, a brain fluid sample, a ductal aspirate sample, a nipple discharge sample, a pleural effusion sample, a fresh frozen tissue sample or a paraffin embedded tissue sample. For example, a patient peripheral blood sample can be initially processed to extract an epithelial cell population, and this extract can then be assayed. A microdissection of the tissue sample to obtain a cellular sample enriched with suspected tumor cells can also be used. The preferred tissue samples for use herein are peripheral blood, tumor tissue or suspected tumor tissue, including fine needle aspirates, fresh frozen tissue and paraffin embedded tissue, and bone marrow.

The tissue sample can be processed by any desirable method for performing in situ hybridization or other nucleic acid assays. For the preferred in situ hybridization assays, a paraffin embedded tumor tissue sample or bone marrow sample is fixed on a glass microscope slide and deparaffinized with a solvent, typically xylene. Useful protocols for tissue deparaffinization and in situ hybridization are available from Abbott Molecular Inc. (Des Plaines, Illinois). Any suitable instrumentation or automation can be used in the performance of the inventive assays. PCR based assays
can be performed on the m2000 instrument system (Abbott Molecular, Des Plaines, IL). Automated imaging can be employed for the preferred fluorescence in situ hybridization assays.

In one embodiment, the sample comprises a peripheral blood sample from a patient which is processed to produce an extract of circulating tumor cells having increased chromosomal copy number of at least one of 18q21-q22 and 14q11.2. The circulating tumor cells can be separated by immunomagnetic separation technology such as that available from Immunicon (Huntingdon Valley, Pennsylvania). The number of circulating tumor cells showing at least one copy number gain is then compared to the baseline level of circulating tumor cells having increased copy number determined preferably at the start of therapy. Increases in the number of such circulating tumor cells can indicate therapy failure.

Test samples can comprise any number of cells that is sufficient for a clinical diagnosis, and typically contain at least about 100 cells. In a typical FISH assay, the hybridization pattern is assessed in about 25-1,000 cells. Test samples are typically considered "test positive" when found to contain the chromosomal gain in a sufficient proportion of the sample. The number of cells identified with chromosomal copy number and used to classify a particular sample as positive, in general will vary with the number of cells in the sample. The number of cells used for a positive classification is also known as the cut-off value. Examples of cutoff values that can be used in the determinations include about 5, 25, 50, 100 and 250 cells, or 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50% and 60% of cells in the sample population. As low as one cell may be sufficient to classify a sample as positive. In a typical paraffin embedded tissue sample, it is preferred to identify at least 30 cells as positive and more preferred to identify at least 20 cells as positive for having the chromosomal copy number gain. For example, detection in a typical paraffin embedded small cell lung cancer tissue of 30 cells having gain of 18q21-q22 would be sufficient to classify the tissue as positive and eligible for treatment with ABT-737.

V. Assay Kits
In another aspect, the invention comprises kits for the detection of the genomic biomarkers that comprise containers containing at least one probe specific for binding to at least one of 18q21-q22 or 14q11. These kits may also include containers with other associated reagents for the assay. Preferred kits of the invention comprise containers containing, respectively, at least two FISH probes capable of binding specifically to each of 18q21-q22 and 14q21, and more preferred kits include a FISH probe to the Bcl-2 locus at 18q21.3. The inventive kits can comprise nucleic acid probe analogs, such as peptide nucleic acid probes.

VI. Experimental

The following describes Applicants' performance of a series of experiments. First, a whole-genome screen with high-density SNP genotyping arrays identified recurrent gene amplifications/deletions in SCLC cells. Novel recurrent chromosomal copy number gains were identified, were confirmed by real-time qPCR, and were then validated as present in an independent SNP analysis dataset of 19 SCLC tumors obtained from Zhao et al. One of these copy number gains, on 18q, was correlated with sensitivity of SCLC cell lines to the targeted cancer drug ABT-737. The clinical relevance of the 18q21 gain was then verified by FISH analysis of SCLC tumors. The genes residing in the 18q21 marker region were shown to be overexpressed in the sensitive cell lines.

Materials and Methods

Cell culture.

humidified atmosphere containing 5% CO₂. Genomic DNA was isolated from the cell lines using a DNAeasy kit (Qiagen, Valencia, CA).

