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(71) Applicant (for all designated States except US): **BRISTOL-MYERS SQUIBB COMPANY** [US/US]; Route 206 and Province Line Road, Princeton, New Jersey 08543-4000 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HUANG, Fei** [CN/US]; c/o Bristol-Myers Squibb Co., 311 Pennington-Rocky Hill Road, Hopewell, New Jersey 08534 (US). **CLARK, Edwin, A.** [US/US]; c/o Bristol-Myers Squibb

Co., 311 Pennington-Rocky Hill Road, Hopewell, New Jersey 08534 (US). **WANG, Xi-de** [CN/US]; c/o Bristol-Myers Squibb Co., 311 Pennington-Rocky Hill Road, Hopewell, New Jersey 08534 (US).

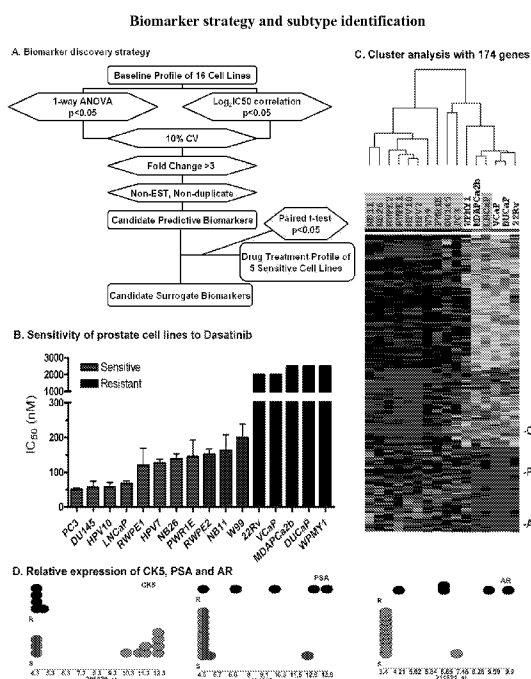
(74) Agents: **D'AMICO, Stephen, C.** et al.; Bristol-Myers Squibb Company, P.O. Box 4000, Princeton, New Jersey 08543-4000 (US).

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(54) Title: IDENTIFICATION OF POLYNUCLEOTIDES FOR PREDICTING ACTIVITY OF COMPOUNDS THAT INTERACT WITH AND/OR MODULATE PROTEIN TYROSINE KINASES AND/OR PROTEIN TYROSINE KINASE PATHWAYS IN PROSTATE CELLS





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**IDENTIFICATION OF POLYNUCLEOTIDES FOR PREDICTING
ACTIVITY OF COMPOUNDS THAT INTERACT WITH AND/OR
MODULATE PROTEIN TYROSINE KINASES AND/OR PROTEIN
TYROSINE KINASE PATHWAYS IN PROSTATE CELLS**

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[0001] This application claims benefit to provisional application U.S. Serial No. 60/879,374 filed January 9, 2007; and to provisional application U.S. Serial No. 60/899,260, filed February 2, 2007; under 35 U.S.C. 119(e). The entire teachings of the referenced applications are incorporated herein by reference.

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FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of pharmacogenomics, and more specifically to new and alternative methods and procedures to determine drug sensitivity in patients, and particularly in patients with prostate cancer. This invention allows the development of individualized genetic profiles which aid in treating diseases and disorders based on patient response at a molecular level.

BACKGROUND OF THE INVENTION

[0003] Prostate cancer is the most common type of cancer in men of western countries. It is estimated that each year about 230,000 men in the US alone are diagnosed with prostate cancer and ~30,000 die of this disease (1). Although targeted therapeutics have delivered promises for cancer patients, their use in treating prostate cancer is still few. Current regimens available for prostate cancer patients include conventional surgery, radiation and hormonal therapies for early stage tumors, and TAXOL[®]-based chemotherapy for late stage metastatic tumors (2, 3). There is a clear unmet medical need to develop targeted therapeutics for prostate cancer, and a need for methodologies that can accurately foretell a patient's response to drugs in the clinic and thus facilitate the individualization of therapy for each patient.

[0004] The problem may be solved by the identification of new parameters that can better predict a patient's sensitivity to treatment or therapy. The classification of patient samples is a crucial aspect of cancer diagnosis and treatment. The association of a patient's response to drug treatment with molecular and genetic markers can open

up new opportunities for drug development in non-responding patients, or distinguish a drug's indication among other treatment choices because of higher confidence in the efficacy. Further, the pre-selection of patients who are likely to respond well to a medicine, drug, or combination therapy may reduce the number of patients needed in a clinical study or accelerate the time needed to complete a clinical development program (M. Cockett et al., *Current Opinion in Biotechnology*, 11:602-609 (2000)).

[0005] The major goal of pharmacogenomics research is to identify genetic markers that accurately predict a given patient's response to drugs in the clinic; such individualized genetic assessment would greatly facilitate personalized treatment. An approach of this nature is particularly needed in cancer treatment and therapy, where commonly used agents are ineffective in many patients, and side effects are frequent. The ability to predict drug sensitivity in patients is particularly challenging because drug responses reflect both the properties intrinsic to the target cells and also a host's metabolic properties. Efforts by those in the art to use genetic information to predict drug sensitivity have primarily focused on individual polynucleotides that have broad effects, such as the multidrug resistant polynucleotides, *mdr1* and *mrp1* (P. Sonneveld, *J. Intern. Med.*, 247:521-534 (2000)).

[0006] The development of microarray technologies for large scale characterization of polynucleotide expression pattern makes it possible to systematically search for multiple molecular markers and to categorize cancers into distinct subgroups that are not evident by traditional histopathological methods (J. Khan et al., *Cancer Res.*, 58:5009-5013 (1998); A.A. Alizadeh et al., *Nature*, 403:503-511 (2000); M. Bittner et al., *Nature*, 406:536-540 (2000); J. Khan et al., *Nature Medicine*, 7(6):673-679 (2001); and T.R. Golub et al., *Science*, 286:531-537 (1999); U. Alon et al., *Proc. Natl. Acad. Sci. USA*, 96:6745-6750 (1999)). Such technologies and molecular tools have made it possible to monitor the expression levels of a large number of transcripts within a cell at any given time (see, e.g., Schena et al., *Science*, 270:467-470 (1995); Lockhart et al., *Nature Biotechnology*, 14:1675-1680 (1996); Blanchard et al., *Nature Biotechnology*, 14:1649 (1996); and U.S. Pat. No. 5,569,588, issued Oct. 29, 1996 to Ashby et al.).

[0007] How differential polynucleotide expression is associated with health and disease is a basis of functional genomics, which is defined as the study of all of the

polynucleotides expressed by a specific cell or a group of cells and the changes in their expression pattern during development, disease, or environmental exposure. Hybridization arrays, used to study polynucleotide expression, allow polynucleotide expression analysis on a genomic scale by permitting the examination of changes in expression of literally thousands of polynucleotides at one time. In general, for hybridization arrays, gene-specific sequences (probes) are immobilized on a solid state matrix. These sequences are then queried with labeled copies of nucleic acids from biological samples (targets). The underlying theory is that the greater the expression of a gene, the greater the amount of labeled target and thus, the greater output of signal. (W.M. Freeman et al., *BioTechniques*, 29:1042-1055 (2000)).

[0008] Recent studies have demonstrated that polynucleotide expression information generated by microarray analysis of human tumors can predict clinical outcome (L.J. van't Veer et al., *Nature*, 415:530-536 (2002); M. West et al., *Proc. Natl. Acad. Sci. USA*, 98:11462-11467 (2001); T. Sorlie et al., *Proc. Natl. Acad. Sci. USA*, 98:10869-10874 (2001); M. Shipp et al., *Nature Medicine*, 8(1):68-74 (2002)). These findings bring hope that cancer treatment will be vastly improved by better predicting the response of individual tumors to therapy.

[0009] Biomarkers can dictate the success of clinical development of novel anti-cancer drugs and the clinical benefits that patients can derive from such specific targeted therapeutics. Using expression of HER2 as a patient selection criteria has allowed the successful development of HERCEPTIN[®], a monoclonal antibody therapy targeting HER2 in breast cancer. Breast cancer patients that are identified to over-express HER2 and subsequently receive this therapy show a remarkable response rate and profound clinical benefits (10). In contrast, failure of using robust biomarkers in trials for innovative medicine, e.g., Gefitinib, a small molecule targeting epidermal growth factor receptor (EGFR), resulted in a setback in its clinical development (11, 12), even though its efficacy in inhibiting tumor cell growth *in vitro* and/or in animal models had been fully demonstrated (13, 14). It has become increasingly important in clinical development to identify molecular biomarkers that could guide patient selection and enable monitoring of drug efficacy at the molecular level.

- [0010] Dasatinib is a potent, orally available small molecule inhibitor that targets multiple cytosolic or membrane-bound tyrosine kinases including Src, BCR-Abl, c-kit, PDGFR β and EphA2 (4, 5). Due to its potency against a large panel of leukemic cancer cell lines harboring BCR-ABL mutations (6) and the clear and imminent need for overcoming the imatinib resistance, this compound has been expedited in investigational trials for leukemias. Based on the profound clinical benefits demonstrated in these trials, dasatinib was recently approved for use in chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL) that are resistant or intolerant to imatinib (7). The involvement of Src kinase in a number of cellular processes such as cell migration, adhesion and angiogenesis, participation of Src in a number of pathways such as EGFR, and the potential role of those tyrosine kinase targets in tumorigenesis (8, 9) have prompted investigations for potential use of dasatinib in other types of malignancy including solid tumors.
- [0011] Needed in the art are new and alternative methods and procedures to determine drug sensitivity in patients and which are necessary to treat diseases and disorders, particularly cancers such as prostate cancer, based on patient response to protein tyrosine kinase inhibitors at a molecular level. By using cultured cells as a model of *in vivo* effects, the present invention advantageously focuses on cell-intrinsic properties that are exposed in cell culture and involves identified polynucleotides that correlate with drug sensitivity. The presently described discovery and identification of polynucleotides/marker polynucleotides (predictor polynucleotides and polynucleotide sets) in cell lines assayed *in vitro* can be used to correlate with drug responses *in vivo*, and thus can be extended to clinical situations in which the same polynucleotides are used to predict responses to drugs and/or chemotherapeutic agents, including protein tyrosine kinase inhibitors, by patients, with particular regard to prostate cancer patients.

SUMMARY OF THE INVENTION

- [0012] The present invention describes the identification of marker polynucleotides whose expression levels are highly correlated with drug sensitivity in prostate cell lines that are either sensitive or resistant to protein tyrosine kinase

inhibitor compounds. More particularly, the protein tyrosine kinases that are inhibited in accordance with the present invention include members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors. For a review of these and other protein tyrosine kinases, see, for example, P. Blume-Jensen and T. Hunter, "Oncopolynucleotide Kinase Signaling", *Nature*, 411:355-365 (2001). Some of these polynucleotides are also modulated by the tyrosine kinase inhibitor compounds, in particular, src tyrosine kinase inhibitor compounds, which indicates their involvement in the protein tyrosine kinase signaling pathway. These polynucleotides or "markers" show utility in predicting a host's response to a drug and/or drug treatment.

[0013] It is an aspect of this invention to provide a cell culture model to identify polynucleotides whose expression levels correlate with drug sensitivity of cells associated with a disease state, or with a host having a disease. In accordance with the present invention, oligonucleotide microarrays were utilized to measure the expression levels of a large number of polynucleotides in a panel of untreated cell lines, particularly prostate cell lines, for which drug sensitivity to a protein tyrosine kinase inhibitor compound was determined. The determination of the polynucleotide expression profiles in the untreated cells allowed a prediction of chemosensitivity and the identification of marker polynucleotides whose expression levels highly correlated with sensitivity to drugs or compounds that modulate, preferably inhibit, protein tyrosine kinase or the pathway in which the protein tyrosine kinase, e.g., src tyrosine kinase, is involved. The marker polynucleotides are thus able to be utilized as one or more predictors to foresee a patient's response to drugs or drug treatments that directly or indirectly affect protein tyrosine kinase activity.

[0014] It is another aspect of the present invention to provide a method of determining or predicting if an individual requiring drug or chemotherapeutic treatment or therapy for a disease state, or a cancer or tumor of a particular type, e.g., a prostate cancer or prostate tumor, will successfully respond or will not respond to the drug or chemotherapeutic treatment or therapy prior to the administration of such treatment or chemotherapy. Preferably, the treatment or therapy involves a protein tyrosine kinase modulating agent, e.g., an inhibitor of the protein tyrosine kinase

activity. The protein tyrosine kinases whose activities can be inhibited by inhibitor compounds according to this invention include, for example, members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors. Also in accordance with the present invention, cells from a patient tissue sample, e.g., a prostate tumor or cancer biopsy, are assayed to determine their polynucleotide expression pattern prior to treatment with a protein tyrosine kinase modulating compound or drug, preferably a src tyrosine kinase inhibitor. The resulting polynucleotide expression profile of the test cells before exposure to the compound or drug is compared with the polynucleotide expression pattern of the predictor set of polynucleotides that have been described and shown herein (Table 2). In addition, in such a method, the polynucleotide expression pattern of one or more predictor polynucleotides, i.e., such as the 174, 14, 10 and 5 polynucleotide predictor sets as set forth in Tables 2, 3, 4, and 5, respectively, and/or the measurement of polynucleotide expression pattern of uPA, CK5, EPHA2, AR, and/or PSA, individually or any combination thereof, can also be used. These polynucleotides are derived from the control panel of the untreated cells that have been determined to be either resistant or sensitive to the drug or compound.

[0015] Success or failure of treatment with a drug can be determined based on the polynucleotide expression pattern of cells from the test tissue (test cells), e.g., a tumor or cancer biopsy, as being relatively similar to or different from the polynucleotide expression pattern of the predictor set of polynucleotides. Thus, if the test cells show a polynucleotide expression profile which corresponds to that of the predictor set of polynucleotides in the control panel of cells which are sensitive to the drug or compound, it is highly likely or predicted that the individual's cancer or tumor will respond favorably to treatment with the drug or compound. By contrast, if the test cells show a polynucleotide expression pattern corresponding to that of the predictor set of polynucleotides of the control panel of cells which are resistant to the drug or compound, it is highly likely or predicted that the individual's cancer or tumor will not respond to treatment with the drug or compound.

[0016] It is a further aspect of this invention to provide screening assays for determining if a cancer patient will be susceptible or resistant to treatment with a drug

or compound, particularly, a drug or compound directly or indirectly involved in a protein tyrosine kinase activity or a protein tyrosine kinase pathway. Such protein tyrosine kinases include, without limitation, members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other
5 protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors.

[0017] In a more particular aspect, the present invention provides screening assays for determining if a cancer patient will be susceptible or resistant to treatment with a drug or compound, particularly, a drug or compound directly or indirectly involved in src tyrosine kinase activity or the src tyrosine kinase pathway.

10 **[0018]** It is another aspect of the present invention to provide a method of monitoring the treatment of a patient having a disease treatable by a compound or agent that modulates a protein tyrosine kinase, including members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph
15 receptors. This can be accomplished by comparing the resistance or sensitivity polynucleotide expression profile of cells from a patient tissue sample, e.g., a tumor or cancer biopsy, e.g., a prostate cancer or tumor sample, prior to treatment with a drug or compound that inhibits the protein tyrosine kinase activity and again following treatment with the drug or compound. The isolated test cells from the
20 patient's tissue sample are assayed to determine their polynucleotide expression pattern before and after exposure to a compound or drug, such as, e.g., a src tyrosine kinase inhibitor. The resulting polynucleotide expression profile of the test cells before and after treatment is compared with the polynucleotide expression pattern of the predictor set, predictor subsets, or any one of the predictor polynucleotides
25 individually or any combination thereof. Thus, if a patient's response becomes one that is sensitive to treatment by a protein tyrosine kinase inhibitor compound, based on a correlation of the expression profile of the predictor polynucleotides, the patient's treatment prognosis can be qualified as favorable and treatment can continue. Also, if after treatment with a drug or compound, the test cells do not show
30 a change in their polynucleotide expression profile that corresponds to the control panel of cells that are sensitive to the drug or compound, this can serve as an indicator that the current treatment should be modified, changed, or even discontinued. Such a

monitoring process can indicate success or failure of a patient's treatment with a drug or compound, and the monitoring processes can be repeated as necessary or desired.

[0019] It is a further aspect of the present invention to provide predictor polynucleotides and predictor sets of polynucleotides having both diagnostic and prognostic value in disease areas in which signaling through a protein tyrosine kinase or a protein tyrosine kinase pathway is of importance, e.g., in cancers and tumors, in immunological disorders, conditions or dysfunctions, or in disease states in which cell signaling and/or proliferation controls are abnormal or aberrant. Such protein tyrosine kinases whose direct or indirect modulation can be associated with a disease state or condition, include members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors. In accordance with this invention, the use of one or more predictor polynucleotides, or a predictor polynucleotide set or subset (such as the 174, 14, 10 and 5 polynucleotide predictor sets as set forth in Tables 2, 3, 4, and 5, respectively, and/or the measurement of polynucleotide expression pattern of uPA, CK5, EPHA2, AR, and/or PSA, individually or any combination thereof) is to forecast or foretell an outcome prior to having any knowledge about a biological system, or a cellular response.