**Comparative Genomic Hybridization.**

Genomic DNA from the SCLC cell lines was run on IOOK SNP genotyping array sets (Affymetrix, Santa Clara, CA). Each IOOK set consists of two 5OK arrays, HindIII and Xbal. Briefly, 250 ng of genomic DNA from each cell line was digested with the corresponding restriction enzyme (HindIII or Xbal, New England Biolabs, Boston, MA). Adapters were ligated to the digested DNA, followed by PCR amplification with Pfx DNA polymerase (Invitrogen, Carlsbad, California). The PCR products were purified, fragmented, labeled, and hybridized to the SNP microarray according to the manufacturer's protocol. After a 16-hour hybridization, the arrays were scanned, and the data were processed using the Affymetrix GTYPE software to create copy number (.cnf) files containing information on the inferred copy number for each probeset (SNP). The GTYPE software generates an inferred copy number for each SNP by comparing the signal intensity for the sample with an internal data set from a healthy population, which is included in the GTYPE software. The .cnt files contained combined information from both arrays in the set. These files were converted into .txt files and loaded into an internally developed software program for further analysis.

Applicants' program was used for the graphical display and analysis of multiple .txt files. The data were displayed chromosome by chromosome as a histogram of copy number versus SNP's ordered sequentially along the chromosome. For each SNP, the predicted cytogenetic band as well as any genes between this and the next adjacent SNP were reported. The gene coordinates and cytogenetic band positions were inferred from the Build 35 of the Human Genome. From a selected region of the histogram, for example, 18q21, a summary file can be produced that contains the coordinates of all probesets on the microarray for that region (individual SNP's) with the corresponding copy numbers, cytogenetic bands, gene IDs, names, and the coordinates of all the genes residing in the region (regardless of whether a gene is actually represented by SNP's on
In the analysis, contiguous SNP's with a small p-value (p-value < 0.08) were considered to be one region.

To facilitate identification of recurrent aberrations, the frequency of copy number change was calculated and plotted for each probeset (SNP) on the microarray, using a threshold of >2.8 copies for copy number gains and of <1.5 copies for copy number losses. The cell lines were then classified as sensitive and resistant to ABT-737. Fisher's Exact Test was used to identify aberrations in the copy number data that were associated with the sensitivity of cell lines to the Bcl-2 inhibitor. For each SNP, a 2x2 contingency table was constructed for testing the significance of an increase or decrease in copy number in the two groups.

Applicants also obtained from the authors of Zhao et al. study of SCLC, a copy of their raw microarray hybridization data produced in the study reported on in Zhao et al. Applicants analyzed the Zhao et al. raw data for copy number aberrations, and compared the copy number changes identified by Applicants as present in the Zhao data to those identified in Applicants’ study of the SCLC cell lines.

**Real-time quantitative PCR (qPCR).**

Primers were designed using the Vector NTI software (Invitrogen) and tested to ensure amplification of single discrete bands with no primer dimers. All primers were synthesized by IDT (Coraville, Iowa). Two independent forward and reverse primer pairs were used for each of the six loci within the 18q21-q22 discriminant region. The primer sequences used are listed in pairs with each pair’s approximate location from the 18p terminus, with the forward primers having odd Sequence Identification Numbers (SEQ ID NO’s) and the reverse primers having even SEQ ID NO’s, and were:
<table>
<thead>
<tr>
<th>From 18p</th>
<th>Sequence</th>
<th>SEO ID NO</th>
</tr>
</thead>
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<tr>
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<td>62MB</td>
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Real-time, quantitative PCR was conducted on an iCycler thermocycler (Bio-Rad, Hercules, California) using SYBR Green qPCR supermix UDG (Invitrogen). Each reaction was run in triplicate and contained 10 ng of purified genomic DNA along with 300 nM of each primer in a final volume of 50 µl. The cycling parameters used were: 95°C for 3 min.; 35 cycles of 95°C for 10 sec; 57°C for
45 sec. Melting curves were performed to ensure that only a single amplicon was produced and samples were run on a 4% agarose gel (Invitrogen) to confirm specificity. Data analysis was performed in the linear regression software DART-PCR v1.O, see Peirson, S. N., et al., "Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis", Nucleic Acids Res., 31: e73, 2003, using raw thermocycler values. Normalization of sample input was conducted using geometric averaging software GeNorm v3.3 (23) to GAPDH, β-2 microglobulin, YWHAZ, RPL13a, and PLP-I, see Vandesompele, J, De Preter K et.al., "Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes", Genome Biol.,2002 Jun 18; 3 (7):RESEARCH0034, Epub 2002 Jun. 18, PMID 12184808 [PubMed - indexed for MEDLINE].