[0020] It is yet another aspect of the present invention to assemble one or more polynucleotides, such as those listed Tables 2, 3, 4, and 5, respectively, and/or the measurement of polynucleotide expression pattern of uPA, CK5, EPHA2, AR, and/or PSA, individually or any combination thereof, that highly correlate with resistance or sensitivity to protein tyrosine kinase inhibitor drugs or compounds, into predictor polynucleotide sets, so as to predict, or reasonably foretell the effect of either the protein tyrosine inhibitor compounds, or compounds that affect the protein tyrosine kinase signaling pathway(s) in different biological systems, or for cellular responses. The predictor polynucleotide sets can be used in *in vitro* assays of drug response by test cells to predict *in vivo* outcome. In accordance with this invention, the various predictor polynucleotide sets described herein, or the combination of these predictor sets with other polynucleotides or other co-variants of these polynucleotides, can be used, for example, to predict how patients with cancer or a tumor might respond to therapeutic intervention with compounds that modulate protein tyrosine kinases, or

modulate signaling through an entire protein tyrosine kinase regulatory pathway. The predictor sets of polynucleotides, or co-variants of these polynucleotides, can be used to predict how patients with a cancer or tumor respond to therapy employing compounds that modulate a tyrosine kinase, or the activity of a tyrosine kinase, such as protein tyrosine kinase members of the Src family, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors.

[0021] Another object of the present invention is to provide one or more specialized microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, comprising those polynucleotides or combinations thereof, as described herein, showing expression profiles that correlate with either sensitivity or resistance to protein tyrosine kinase inhibitor compounds. Such microarrays can be employed in *in vitro* assays for assessing the expression level of the polynucleotides on the microarrays in the test cells from tumor biopsies, for example, and determining whether these test cells will be likely to be resistant or sensitive to the protein tyrosine kinase inhibitor compound(s). For example, a specialized microarray can be prepared using some or all of the polynucleotides, polynucleotide subsets, or combinations thereof, as described herein and shown in Tables 2, 3, 4, and 5, respectively, and/or the measurement of the polynucleotide expression pattern of uPA, CK5, EPHA2, AR, and/or PSA, individually or any combination thereof. Cells from a tissue or organ biopsy can be isolated and exposed to one or more inhibitor compounds. Following application of nucleic acids isolated from both untreated and treated cells to one or more of the specialized microarrays, the pattern of polynucleotide expression of the tested cells can be determined and compared with that of the predictor polynucleotide pattern from the control panel of cells used to create the predictor polynucleotide set on the microarray. Based upon the polynucleotide expression pattern results from the cells undergoing testing, it can be determined if the cells show a resistant or a sensitive profile of polynucleotide expression. Whether or not the tested cells from a tissue or organ biopsy will respond to a protein tyrosine kinase inhibitor compound, and the course of treatment or therapy, can then be determined or evaluated based on the information gleaned from the results of the specialized microarray analysis.

[0022] It is a further aspect of the present invention to provide a kit for determining or predicting drug susceptibility or resistance by a patient having a disease, with particular regard to a cancer or tumor, namely, a prostate cancer or tumor. Such kits are useful in a clinical setting for testing a patient's biopsied tumor or cancer sample, for example, to determine or predict if the patient's tumor or cancer will be resistant or sensitive to a given treatment or therapy with a drug, compound, chemotherapy agent, or biological agent that is directly or indirectly involved with modification, preferably, inhibition, of the activity of a protein tyrosine kinase or a cell signaling pathway involving protein tyrosine kinase activity. Provided in the kit are one or more microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, comprising those polynucleotides that correlate with resistance and sensitivity to protein tyrosine kinase modulators, particularly, inhibitors of members of the Src family of protein tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as inhibitors of the Bcr-abl, Jak, PDGFR, c-kit and Eph receptors protein tyrosine kinases; and, in suitable containers, the modulator agents/compounds for use in testing cells from patient tissue specimens or patient samples; and instructions for use. In addition, kits contemplated by the present invention can include reagents or materials for the monitoring of the expression of the predictor or marker polynucleotides of the invention at the level of mRNA or protein, using other techniques and systems practiced in the art, e.g., RT-PCR assays, which employ primers designed on the basis of one or more of the predictor polynucleotides described herein, immunoassays, such as enzyme linked immunosorbent assays (ELISAs), immunoblotting, e.g., Western blots, or *in situ* hybridization, and the like, as further described herein. The kits according to the present invention can also comprise one or more predictor polynucleotides as set forth in Table 2, including subsets of predictor polynucleotides which include, but are not limited to the predictor polynucleotide subsets as presented in such as the 174, 14, 10 and 5 polynucleotide predictor sets set forth in Tables 3, 4, and 5, respectively, and/or the measurement of polynucleotide expression pattern of uPA, CK5, EPHA2, AR, and/or PSA, individually or any combination thereof herein.

[0023] Another aspect of the present invention is to provide one or more polynucleotides among those of the predictor polynucleotides identified herein that

can serve as targets for the development of drug therapies for disease treatment. Such targets can be particularly applicable to treatment of prostate disease, such as prostate cancers or tumors. Because these predictor polynucleotides are differentially expressed in sensitive and resistant cells, their expression pattern is correlated with the relative intrinsic sensitivity of cells to treatment with compounds that interact with and/or inhibit protein tyrosine kinases, including members of the Src family of protein tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as the Bcr-abl, Jak, PDGFR, c-kit and Eph receptors protein tyrosine kinases. Accordingly, the polynucleotides highly expressed in resistant cells can serve as targets for the development of new drug therapies for those tumors which are resistant to protein tyrosine kinase inhibitor compounds.

[0024] Yet another object of the present invention is to provide antibodies, either polyclonal or monoclonal, directed against one or more of the protein tyrosine kinase biomarker polypeptides, or peptides thereof, encoded by the predictor polynucleotides. Such antibodies can be used in a variety of ways, for example, to purify, detect, and target the protein tyrosine kinase biomarker polypeptides of the present invention, including both *in vitro* and *in vivo* diagnostic, detection, screening, and/or therapeutic methods, and the like. Included among the protein tyrosine kinase biomarker polypeptides of this invention are members of the Src family of protein tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as the Bcr-abl, Jak, PDGFR, c-kit and Eph receptors protein tyrosine kinases.

[0025] Yet another object of the present invention is to provide antisense reagents, including siRNA, RNAi, and ribozyme reagents, directed against one or more of the protein tyrosine kinase biomarker polypeptides, or peptides thereof, encoded by the predictor polynucleotides. Such antisense reagents can be used in a variety of ways, for example, to detect, to target, and inhibit the expression of the protein tyrosine kinase biomarker polypeptides of the present invention, including both *in vitro* and *in vivo* diagnostic, detection, screening, and/or therapeutic methods, and the like. Included among the protein tyrosine kinase biomarker polypeptides of this invention are members of the Src family of protein tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as the Bcr-abl, Jak, PDGFR, c-kit and Eph receptors protein tyrosine kinases.

[0026] The invention also relates to an antisense compound at least 8 to 30 nucleotides in length that specifically hybridizes to a nucleic acid molecule encoding the human protein tyrosine kinase biomarker polypeptides of the present invention, wherein said antisense compound inhibits the expression of the human protein tyrosine kinase biomarker polypeptides.

[0027] The invention further relates to a method of inhibiting the expression of the human protein tyrosine kinase biomarker polypeptides of the present invention in human cells or tissues comprising contacting said cells or tissues *in vitro*, or *in vivo*, with an antisense compound of the present invention so that expression of the protein tyrosine kinase biomarker polypeptides is inhibited.

[0028] The present invention is also directed to a method of identifying a compound that modulates the biological activity of protein tyrosine kinase biomarker polypeptides, comprising the steps of: (a) combining a candidate modulator compound with protein tyrosine kinase biomarker polypeptides in the presence of an antisense molecule that antagonizes the activity of the protein tyrosine kinase biomarker polypeptides, and (b) identifying candidate compounds that reverse the antagonizing effect of the peptide.

[0029] The present invention is also directed to a method of identifying a compound that modulates the biological activity of protein tyrosine kinase biomarker polypeptides, comprising the steps of: (a) combining a candidate modulator compound with protein tyrosine kinase biomarker polypeptides in the presence of a small molecule that antagonizes the activity of the protein tyrosine kinase biomarker polypeptides, and (b) identifying candidate compounds that reverse the antagonizing effect of the peptide.

[0030] The present invention is also directed to a method of identifying a compound that modulates the biological activity of protein tyrosine kinase biomarker polypeptides, comprising the steps of: (a) combining a candidate modulator compound with protein tyrosine kinase biomarker polypeptides in the presence of a small molecule that agonizes the activity of the protein tyrosine kinase biomarker polypeptides, and (b) identifying candidate compounds that reverse the agonizing effect of the peptide.

[0031] The present invention is also directed to a predictor set comprising a plurality of polynucleotides whose expression pattern is predictive of the response of cells to treatment with a compound that modulates protein tyrosine kinase activity or members of the protein tyrosine kinase pathway; wherein the polynucleotides are
5 selected from the group consisting of: the polynucleotides provided in Table 2; the sensitive predictor polynucleotides provided in Table 2; and the resistant predictor polynucleotides provided in Table 2.