The copy number for each locus evaluated was determined by establishing the normalized qPCR output for the sample and dividing this value by the normalized qPCR output of a control genomic DNA (Clontech, Mountain View, California) and multiplying this value by two. Each qPCR copy number estimate is the average value for two independent primer sets (mean CV 11.5%).

**Fluorescent in situ** hybridization.

A tissue microarray containing primary SCLC tumors from 62 patients provided by Dr. Guido Sauter of the Department of Pathology, University Medical Center, Hamburg-Eppendorf, was analyzed by FISH using a commercially available dual-color FISH probe targeting 18q21 (LSI Bcl-2 Break-apart probe, Abbott Molecular). This LSI Bcl-2 FISH probe contains two probes labeled in different fluorescent colors that hybridize adjacent to each side of the Bcl-2 locus at 18q21.3, but does not hybridize to any of the genomic sequence of Bcl-2. The slides were deparaffinized for 10 minutes in Xylol, rinsed in 95% EtOH, air-dried, incubated in a Pretreatment Solution (Abbott Molecular) for 15 minutes at 80°C, rinsed in water, incubated in a Protease Buffer (Abbott Molecular) for 2.5 to 5 hours, rinsed in water, dehydrated for 3 min each in 70, 80, and 95% EtOH, and air-dried. 10 µl of the probe mix was applied onto the slide, and the slide was covered, sealed, heated to 72°C for 5 minutes, and hybridized
overnight at 37°C in a wet chamber. The slides were then washed with a wash buffer containing 2xSSC and 0.3% NP40 (pH 7.7-7.5) for 2 minutes at 75°C, rinsed in water at room temperature, air-dried, mounted with a DAPI solution and a 24x50 mm coverslip, and examined under an epifluorescence microscope. For each tissue sample, the range of red and green FISH signals corresponding to the Bcl-2 locus was recorded. An average copy number per spot was then calculated based on the minimal and maximal number of FISH signals per cell nucleus in each tissue spot. Copy number groups were then built according to the following criteria:

(1) 1-2 signals = average copy number <2.5;
(2) 3-4 signals = average copy number <4.5;
(3) 5-6 signals = average copy number <6.5; and
(4) 7-10 signals = average copy number >6.5.

Microarray analysis of gene expression.

Total RNA was isolated by using the Trizol reagent (Invitrogen,) and purified on RNeasy columns (Qiagen, Valencia, California). Labeled cRNA was prepared according to the microarray manufacturer's protocol and hybridized to human U133A 2.0 arrays (Affymetrix, Santa Clara, California). The U133A 2.0 chips contain 14,500 well-characterized genes, as well as several thousand ESTs. The microarray data files were loaded into the Rosetta Resolver™ software for analysis and the intensity values for all probesets were normalized using the Resolver's Experimental Definition. The intensity values for the probesets corresponding to genes within the amplified regions were normalized across each gene and compared in heatmaps using the Spotfire™ software.

RESULTS

Table 1 summarizes all copy number abnormalities that Applicants identified as (i) present in >40% of the tested cell lines, and (ii) present in >40% of the 19 SCLC tumors from the dataset of Zhao et al., and (iii) as not previously reported in the literature, including not reported by Zhao et al. The list of identified novel aberrations
includes gains of 2q, 6p, 7p, 9q, lip, Hq, 12p, 12q, 13q, 14q, 17q, 18q, 20p, 20q, 21q, and 22q and losses of 10q21.1. All of these were confirmed by real-time qPCR in selected cell lines. As can be seen in Table 1, all of these identified novel aberrations are relatively short (about 70 kb to about 3.6 Mb). The mean spacing between the SNPs on the IOOK SNP array used in this study is 23.6 kb, thus permitting identification of very short regions of gains and losses. It is possible that some of the newly detected recurrent copy number changes represent copy number polymorphisms, as opposed to disease driven changes. However, this is only a remote possibility, because the copy number was determined relative to a panel of 110 normal individuals, see Huang, J., et al., "Whole genome DNA copy number changes identified by high density oligonucleotide arrays", Hum. Genomics, 1: 287-299, 2004.
<table>
<thead>
<tr>
<th>Copy Number Abnormality</th>
<th>Length</th>
<th>Frequency in cell lines</th>
<th>Frequency in tumors</th>
<th>Genes in this locus with reported association with cancer</th>
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Table 1 (Continued)

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<th>Copy Number Abnormality</th>
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<th>Frequency in tumors</th>
<th>Genes in this locus with reported association with cancer</th>
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<td>Frequency in cell lines</td>
<td>Frequency in tumors</td>
<td>Genes in this locus with reported association with cancer</td>
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</tr>
</tbody>
</table>
The 23 SCLC cell lines were tested for sensitivity to ABT-737 using the procedure described in Oltersdorf, T., "An inhibitor of Bcl-2 family proteins induces regression of solid tumours", Nature, 435: 677-681, 2005, with a cell line classified as sensitive if its EC50 < 1 µM and as resistant if its EC50 > 10 µM. The sensitive cell

<table>
<thead>
<tr>
<th>Copy Number Abnormality</th>
<th>Length</th>
<th>Frequency in cell lines</th>
<th>Frequency in tumors</th>
<th>Genes in this locus with reported association with cancer</th>
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To identify potential genomic correlates of the sensitivity of SCLC cells to ABT-737, we developed a bioinformatics approach that identifies regions of chromosomal aberrations that discriminate between the sensitive and resistant groups. Our program tested for statistical significance using Fisher's Exact Test to determine if a SNP shows preferential gain/loss in the sensitive or resistant group. The copy number thresholds for amplifications and deletions were set at 2.8 and 1.5, respectively. Contiguous regions of probesets (SNPs) with low table and two-sided p-values were subjected to further analysis. The top discriminating aberration represents a long region of chromosome 18, starting at nucleotide position 45704096 and ending at nucleotide position 74199087 and spanning the chromosomal bands 18q21.1 through 18q22.1 (nucleotide positions are from Build 35 of the Human Genome Map).

Real-time qPCR was then applied to validate the 18q21 region identified in the copy number analysis as a potential stratification marker. Two different primer sets run in triplicate were used to evaluate six loci starting at 48 Mb from the chromosome 18p terminus (18q21.1) and ending at 62 Mb from the chromosome 18p terminus (18q22). The qPCR results are shown in Figure 1, with the copy number measured at each locus plotted against sensitivity to ABT-737. Figure 1 shows segregation between the sensitive and resistant lines based on the copy number of the test locus (ANOVA test p-value < 0.0001), thus confirming the copy number analysis. The sensitive lines carry an amplification of the region under consideration (3 to 7 copies), whereas the resistant lines display a normal copy number. Further, the most sensitive lines (H889, H1963, H1417, and H146) have the highest Bcl-2 copy number (4 or 5 copies).

Notably, the Bcl-2 gene (p-value 0.04), the target of ABT-737, is located within the 18q21-q22 discriminant region at 18q21.3, which led to investigation of whether the sensitivity of a cell line to the drug may be determined by the amplification status of the
Bcl-2 gene. Figure 2 illustrates the relationship between the Bcl-2 gene copy number and the sensitivity of the SCLC cell lines. The cell lines are arranged from left to right in the order of decreasing sensitivity to the drug, as determined by the EC_{50} values for the cell lines from Oltersdorf, T., et al., "An inhibitor of Bcl-2 family proteins induces regression of solid tumours", Nature, 435: 677-681, 2005.