[0032] The present invention is also directed to a predictor set comprising a plurality of polynucleotides whose expression pattern is predictive of the response of
10 cells to treatment with a compound that modulates protein tyrosine kinase activity or members of the protein tyrosine kinase pathway; wherein the polynucleotides are selected from the group consisting of: the polynucleotides provided in Table 2; the sensitive predictor polynucleotides provided in Table 2; the resistant predictor polynucleotides provided in Table 2; one or more of uPA, CK5, EPHA2, AR, and/or
15 PSA, individually or any combination thereof, wherein the plurality of polynucleotides comprise the number of polynucleotides selected from the group consisting of: at least about 1 polynucleotides; at least about 2 polynucleotides; at least about 3 polynucleotides; at least about 4 polynucleotides; at least about 5 polynucleotides; at least about 6 polynucleotides; at least about 7 polynucleotides; at
20 least about 10 polynucleotides; at least about 15 polynucleotides; at least about 20 polynucleotides; at least about 25 polynucleotides; and at least about 30 polynucleotides.

[0033] The present invention is also directed to a predictor set comprising a plurality of polynucleotides whose expression pattern is predictive of the response of
25 cells to treatment with a compound that modulates protein tyrosine kinase activity or members of the protein tyrosine kinase pathway; wherein the polynucleotides are selected from the group consisting of: the polynucleotides provided in Table 2; the sensitive predictor polynucleotides provided in Table 2; the resistant predictor polynucleotides provided in Table 2, one or more of uPA, CK5, EPHA2, AR, and/or
30 PSA, individually or any combination thereof; wherein the plurality of polynucleotides comprise the number of polynucleotides selected from the group consisting of: at least about 1 polynucleotides; at least about 2 polynucleotides; at

least about 3 polynucleotides; at least about 4 polynucleotides; at least about 5 polynucleotides; at least about 6 polynucleotides; at least about 7 polynucleotides; at least about 10 polynucleotides; at least about 15 polynucleotides; at least about 20 polynucleotides; at least about 25 polynucleotides; and at least about 30 polynucleotides; wherein the plurality of polynucleotides comprise a member of the group consisting of: the polynucleotides provided in Table 3; the sensitive predictor polynucleotides provided in Table 3; the resistant predictor polynucleotides provided in Table 3; the polynucleotides provided in Table 4; the sensitive predictor polynucleotides provided in Table 4; the resistant predictor polynucleotides provided in Table 4; the polynucleotides provided in Table 5; the sensitive predictor polynucleotides provided in Table 5; and the resistant predictor polynucleotides provided in Table 5.

[0034] The present invention is also directed to a predictor set comprising a plurality of polynucleotides whose expression pattern is predictive of the response of cells to treatment with a compound that modulates protein tyrosine kinase activity or members of the protein tyrosine kinase pathway; wherein the polynucleotides are selected from the group consisting of: the polynucleotides provided in Table 2; the sensitive predictor polynucleotides provided in Table 2; and the resistant predictor polynucleotides provided in Table 2; wherein the plurality of polynucleotides comprise the number of polynucleotides selected from the group consisting of: at least about 1 polynucleotides; at least about 2 polynucleotides; at least about 3 polynucleotides; at least about 4 polynucleotides; at least about 5 polynucleotides; at least about 6 polynucleotides; at least about 7 polynucleotides; at least about 10 polynucleotides; at least about 15 polynucleotides; at least about 20 polynucleotides; at least about 25 polynucleotides; and at least about 30 polynucleotides; wherein the plurality of polynucleotides comprise a member of the group consisting of: the polynucleotides provided in Table 3; the sensitive predictor polynucleotides provided in Table 3; the resistant predictor polynucleotides provided in Table 3; the polynucleotides provided in Table 4; the sensitive predictor polynucleotides provided in Table 4; the resistant predictor polynucleotides provided in Table 4; the polynucleotides provided in Table 5; the sensitive predictor polynucleotides provided in Table 5; the resistant predictor polynucleotides provided in Table 5, and any one or

more uPA, CK5, EPHA2, AR, and/or PSA, individually or any combination thereof; wherein the compound is selected from the group consisting of: antisense reagents directed to said polynucleotides, or one or more members of the protein tyrosine kinase pathway; RNAi reagents directed to said polynucleotides, or one or more
5 members of the protein tyrosine kinase pathway; antibodies directed against polypeptides encoded by said polynucleotides, or one or more members of the protein tyrosine kinase pathway; and small molecule compounds.

[0035] The present invention is also directed to a predictor set comprising a plurality of polynucleotides whose expression pattern is predictive of the response of
10 cells to treatment with a compound that modulates protein tyrosine kinase activity or members of the protein tyrosine kinase pathway; wherein the polynucleotides are selected from the group consisting of: the polynucleotides provided in Table 2; the sensitive predictor polynucleotides provided in Table 2; the resistant predictor polynucleotides provided in Table 2, and any one or more of uPA, CK5, EPHA2, AR,
15 and/or PSA, individually or any combination thereof; wherein the plurality of polynucleotides comprise the number of polynucleotides selected from the group consisting of: at least about 1 polynucleotides; at least about 2 polynucleotides; at least about 3 polynucleotides; at least about 4 polynucleotides; at least about 5 polynucleotides; at least about 6 polynucleotides; at least about 7 polynucleotides; at
20 least about 10 polynucleotides; at least about 15 polynucleotides; at least about 20 polynucleotides; at least about 25 polynucleotides; and at least about 30 polynucleotides; wherein the plurality of polynucleotides comprise a member of the group consisting of: the polynucleotides provided in Table 3; the sensitive predictor polynucleotides provided in Table 3; the resistant predictor polynucleotides provided
25 in Table 3; the polynucleotides provided in Table 4; the sensitive predictor polynucleotides provided in Table 4; the resistant predictor polynucleotides provided in Table 4; the polynucleotides provided in Table 5; the sensitive predictor polynucleotides provided in Table 5; and the resistant predictor polynucleotides provided in Table 5, wherein the compound is selected from the group consisting of:
30 antisense reagents directed to said polynucleotides, or one or more members of the protein tyrosine kinase pathway; RNAi reagents directed to said polynucleotides, or one or more members of the protein tyrosine kinase pathway; antibodies directed

against polypeptides encoded by said polynucleotides, or one or more members of the protein tyrosine kinase pathway; and small molecule compounds; wherein the compound is BMS-A.

5 **[0036]** A predictor set according to the present invention wherein said cells are a member of the group consisting of: cancer cells, prostate cells, prostate cancer cells, breast cells, breast cancer cells, lung cells, lung cancer cells.

10 **[0037]** The present invention is also directed to a predictor set comprising a plurality of polypeptides whose expression pattern is predictive of the response of cells to treatment with compounds that modulate protein tyrosine kinase activity or members of the protein tyrosine kinase pathway.

15 **[0038]** The present invention is also directed to a predictor set comprising a plurality of polypeptides whose expression pattern is predictive of the response of cells to treatment with compounds that modulate protein tyrosine kinase activity or members of the protein tyrosine kinase pathway; wherein the polypeptides are selected from the group consisting of: the polypeptides provided in Table 2; the sensitive predictor polypeptides provided in Table 2; the resistant predictor polypeptides provided in Table 2; the polypeptides provided in Table 3; the sensitive predictor polypeptides provided in Table 3; the resistant predictor polypeptides provided in Table 3; the polypeptides provided in Table 4; the sensitive predictor polypeptides provided in Table 4; the resistant predictor polypeptides provided in Table 4; the polypeptides provided in Table 5; the sensitive predictor polypeptides provided in Table 5; the resistant predictor polypeptides provided in Table 5; and any one or more of uPA, CK5, EPHA2, AR, and/or PSA polypeptides, individually or any combination thereof

25 **[0039]** The present invention is also directed to a predictor set comprising a plurality of polypeptides whose expression pattern is predictive of the response of cells to treatment with compounds that modulate protein tyrosine kinase activity or members of the protein tyrosine kinase pathway; wherein the polypeptides are selected from the group consisting of: the polypeptides provided in Table 2; the sensitive predictor polypeptides provided in Table 2; the resistant predictor polypeptides provided in Table 2; and any one or more of uPA, CK5, EPHA2, AR, and/or PSA polypeptide, individually or any combination thereof wherein the

30

plurality of polypeptides comprise the number of polypeptides selected from the group consisting of: at least about 1 polypeptides; at least about 2 polypeptides at least about 3 polypeptides; at least about 4 polypeptides at least about 5 polypeptides; at least about 6 polypeptides at least about 7 polypeptides; at least about 10
5 polypeptides; at least about 15 polypeptides; at least about 20 polypeptides; at least about 25 polypeptides; and at least about 30 polypeptides.

[0040] The present invention is also directed to a predictor set comprising a plurality of polypeptides whose expression pattern is predictive of the response of cells to treatment with compounds that modulate protein tyrosine kinase activity or
10 members of the protein tyrosine kinase pathway; wherein the polypeptides are selected from the group consisting of: the polypeptides provided in Table 2; the sensitive predictor polypeptides provided in Table 2; the resistant predictor polypeptides provided in Table 2; and any one or more of uPA, CK5, EPHA2, AR, and/or PSA polypeptide, individually or any combination thereof wherein the
15 plurality of polypeptides comprise the number of polypeptides selected from the group consisting of: at least about 1 polypeptides; at least about 2 polypeptides at least about 3 polypeptides; at least about 4 polypeptides at least about 5 polypeptides; at least about 6 polypeptides at least about 7 polypeptides; at least about 10 polypeptides; at least about 15 polypeptides; at least about 20 polypeptides; at least
20 about 25 polypeptides; and at least about 30 polypeptides; wherein the plurality of polypeptides comprise a member of the group consisting of: polypeptides provided in Table 3; the sensitive predictor polypeptides provided in Table 3; the resistant predictor polypeptides provided in Table 3; the polypeptides provided in Table 4; the sensitive predictor polypeptides provided in Table 4; the resistant predictor
25 polypeptides provided in Table 4; the polypeptides provided in Table 5; the sensitive predictor polypeptides provided in Table 5; and the resistant predictor polypeptides provided in Table 5.

[0041] The present invention is also directed to a predictor set comprising a plurality of polypeptides whose expression pattern is predictive of the response of
30 cells to treatment with compounds that modulate protein tyrosine kinase activity or members of the protein tyrosine kinase pathway; wherein the polypeptides are selected from the group consisting of: the polypeptides provided in Table 2; the

sensitive predictor polypeptides provided in Table 2; the resistant predictor polypeptides provided in Table 2; and any one or more of uPA, CK5, EPHA2, AR, and/or PSA polypeptide, individually or any combination thereof; wherein the plurality of polypeptides comprise the number of polypeptides selected from the group consisting of: at least about 1 polypeptides; at least about 2 polypeptides at least about 3 polypeptides; at least about 4 polypeptides at least about 5 polypeptides; at least about 6 polypeptides at least about 7 polypeptides; at least about 10 polypeptides; at least about 15 polypeptides; at least about 20 polypeptides; at least about 25 polypeptides; and at least about 30 polypeptides; wherein the plurality of polypeptides comprise a member of the group consisting of: polypeptides provided in Table 3; the sensitive predictor polypeptides provided in Table 3; the resistant predictor polypeptides provided in Table 3; the polypeptides provided in Table 4; the sensitive predictor polypeptides provided in Table 4; the resistant predictor polypeptides provided in Table 4; the polypeptides provided in Table 5; the sensitive predictor polypeptides provided in Table 5; and the resistant predictor polypeptides provided in Table 5; wherein the compound is selected from the group consisting of: antisense reagents directed against polynucleotides encoding said polypeptides, or one or more members of the protein tyrosine kinase pathway; antibodies directed against said polypeptides, or one or more members of the protein tyrosine kinase pathway; and small molecule compounds.

[0042] The present invention is also directed to a predictor set comprising a plurality of polypeptides whose expression pattern is predictive of the response of cells to treatment with compounds that modulate protein tyrosine kinase activity or members of the protein tyrosine kinase pathway; wherein the polypeptides are selected from the group consisting of: the polypeptides provided in Table 2; the sensitive predictor polypeptides provided in Table 2; the resistant predictor polypeptides provided in Table 2; and any one or more of uPA, CK5, EPHA2, AR, and/or PSA, individually or any combination thereof, wherein the plurality of polypeptides comprise the number of polypeptides selected from the group consisting of: at least about 1 polypeptides; at least about 2 polypeptides at least about 3 polypeptides; at least about 4 polypeptides at least about 5 polypeptides; at least about 6 polypeptides at least about 7 polypeptides; at least about 10 polypeptides; at least

about 15 polypeptides; at least about 20 polypeptides; at least about 25 polypeptides; and at least about 30 polypeptides; wherein the plurality of polypeptides comprise a member of the group consisting of: polypeptides provided in Table 3; the sensitive predictor polypeptides provided in Table 3; the resistant predictor polypeptides provided in Table 3; the polypeptides provided in Table 4; the sensitive predictor polypeptides provided in Table 4; the resistant predictor polypeptides provided in Table 4; the polypeptides provided in Table 5; the sensitive predictor polypeptides provided in Table 5; and the resistant predictor polypeptides provided in Table 5; wherein the compound is selected from the group consisting of: antisense reagents directed against polynucleotides encoding said polypeptides, or one or more members of the protein tyrosine kinase pathway; antibodies directed against said polypeptides, or one or more members of the protein tyrosine kinase pathway; and small molecule compounds; wherein the compound is BMS-A.

[0043] The present invention is also directed to a method for predicting whether a compound is capable of modulating the activity of cells, comprising the steps of: obtaining a sample of cells; determining whether said cells express a plurality of markers; and correlating the expression of said markers to the compounds ability to modulate the activity of said cells.

[0044] The present invention is also directed to a method for predicting whether a compound is capable of modulating the activity of cells, comprising the steps of: obtaining a sample of cells; determining whether said cells express a plurality of markers; and correlating the expression of said markers to the compounds ability to modulate the activity of said cells wherein the plurality of markers are polynucleotides.

[0045] The present invention is also directed to a method for predicting whether a compound is capable of modulating the activity of cells, comprising the steps of: obtaining a sample of cells; determining whether said cells express a plurality of markers; and correlating the expression of said markers to the compounds ability to modulate the activity of said cells wherein the plurality of markers are polynucleotides; wherein the polynucleotides, compounds, and cells are the polynucleotides, compounds, and cells disclosed herein.

[0046] The present invention is also directed to a method for predicting whether a compound is capable of modulating the activity of cells, comprising the steps of: obtaining a sample of cells; determining whether said cells express a plurality of markers; and correlating the expression of said markers to the compounds ability to modulate the activity of said cells wherein the plurality of markers are polypeptides.

[0047] The present invention is also directed to a plurality of cell lines for identifying polynucleotides and polypeptides whose expression levels correlate with compound sensitivity or resistance of cells associated with a disease state; wherein said cell lines are prostate cancer cell lines, breast cancer cell lines, or lung cancer cell lines.

[0048] The present invention is also directed to a method of identifying polynucleotides and polypeptides that predict compound sensitivity or resistance of cells associated with a disease state, comprising the steps of: subjecting the plurality of cell lines to one or more compounds; analyzing the expression pattern of a microarray of polynucleotides or polypeptides; and selecting polynucleotides or polypeptides that predict the sensitivity or resistance of cells associated with a disease state by using said expression pattern of said microarray; wherein said compounds are compounds disclosed herein, and wherein said disease is prostate cancer, breast cancer, and/or lung cancer.

[0049] The present invention is also directed to a method for predicting whether an individual requiring treatment for a disease state, will successfully respond or will not respond to said treatment comprising the steps of: obtaining a sample of cells from said individual; determining whether said cells express a plurality of markers; and correlating the expression of said markers to the individuals ability to respond to said treatment.

[0050] The present invention is also directed to a method for predicting whether an individual requiring treatment for a disease state, will successfully respond or will not respond to said treatment comprising the steps of: obtaining a sample of cells from said individual; determining whether said cells express a plurality of markers; and correlating the expression of said markers to the individuals ability to respond to said treatment; wherein the plurality of markers are polynucleotides; wherein the polynucleotides, and compounds are disclosed herein.

[0051] The present invention is also directed to a method for predicting whether an individual requiring treatment for a disease state, will successfully respond or will not respond to said treatment comprising the steps of: obtaining a sample of cells from said individual; determining whether said cells express a plurality of markers; and correlating the expression of said markers to the individuals ability to respond to said treatment; wherein the plurality of markers are polypeptides; wherein the polypeptides, and compounds are disclosed herein.

[0052] The present invention is also directed to a method of screening for candidate compounds capable of binding to and/or modulating the activity of a protein tyrosine kinase biomarker polypeptide, comprising contacting a test compound with a polypeptide described herein; and selecting as candidate compounds those test compounds that bind to and/or modulate activity of the polypeptide.

[0053] The present invention is also directed to a method of treating prostate cancer in a subject, comprising administering a modulator of one or more protein tyrosine kinase biomarker polypeptides, wherein said polypeptide(s) is selected from the group consisting of: polypeptides provided in Table 2; the sensitive predictor polypeptides provided in Table 2; the resistant predictor polypeptides provided in Table 2; polypeptides provided in Table 3; the sensitive predictor polypeptides provided in Table 3; the resistant predictor polypeptides provided in Table 3; the polypeptides provided in Table 4; the sensitive predictor polypeptides provided in Table 4; the resistant predictor polypeptides provided in Table 4; the polypeptides provided in Table 5; the sensitive predictor polypeptides provided in Table 5; the resistant predictor polypeptides provided in Table 5; and any one or more of uPA, CK5, EPHA2, AR, and/or PSA, individually or any combination thereof.