The copy number for each cell line in Figure 2 is the average of the copy numbers for 17 SNP's within the Bcl-2 gene measured by the IOOK mapping array set. The copy number for the NOXA and Bcl-w genes was the number determined for at least three contiguous SNP's surrounding their gene loci. It is clear from the plot that the sensitivity of the SCLC cell lines correlates with the Bcl-2 copy number. The most sensitive lines (H889, H1963, H1417, and H146) have the highest Bcl-2 copy number (4 or 5 copies). Another apoptosis-related gene (NOXA), whose product promotes degradation of Mcl-I, is located next to Bcl-2 and has a similar copy number profile. There are two outliers in this dataset, which are sensitive, but have a normal copy number of the Bcl-2 gene (H187 and H526). However, both H187 and H526 cell lines have copy number gain of the Bcl-w gene at 14ql 1.2, which is also a target of the drug. Their sensitivity to ABT-737 is attributed to the extra copy of the Bcl-w gene at 14ql 1.2. A similar plot did not show any correlation of sensitivity to Bcl-XL copy number gain, although copy number gain was seen in some cell lines. Thus, we established a correlation between the amplification of Bcl-2 and NOXA on 18q21.3 and the sensitivity of SCLC cell lines to ABT-737. This observation is consistent with the mechanism of action of the drug and suggests that the single-agent sensitivity of a cell line to the drug may be determined by the copy number status of 18q21, particularly the 18q21.3 locus of Bcl-2 and NOXA.

The relative expression of the 18q genes in the ABT-737 sensitive and resistant SCLC cell lines was profiled with expression microarrays as described above. The 12 most sensitive cell lines and four resistant lines were analyzed for expression of all genes located on the discriminant region on 18q21-q22 and present on the Affymetrix U133A microarray used. The genes in the amplified region were found overexpressed in the sensitive lines relative to the resistant ones. Overall, the finding of
overexpression of the 18q21-q22 genes implies a significant degree of correlation between gene amplification and gene overexpression. These data further support for the selection of the 18q21-q22 copy number gain as a patient stratification biomarker in SCLC.

To determine the clinical relevance of the 18q21-q22 marker, the Bcl-2 copy number in SCLC tumors using FISH with a commercially available Bcl-2 locus probe set. Although the commercial FISH probe used did not contain any of the Bcl-2 gene sequence itself, the probe used contain sequences that hybridize on both sides of the gene, and a contiguous copy number increase seen with both parts of this probe is believed by Applicants to include a gain of the Bcl-2 locus also. Applicants' analysis included SCLC tumors from 62 patients arrayed on a tissue microarray. The data is shown in Figure 3. Copy number gains were seen in 48% of the cohort, with low-level amplifications of the Bcl-2 gene present in 40% of the patients (25 out of 62) and high-level amplifications in 8% of the tumors (5 out of 62). This finding is consistent with the copy number data from the SCLC cell lines, as most copy number changes in the cell lines were also low-level gains. The percentage of lines carrying the aberration was also similar (40%).
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The above-described exemplary embodiments are intended to be illustrative in all respects, rather than restrictive, of the present invention. Thus, the present invention is capable of implementation in many variations and modifications that can be derived from the description herein by a person skilled in the art. All such variations and modifications are considered to be within the scope and spirit of the present invention as defined by the following claims.
WHAT I S CLAIMED IS:

1. A method of classifying a patient for eligibility for cancer therapy comprising:

   (a) providing a tissue sample from a patient;

   (b) determining presence or absence of chromosomal copy number gain at chromosome locus 18q21-q22; and

   (c) classifying the patient as eligible to receive a cancer therapy based on the presence or absence of 18q21-q22 copy number gain.

2. The method of claim 1, wherein the tissue sample comprises a peripheral blood sample, a tumor tissue or a suspected tumor tissue, a thin layer cytological sample, a fine needle aspirate sample, a bone marrow sample, a lymph node sample, a urine sample, an ascites sample, a lavage sample, an esophageal brushing sample, a bladder or lung wash sample, a spinal fluid sample, a brain fluid sample, a ductal aspirate sample, a nipple discharge sample, a pleural effusion sample, a fresh frozen tissue sample, a paraffin embedded tissue sample or an extract or processed sample produced from any of a peripheral blood sample, a tumor tissue or a suspected tumor tissue, a thin layer cytological sample, a fine needle aspirate sample, a bone marrow sample, a urine sample, an ascites sample, a lavage sample, an esophageal brushing sample, a bladder or lung wash sample, a spinal fluid sample, a brain fluid sample, a ductal aspirate sample, a nipple discharge sample, a pleural effusion sample, a fresh frozen tissue sample or a paraffin embedded tissue sample.

3. The method of claim 2, wherein the tissue sample is a paraffin embedded fixed tissue sample, a fine needle aspirate or a fresh frozen tissue sample.

4. The method of claim 1, wherein the determining step (b) is performed by in situ hybridization.
5. The method of claim 4, wherein the in situ hybridization is performed with a nucleic acid probe that is fluorescently labeled.

6. The method of claim 4, wherein the in situ hybridization is performed with at least two nucleic acid probes.

7. The method of claim 4, wherein the in situ hybridization is performed with a peptide nucleic acid probe.

8. The method of claim 3, wherein the determining step (b) is performed by in situ hybridization.

9. The method of claim 4, wherein the in situ hybridization is performed with a nucleic acid probe or peptide nucleic acid probe that hybridizes under selected hybridization conditions to at least part of chromosomal locus 18q21.3.

10. The method of claim 1, wherein the determining step (b) is performed by polymerase chain reaction.

11. The method of claim 3, wherein the determining step (b) is performed by polymerase chain reaction.

12. The method of claim 1, wherein the polymerase chain reaction is performed with at least one primer that hybridizes under selected hybridization conditions to at least part of a nucleic acid sequence at chromosomal locus at 18q21.3.

13. The method of claim 1, wherein the determining step (b) is performed by a nucleic acid microarray assay.

14. The method of claim 3, wherein the determining step (b) is performed by a nucleic acid microarray assay.
15. The method of claim 1, wherein the classifying step (c) is based on the presence or absence of a copy number gain of at least two additional copies.

16. The method of claim 1, wherein the cancer therapy comprises chemotherapy with a Bcl-2 family inhibitor.

17. A method for identifying a patient with cancer as eligible to receive Bcl-2-family inhibitor therapy comprising:

(a) providing a tissue sample from a patient;

(b) determining presence or absence in the tissue sample of chromosomal copy number gain at chromosome locus 18q21-q22; and

(c) classifying the patient as eligible to receive Bel-family inhibitor therapy where the tissue sample has a chromosomal copy number gain at chromosome locus 18q21-q22.

18. The method of claim 17, wherein the tissue sample comprises a peripheral blood sample, a tumor or suspected tumor tissue, a thin layer cytological sample, a fine needle aspirate sample, a bone marrow sample, a lymph node sample, a urine sample, an ascites sample, a lavage sample, an esophageal brushing sample, a bladder or lung wash sample, a spinal fluid sample, a brain fluid sample, a ductal aspirate sample, a nipple discharge sample, a pleural effusion sample, a fresh frozen tissue sample, a paraffin embedded tissue sample or an extract or processed sample produced from any of a peripheral blood sample, a tumor or suspected tumor tissue, a thin layer cytological sample, a fine needle aspirate sample, a bone marrow sample, a lymph node sample, a urine sample, an ascites sample, a lavage sample, an esophageal brushing sample, a bladder or lung wash sample, a spinal fluid sample, a brain fluid sample, a ductal aspirate sample, a nipple discharge sample, a pleural effusion sample, a fresh frozen tissue sample or a paraffin embedded tissue sample.
19. The method of claim 17, wherein the tissue sample is from a patient with a cancer selected from the group consisting of small cell lung carcinoma and a lymphoma.

20. The method of claim 17, wherein the patient is classified as eligible to receive \( N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohex-1-en-1-yl)methyl)piperazin-1-yl)benzoyl)-4-(((lR)-3-(morpholin-4-yl)-l-((phenylsulfanyl)methyl)propyl)amino)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide. \)

21. The method of claim 17, wherein the patient is classified as eligible to receive an anti-sense therapy compound designed to bind to one of Bcl-2, Bcl-w, and Bcl-xl.

22. The method of claim 17, wherein the determining step (b) is performed by in situ hybridization.

23. The method of claim 22, wherein the in situ hybridization is performed with a nucleic acid probe that is fluorescently labeled.

24. The method of claim 22, wherein the in situ hybridization is performed with at least two nucleic acid probes.

25. The method of claim 24, wherein one of the nucleic acid probes is designed to hybridize to chromosome locus 14ql 1.2.

26. The method of claim 22, wherein the in situ hybridization is performed with a peptide nucleic acid probe.
27. The method of claim 17, wherein chromosomal copy number at chromosome locus 18q21.3 is determined.