[0054] The present invention is also directed to a method of predicting whether patients may be resistant to a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, by measuring the expression level of kallikrein 3 and androgen receptor in a patient sample, wherein an elevated expression level (e.g., about 2-fold or more above standard) of kallikrein 3 and/or androgen receptor relative to the level observed in a standard sample, is indicative of

at least partial resistance to said protein tyrosine kinase inhibitor, and wherein decreased expression (e.g., about 2-fold or more less than standard) of kallikrein 3 and/or androgen receptor relative to the level observed in a standard sample, is indicative of sensitivity to said protein tyrosine kinase inhibitor. The expression
5 profiling measurement may be at either the mRNA or protein level.

[0055] The present invention is also directed to a method of predicting whether patients may be resistant to a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, by measuring the expression level of
10 cytokeratin 5 in a patient sample, wherein a decreased expression level of cytokeratin 5 relative to the level observed in a standard sample (e.g., about 2-fold or more below standard), is indicative of at least partial resistance to said protein tyrosine kinase inhibitor, and wherein elevated expression of cytokeratin 5 relative to the level observed in a standard sample, is indicative of sensitivity to said protein tyrosine
15 kinase inhibitor. The expression profiling measurement may be at either the mRNA or protein level.

[0056] The present invention is also directed to a method of predicting whether patients may be resistant to a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, by determining whether a patient sample
20 shows signs of basal cell type phenotype for the prostatic cell lineage, wherein the presence of such signs is indicative of sensitivity to said protein tyrosine kinase inhibitor. Such signs may constitute expression profile signatures of such a phenotype, cellular morphology, histochemical signature, or other sign known in the
25 art to be associated with basal cell type phenotype for the prostatic cell lineage.

[0057] The present invention is also directed to a method of predicting whether patients may be resistant to a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, by measuring the expression level of
30 kallikrein 3, androgen receptor, and cytokeratin 5 in a patient sample, wherein an elevated expression level of kallikrein 3 and/or androgen receptor relative to the level observed in a standard sample (e.g., about 2-fold or more above standard)

accompanied by a decreased expression level of cytokeratin 5 relative to the level observed in a standard sample (e.g., about 2-fold or more below standard), is indicative of at least partial resistance to said protein tyrosine kinase inhibitor, and wherein decreased expression of kallikrein 3 and/or androgen receptor relative to the level observed in a standard sample (e.g., about 2-fold or more below standard) accompanied by an elevated expression level of cytokeratin 5 relative to the level observed in a standard sample (e.g., about 2-fold or more above standard), is indicative of sensitivity to said protein tyrosine kinase inhibitor. The expression profiling measurement may be at either the mRNA or protein level.

- 10 **[0058]** The present invention is also directed to a method of predicting whether patients may be resistant to a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, by measuring the expression level of SCCL, ANXA3, CST6, LAMC2, ZBED2, EREG, AXL, FHL2, PLAU, and/or
- 15 ARNTL2 in a patient sample, wherein a decreased expression level of SCCL, ANXA3, CST6, LAMC2, ZBED2, EREG, AXL, FHL2, PLAU, and/or ARNTL2 relative to the level observed in a standard sample (e.g., about 2-fold or more below standard), is indicative of at least partial resistance to said protein tyrosine kinase inhibitor, and wherein elevated expression of SCCL, ANXA3, CST6, LAMC2,
- 20 ZBED2, EREG, AXL, FHL2, PLAU, and/or ARNTL2 relative to the level observed in a standard sample (e.g., about 2-fold or more above standard), is indicative of sensitivity to said protein tyrosine kinase inhibitor. The expression profiling measurement may be at either the mRNA or protein level.

- [0059]** The present invention is also directed to a method of predicting whether
- 25 patients may be resistant to a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, by measuring the expression level of EPHA2 in a patient sample, wherein a decreased expression level of EPHA2 relative to the level observed in a standard sample (e.g., about 2-fold or more below standard),
- 30 is indicative of at least partial resistance to said protein tyrosine kinase inhibitor, and wherein elevated expression of EPHA2 relative to the level observed in a standard sample (e.g., about 2-fold or more above standard), is indicative of sensitivity to said

protein tyrosine kinase inhibitor. The expression profiling measurement may be at either the mRNA or protein level.

[0060] The present invention is also directed to a method of predicting whether patients may be resistant to a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, by measuring the expression level of UPA in a patient sample, wherein a decreased expression level of UPA relative to the level observed in a standard sample (e.g., about 2-fold or more below standard), is indicative of at least partial resistance to said protein tyrosine kinase inhibitor, and wherein elevated expression of UPA relative to the level observed in a standard sample (e.g., about 2-fold or more above standard), is indicative of sensitivity to said protein tyrosine kinase inhibitor. The expression profiling measurement may be at either the mRNA or protein level.

[0061] The present invention is also directed to a method of using a surrogate marker to predict the responsiveness of a patient suffering from cancer to a protein tyrosine kinase inhibitor, comprising the step of comparing the expression level of a predictive polynucleotide or polypeptide marker both prior to and subsequent to the administration of a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, wherein a decrease in expression of said predictive marker subsequent to said administration relative to the absence of said administration is indicative of said patient being responsive to said protein tyrosine kinase inhibitor, wherein said predictive marker is represented by one or more sensitive markers provided in Table 2, 3, 4, or 5, including, but not limited to SCCL, ANXA3, CST6, LAMC2, ZBED2, EREG, AXL, FHL2, PLAU, and/or ARNTL2.

[0062] The present invention is also directed to a method of using a surrogate marker to predict the responsiveness of a patient suffering from cancer to a protein tyrosine kinase inhibitor, comprising the step of comparing the expression level of a predictive polynucleotide or polypeptide marker both prior to and subsequent to the administration of a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, wherein a decrease in expression of said

predictive marker subsequent to said administration relative to the absence of said administration is indicative of said patient being responsive to said protein tyrosine kinase inhibitor, wherein said predictive marker is represented by one or more sensitive markers provided in Table 2, 3, 4, or 5, including, but not limited to SCEL, ANXA3, CST6, LAMC2, ZBED2, EREG, AXL, FHL2, PLAU, and/or ARNTL2.

[0063] The present invention is also directed to a method of determining an efficacious dose of a protein tyrosine kinase inhibitor, comprising the step of comparing the expression level of a predictive sensitive polynucleotide or polypeptide marker both prior to and subsequent to the administration of a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, wherein a decrease in expression of said predictive marker to a specified level below the standard expression level is indicative of said patient reaching an efficacious dose of said protein tyrosine kinase inhibitor, wherein said predictive sensitive marker is represented by one or more sensitive markers provided in Table 2, 3, 4, or 5, including, but not limited to SCEL, ANXA3, CST6, LAMC2, ZBED2, EREG, AXL, FHL2, PLAU, and/or ARNTL2.

[0064] Further aspects, features, and advantages of the present invention will be better appreciated upon a reading of the detailed description of the invention when considered in connection with the accompanying figures or drawings.

DESCRIPTION OF THE FIGURES

[0065] Fig. 1. Identification of biomarkers correlated with sensitivity to dasatinib. (A) Discovery strategy to identify potential predictive and surrogate biomarkers. (B) IC₅₀ determination and sensitivity classification of 16 prostate cancer cell lines to dasatinib. (C) Cluster analysis showing the relative expression pattern of 174 genes that were highly correlated with dasatinib sensitivity/resistance classification of 16 cell lines. The dasatinib-sensitive cell lines were highlighted in red, and the position of three important prostatic cell markers CK5, PSA and AR were marked on the heatmap. These genes have differential expression of more than 3-fold between the sensitive and resistant groups. (D) Relative baseline gene expression of CK5, PSA

and AR in the 16 cell lines. The resistant cells are in black and sensitive cells are in red. The values on X-axes are expression level in log₂-scale.

[0066] Fig. 2. Correlation of EphA2 gene expression with sensitivity to dasatinib.

(A) Negative correlation between the expression levels of EphA2 (●) and the IC₅₀ (◆) values for the 16 cell lines. The coefficient of Pearson correlation is -0.66, indicating a high reverse correlation. (B) Expression of EphA2 protein in 5 sensitive and 3 resistant cell lines. Overall, the protein expression levels in these cell lines correlated well with the mRNA levels detected with microarray.

[0067] Fig. 3. PLAU gene expression and regulation by dasatinib analyzed at

mRNA and protein levels. (A) Differential baseline expression of PLAU gene between resistant (R, in black) and sensitive (S, in red) cell lines. The X-values are the expression level in log₂-scale. The resistant cell line expressing high level of PLAU was WPMY1 and the sensitive cell line expressing low level of PLAU was LNCaP. (B) Down-regulation of PLAU mRNA level by dasatinib-treatment in 5 sensitive cell lines. The cells were treated with 100 nM dasatinib (+D) or DMSO (Ctrl) for 48 hours. The *p* value was 0.048 in paired *t*-test, indicating a significant reduction of PLAU mRNA following dasatinib treatment. (C) Correlation between dasatinib-induced PLAU mRNA down-regulation with the sensitivity of cell lines to dasatinib. In addition to those 5 sensitive cells, three dasatinib-resistant cell lines 22Rv1, MDAPCa2b, and VCaP were also treated with dasatinib same as in Fig. 3B. The extent of PLAU down-regulation by dasatinib (Y-axis) was negatively correlated with the log₂IC₅₀ values (X-axis) of these 8 cell lines. (D) Dose-dependent down-regulation of PLAU mRNA expression in PC3 cells. Cells were treated with or without different concentrations of dasatinib for 4 or 24 hours. The PLAU expression level relative to control was shown in Y-axis. (E) Dose-dependent inhibition of secreted uPA protein in PC3 cells by dasatinib but not by TAXOL[®]. Cells were treated with different doses of dasatinib, TAXOL[®] or DMSO for 24 hours. The amount of uPA protein secreted into the culture medium by 50,000 viable cells was assessed by ELISA assay.

[0068] Fig. 4. Expression pattern of a five-gene model in prostate cell lines and tumors. (A) Hierarchical clustering of five genes AR, PSA, CK5, PLAU, and EphA2 in prostate cell lines. AR and PSA are two luminal cell markers, and CK5 (KRT5) is a

basal cell marker. The resistant cell lines were separated from majority of sensitive cells (labeled in red) by these five genes. Note the high expression of CK5 and/or PLAU, EphA2 expression in sensitive cells and the nearly opposite expression pattern in resistant cells. (B) Expression pattern of these 5 genes in 52 prostate tumors. The gene expression of this published data set were profiled on AFFYMETRIX[®] HG-U95 gene chips. Two AR, three PSA probe sets and one CK5, PLAU and EphA2 probe set are available on the chip and were retrieved for cluster analysis. The tumors samples exhibiting dasatinib-sensitive pattern, i.e., with low AR and PSA expression and high KRT5 and/or PLAU, EphA2 expression were highlighted in red.

10 [0069] Fig. 5. Use of uPA expression levels as a surrogate for predicting tumor sensitivity to BMS-A. uPA protein level in the plasma of mice bearing PC3 xenograft tumors in the absence or presence of dasatinib was measured. Xenograft tumors were established subcutaneously in nu/nu athymic mice. The mice were divided into three groups each with 8 mice after tumors had reached approximately 200mg. Two groups were treated with dasatinib at 15 or 30mg/kg respectively, and the third group as control did not receive dasatinib. Dasatinib was administered orally twice a day on a 5-day-on 2-day-off schedule for 20 doses. The tumor size measurement and blood sampling were performed prior to treatment and weekly for two weeks after treatment initiation. As shown, there was a concordance between BMS-A-dependent uPA expression level modulation with tumor xenograft growth. Experiments were performed as described in Example 2.

DESCRIPTION OF THE TABLES

[0070] Table 1 presents the resistance/sensitivity phenotype classification of the 16 prostate cell lines for the protein tyrosine kinase inhibitor compound BMS-A based on IC₅₀ results. The IC₅₀ for each cell line was assessed in by MTS assays as described in Example 1 (Methods). The mean IC₅₀ values along with standard deviations (SD) were calculated from 2 to 5 individual determinations for each cell line as shown. The IC₅₀ unit is μ M. The mean IC₅₀ for each cell line was log-transformed to log₁₀(IC₅₀) and the mean log₁₀(IC₅₀) across the 16 breast cell lines for BMS-A was calculated and used to normalize the IC₅₀ data for each cell line. The cell lines with an IC₅₀ lower than 0.2 μ m were defined as sensitive to the compound, while

those having an IC₅₀ above 0.2μm were considered to be resistant. The cell lines presented in bold were used in the drug induction study as described herein.

TABLE 1

No.	Cell Lines	mean IC ₅₀ (μM) to BMS-A	SD	Classification
1	PC3	0.051	0.007	Sensitive
2	DU145	0.057	0.03	Sensitive
3	HPV10	0.058	0.022	Sensitive
4	LNCaP	0.068	0.013	Sensitive
5	RWPE1	0.12	0.085	Sensitive
6	HPV7	0.127	0.015	Sensitive
7	NB26	0.139	0.023	Sensitive
8	PWR1E	0.145	0.083	Sensitive
9	RWPE2	0.151	0.027	Sensitive
10	NB11	0.163	0.077	Sensitive
11	W99	0.199	0.068	Sensitive
12	22Rv1	2		Resistant
13	VCaP	2		Resistant
14	MDAPCa2b	2.5		Resistant
15	DUCaP	2.5		Resistant
16	WPMY1	2.5		Resistant

5

[0071] Table 2 shows a polynucleotide list derived from three analysis algorithms that demonstrated a high correlation between expression pattern and resistance/sensitivity classification to BMS-A. For each gene, the DNA and encoded amino acid sequence represented by SEQ ID NOs. in Table 2 are set forth in the

10 Sequence Listing. Genes are listed according to fold change.

TABLE 2
Markers highly correlated to BMS-A in expression pattern and resistance/sensitivity classification

Gene No.	Expressed In	Modulated by BMS-A ($p < 0.05$)	Unigene Title	Gene Symbol	Probe ID	p-value (1-way ANOVA)	p-value (IC50 correlation)	Fold Change*	DNA SEQ ID NO:	GENBANK® Accession No.	Amino Acid SEQ ID NO:	GENBANK® Accession No.
1	Sensitive		serpin peptidase inhibitor, clade B, member 5	SERPINB5	204855_at	1.75E-05	5.24E-04	6.91	11	NM_002639	12	NP_002630.1
2	Sensitive		keratin 6	KRT6	209125_at	3.47E-03	3.01E-02	6.46	13	NM_005554	14	NP_005545.1
3	Sensitive	decreased	scellin	SCEL	206884_s_at	4.11E-04	4.33E-03	5.8	15	NM_003843	16	NP_003834.2
4	Sensitive		prostaglandin-endoperoxide synthase 2	PTGS2	204748_at	1.10E-02	4.22E-02	5.3	17	NM_000963	18	NP_000954.1
5	Sensitive		S100 calcium binding protein A2	S100A2	204268_at	3.15E-04	4.90E-03	5.2	19	NM_005978	20	NP_005969.1
6	Sensitive		keratin 7	KRT7	209016_s_at	8.48E-05	1.38E-03	5.02	21	NM_005556	22	NP_005547.3
7	Sensitive		interleukin 18	IL18	206295_at	2.37E-04	4.76E-03	4.6	23	NM_001562	24	NP_001553.1
8	Sensitive	decreased	annexin A3	ANXA3	209369_at	4.59E-04	4.18E-03	4.46	25	NM_005139	26	NP_005130.1
9	Sensitive		peptidase inhibitor 3, skin-derived	PI3	41469_at	1.69E-02	2.74E-02	4.44	27	NM_002638	28	NP_002629.1
10	Sensitive		G protein-coupled receptor 87	GPR87	219936_s_at	7.86E-04	9.78E-03	4.42	29	NM_023915	30	NP_076404.2
11	Sensitive		keratin 5	KRT5 (CK5)	201820_at	5.67E-03	4.91E-02	4.41	1	NM_000424	2	NP_000415.1
12	Sensitive		amphiregulin	AREG	205239_at	5.32E-05	4.62E-04	4.38	31	NM_001657	32	NP_001648.1
13	Sensitive		serpin peptidase inhibitor, clade B, member 2	SERPINB2	204614_at	2.44E-02	4.85E-02	4.36	33	NM_002575	34	NP_002566.1
14	Sensitive		thrombomodulin	THBD	203887_s_at	5.59E-03	3.21E-02	4.23	35	NM_000361	36	NP_000352.1
15	Sensitive	decreased	cystatin E/M	CST6	206595_at	5.86E-04	8.19E-03	4.21	37	NM_001323	38	NP_001314.1
16	Sensitive		dystonin	DST	204455_at	4.25E-03	3.09E-02	4.19	39	NM_001723	40	NP_001714.1
17	Sensitive		caveolin 2	CAV2	203323_at	1.12E-03	4.32E-03	4.09	41	NM_001233	42	NP_001224.1
18	Sensitive		lipocalin 2	LCN2	212531_at	3.21E-03	7.33E-03	4.03	43	NM_005564	44	NP_005555.2
19	Sensitive		chloride intracellular channel 3	CLIC3	219529_at	1.45E-03	1.15E-02	3.96	45	NM_004669	46	NP_004660.2