28. The method of claim 17, wherein the determining step (b) is performed by polymerase chain reaction.

29. The method of claim 27, wherein the polymerase chain reaction is a multiplex polymerase chain reaction.

30. The method of claim 28, wherein the polymerase chain reaction is performed with at least one primer that hybridizes under selected conditions to a DNA sequence within chromosome locus 18q21.3.

32. The method of claim 17, wherein the determining step (b) is performed by a nucleic acid microarray assay.

33. A method for monitoring a patient being treated with anti-Bcl-2-family therapy comprising:

(a) providing a peripheral blood sample from a cancer patient;

(b) identifying in or extracting from the peripheral blood sample circulating tumor cells;

(c) determining in the circulating tumor cells presence or absence of chromosomal copy number gain at chromosome locus 18q21; and

(d) comparing number of circulating tumor cells having chromosomal copy number gain at chromosome locus 18q21 to baseline level of such circulating tumor cells determined before or at onset of therapy.
34. The method of claim 33 wherein the cancer is selected from the group consisting of small cell lung carcinoma and a lymphoma.

35. The method of claim 33, wherein circulating tumor cells are extracted from the peripheral blood sample by immunomagnetic separation.

36. The method of claim 33, wherein the patient is being treated with N-(4-(4-((2-(4-chlorophenyl)-5,5-dimethyl-1-cyclohex-1-en-1-yl)methyl)piperazin-1-yl)benzoyl)-4-(((IR)-3-(morpholin-4-yl)-l-((phenylsulfanyl)methyl)propyl)amino)-3-((trifluoromethyl)sulfonyl)benzenesulfonamide.

37. The method of claim 33, wherein the patient is being treated with an anti-sense therapy compound designed to bind to at least one of Bcl-2, Bcl-w, and Bcl-xl.

38. The method of claim 33, wherein the determining step (c) is performed by in situ hybridization.

39. The method of claim 38, wherein the in situ hybridization is performed with a nucleic acid probe that is fluorescently labeled.

40. The method of claim 38, wherein the in situ hybridization is performed with at least two nucleic acid probes.

41. The method of claim 40 wherein one of the nucleic acid probes is designed to hybridize to chromosome locus 14q11.2.

42. The method of claim 33, wherein chromosomal copy number gain is determined at chromosome locus 18q21.3.

43. The method of claim 38, wherein the in situ hybridization is performed with a peptide nucleic acid probe.
44. A method of classifying a patient for eligibility for cancer therapy
comprising:

(a) providing a tissue sample from a patient;

(b) determining presence or absence of chromosomal copy number gain at
chromosome locus 14ql1; and

(c) classifying the patient as eligible to receive a cancer therapy based on the
presence or absence of 14ql1 copy number gain.

45. The method of claim 44, wherein the tissue sample comprises a peripheral
blood sample, a tumor tissue or a suspected tumor tissue, a thin layer cytological
sample, a fine needle aspirate sample, a bone marrow sample, a lymph node sample, a
urine sample, an ascites sample, a lavage sample, an esophageal brushing sample, a
bladder or lung wash sample, a spinal fluid sample, a brain fluid sample, a ductal
aspirate sample, a nipple discharge sample, a pleural effusion sample, a fresh frozen
tissue sample, a paraffin embedded tissue sample or an extract or processed sample
produced from any of a peripheral blood sample, a tumor tissue or a suspected tumor
tissue, a thin layer cytological sample, a fine needle aspirate sample, a bone marrow
sample, a urine sample, an ascites sample, a lavage sample, an esophageal brushing
sample, a bladder or lung wash sample, a spinal fluid sample, a brain fluid sample, a
ductal aspirate sample, a nipple discharge sample, a pleural effusion sample, a fresh
frozen tissue sample or a paraffin embedded tissue sample.

46. The method of claim 45, wherein the tissue sample is a paraffin embedded
fixed tissue sample.

47. The method of claim 44, wherein the determining step (b) is performed by
in situ hybridization.
48. The method of claim 47, wherein the in situ hybridization is performed with a nucleic acid probe that is fluorescently labeled.

49. The method of claim 47, wherein the in situ hybridization is performed with at least two nucleic acid probes.

50. The method of claim 47, wherein the in situ hybridization is performed with a peptide nucleic acid probe.

51. The method of claim 46, wherein the determining step (b) is performed by in situ hybridization.

52. The method of claim 44, wherein the in situ hybridization is performed with a nucleic acid probe or peptide nucleic acid probe that hybridizes under selected hybridization conditions to at least part of chromosomal locus of Bcl-w.

53. The method of claim 44, wherein the determining step (b) is performed by polymerase chain reaction.

54. The method of claim 46, wherein the determining step (b) is performed by polymerase chain reaction.

55. The method of claim 44, wherein the polymerase chain reaction is performed with at least one primer that hybridizes under selected hybridization conditions to at least part of a nucleic acid sequence at chromosomal locus of Bcl-w.

56. The method of claim 44, wherein the determining step (b) is performed by a nucleic acid microarray assay.

57. The method of claim 46, wherein the determining step (b) is performed by a nucleic acid microarray assay.
58. A method of classifying a small cell lung cancer sample for therapy comprising:

(a) providing a tissue sample from a patient; and

(b) determining presence or absence of chromosomal copy number change at at least one chromosome locus selected from the group consisting of gain of 2q37.1-q37.2, 6p21.31, 7pl4.3, 7p22.1, 7ql 1.21, 7q22.1, 7q36, 9q34.1, 9q34.2, llql3.2-ql3.3, llql3.4, llql23.3, 12pl3.31, 12ql3.12, 12ql4.2, 12q24.12, 12q24.13, 12q24.33, 13q34, 14q23.2, 14q24.3, 14q24.3-q31, 14q32.12, 14q32.1-q32.2, 14q32.33, 17q21.33, 17q24.2-q25.1, 17q25.3, 18ql2, 18q22-q23, 20pl3, 20pl3-pl2, 20pl l.23,20pl.21, 20ql.23, 20ql2-ql3.1, 20ql3.1-ql3.13, 20ql3.32-ql3.33, 20ql3.3, 21q22.3, and 22ql3.1, and loss of 10q21.1 and llpl 1.12.

59. The method of claim 58, wherein chromosomal copy number change is determined by fluorescence in situ hybridization using at least one fluorescently labeled nucleic acid probe or peptide nucleic acid probe designed to hybridize under selected conditions to at least one of the chromosomal loci listed in claim 58.

60. A method for identifying a patient with small cell lung cancer as eligible to receive imatinib therapy comprising:

(a) providing a small cell lung cancer tissue sample from a patient;

(b) determining presence or absence in the tissue sample of chromosomal copy number gain at chromosome locus 9q34.1; and

(c) classifying the patient as eligible to receive imatinib mesylate therapy where the tissue sample has a chromosomal copy number gain at chromosome locus 9q34.1.

61. The method of claim 60, wherein the tissue sample is a paraffin embedded fixed tissue sample.
62. The method of claim 60, wherein the determining step (b) is performed by in situ hybridization.

63. The method of claim 62, wherein the in situ hybridization is performed with a nucleic acid probe that is fluorescently labeled.

64. The method of claim 62, wherein the in situ hybridization is performed with at least two nucleic acid probes.

65. The method of claim 62, wherein one of the nucleic acid probes is designed to hybridize to chromosome locus of ABL1.

66. The method of claim 62, wherein the in situ hybridization is performed with a peptide nucleic acid probe.

67. The method of claim 60, wherein chromosomal copy number at chromosome locus of ABL1 is determined.

68. The method of claim 60, wherein the determining step (b) is performed by polymerase chain reaction.

69. The method of claim 61, wherein the determining step (b) is performed by polymerase chain reaction.

70. The method of claim 60, wherein the polymerase chain reaction is performed with at least one primer that hybridizes under selected hybridization conditions to at least part of a nucleic acid sequence at chromosomal locus of ABL1.

71. The method of claim 60, wherein the determining step (b) is performed by a nucleic acid microarray assay.
72. The method of claim 62, wherein the determining step (b) is performed by a nucleic acid microarray assay.
FIG. 2

SENSITIVITY TO ABT-737

Bcl-2 GENE COPY NUMBER

FIG. 3

Fraction of Tumors (%)

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