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20	Sensitive		transforming growth factor, beta 2	TGFB2	209909_s_at	6.38E-04	8.25E-03	3.94	47	NM_003238	48	NP_003229.1
21	Sensitive		annexin A8	ANXA8	203074_at	3.50E-03	4.12E-02	3.93	49	NM_001040084	50	NP_001035173.1
22	Sensitive		interferon-induced protein with tetratricopeptide repeats 1	IFIT1	203153_at	1.33E-03	2.56E-03	3.92	51	NM_001001887	52	NP_001001887.1
23	Sensitive		parathyroid hormone-like hormone	PTH1H	211756_at	2.60E-02	2.88E-02	3.91	53	NM_002820	54	NP_002811.1
24	Sensitive		B cell RAG associated protein	GALNAC4S-6ST	203066_at	5.57E-05	3.89E-04	3.91	55	NM_015892	56	NP_056976.2
25	Sensitive		claudin 1	CLDN1	218182_s_at	1.54E-03	1.86E-02	3.91	57	NM_021101	58	NP_066924.1
26	Sensitive		interferon, alpha-inducible protein 27	IFI27	202411_at	5.44E-03	1.76E-02	3.89	59	NM_005532	60	NP_005523.3
27	Sensitive		fibroblast growth factor binding protein 1	FGFBP1	205014_at	5.26E-03	1.58E-02	3.86	61	NM_005130	62	NP_005121.1
28	Sensitive		S100 calcium binding protein A9	S100A9	203535_at	2.55E-02	4.12E-02	3.83	63	NM_002965	64	NP_002956.1
29	Sensitive		interferon-induced protein 44-like	IFI44L	204439_at	1.33E-02	2.40E-02	3.82	65	NM_006820	66	NP_006811.1
30	Sensitive		cytochrome P450, family 24, subfamily A, polypeptide 1	CYP24A1	206504_at	1.01E-02	2.52E-02	3.82	67	NM_000782	68	NP_000773.2
31	Sensitive		EGF-containing fibulin-like extracellular matrix protein 1	EFEMP1	201842_s_at	5.26E-03	2.92E-02	3.81	69	NM_001039348	70	NP_001034437.1
32	Sensitive		interferon induced transmembrane protein 1	IFITM1	214022_s_at	4.05E-04	4.14E-03	3.73	71	NM_003641	72	NP_003632.2
33	Sensitive	decreased	laminin, gamma 2	LAMC2	202267_at	9.70E-04	8.46E-03	3.71	73	NM_005562	74	NP_005553.1
34	Sensitive	decreased	zinc finger, BED-type containing 2	ZBED2	219836_at	2.04E-03	2.13E-02	3.7	75	NM_024508	76	NP_078784.2
35	Sensitive		cytidine deaminase	CDA	205627_at	2.82E-04	1.75E-03	3.69	77	NM_001785	78	NP_001776.1

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36	Sensitive		carbonic anhydrase II	CA2	209301_at	4.60E-03	2.56E-02	3.68	79	NM_000067	80	NP_000058.1
37	Sensitive		interferon-induced protein 44	IFI44	214453_s_at	1.14E-03	3.42E-03	3.67	81	NM_006417	82	NP_006408.2
38	Sensitive		interferon, gamma-inducible protein 16	IFI16	206332_s_at	1.19E-02	3.35E-02	3.63	83	NM_005531	84	NP_005522.2
39	Sensitive		myxovirus resistance 1	MX1	202086_at	2.04E-03	4.92E-03	3.62	85	NM_002462	86	NP_002453.1
40	Sensitive		snail homolog 2	SNAI2	213139_at	9.26E-04	6.16E-03	3.62	87	NM_003068	88	NP_003059.1
41	Sensitive		caveolin 1	CAV1	203065_s_at	2.29E-03	8.77E-03	3.61	89	NM_001753	90	NP_001744.2
42	Sensitive		chemokine (C-X-C motif) ligand 2	CXCL2	209774_x_at	1.52E-02	2.03E-02	3.55	91	NM_002089	92	NP_002080.1
43	Sensitive	decreased	epiregulin	EREG	205767_at	3.84E-03	1.01E-02	3.54	93	NM_001432	94	NP_001423.1
44	Sensitive		interleukin 1 receptor antagonist	IL1RN	212657_s_at	1.22E-02	3.01E-02	3.54	95	NM_000577	96	NP_000568.1
45	Sensitive		sterile alpha motif domain containing 9	SAMD9	219691_at	9.35E-03	4.13E-02	3.51	97	NM_017654	98	NP_060124.2
46	Sensitive		moesin	MSN	200600_at	4.02E-04	1.04E-03	3.48	99	NM_002444	100	NP_002435.1
47	Sensitive		neutrophil cytosolic factor 2	NCF2	209949_at	5.09E-03	1.94E-02	3.45	101	NM_000433	102	NP_000424.2
48	Sensitive		lysosomal-associated membrane protein 3	LAMP3	205569_at	3.17E-03	6.38E-03	3.44	103	NM_014398	104	NP_055213.2
49	Sensitive		lectin, galactoside-binding, soluble, 3	LGALS3	208949_s_at	4.65E-03	1.85E-02	3.4	105	NM_002306	106	NP_002297.1
50	Sensitive		S100 calcium binding protein A14	S100A14	218677_at	2.04E-03	1.72E-02	3.39	107	NM_020672	108	NP_065723.1
51	Sensitive		serpin peptidase inhibitor, clade B, member 7	SERPINF7	206421_s_at	8.26E-03	1.69E-02	3.38	109	NM_001040147	110	NP_001035237.1
52	Sensitive	decreased	AXL receptor tyrosine kinase	AXL	202686_s_at	7.82E-03	2.10E-02	3.36	111	NM_001699	112	NP_001690.2
53	Sensitive		kallikrein 5	KLK5	222242_s_at	7.36E-03	2.71E-02	3.35	113	NM_012427	114	NP_036559.1
54	Sensitive	decreased	four and a half LIM domains 2	FHL2	202949_s_at	6.41E-03	3.23E-02	3.31	115	NM_001039492	116	NP_001034581.1

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55	Sensitive		fascin homolog 1	FSCN1	210933_s_at	1.28E-03	5.84E-03	3.31	117	NM_003088	118	NP_003079.1
56	Sensitive		homeo box A1	HOXA1	214639_s_at	4.05E-04	3.61E-03	3.31	119	NM_005522	120	NP_005513.1
57	Sensitive		2',5'-oligoadenylate synthetase 1	OAS1	202869_at	9.56E-03	1.77E-02	3.31	121	NM_001032409	122	NP_001027581.1
58	Sensitive		dickkopf homolog 1	DKK1	204602_at	8.60E-03	2.22E-02	3.31	123	NM_012242	124	NP_036374.1
59	Sensitive		tissue factor pathway inhibitor 2	TFPI2	209278_s_at	1.77E-02	2.41E-02	3.29	125	NM_006528	126	NP_006519.1
60	Sensitive		inhibin, beta A	INHBA	210511_s_at	1.04E-02	3.94E-02	3.29	127	NM_002192	128	NP_002183.1
61	Sensitive		embryonal Fyn-associated substrate	EFS	204400_at	1.47E-02	3.00E-02	3.28	129	NM_005864	130	NP_005855.1
62	Sensitive		immediate early response 3	IER3	201631_s_at	2.45E-03	9.30E-03	3.28	131	NM_003897	132	NP_003888.1
63	Sensitive	decreased	plasminogen activator, urokinase	PLAU (also referred to as uPA)	205479_s_at	5.66E-04	8.12E-04	3.27	3	NM_002658	4	NP_002649.1
64	Sensitive		met proto-oncogene	MET	213816_s_at	1.54E-03	8.71E-03	3.26	133	NM_000245	134	NP_000236.2
65	Sensitive		laminin, beta 3	LAMB3	209270_at	4.23E-05	5.06E-04	3.26	135	NM_000228	136	NP_000219.2
66	Sensitive		complement component 3	C3	217767_at	1.94E-03	1.93E-02	3.25	137	NM_000064	138	NP_000055.1
67	Sensitive		bone marrow stromal cell antigen 2	BST2	201641_at	9.99E-03	4.78E-02	3.25	139	NM_004335	140	NP_004326.1
68	Sensitive		transforming growth factor, alpha	TGFA	205016_at	5.51E-03	2.83E-02	3.25	141	NM_003236	142	NP_003227.1
69	Sensitive		transforming growth factor beta 1 induced transcript 1	TGFB1I1	209651_at	1.01E-02	4.60E-02	3.24	143	NM_001042454	144	NP_001035919.1
70	Sensitive		supervillin	SVIL	202565_s_at	5.86E-04	7.34E-03	3.23	145	NM_003174	146	NP_003165.1
71	Sensitive		fer-1-like 3, myoferlin	FER1L3	211864_s_at	5.16E-03	1.65E-02	3.23	147	NM_013451	148	NP_038479.1
72	Sensitive		aldo-keto reductase family 1, member B10	AKR1B10	206561_s_at	1.65E-02	1.56E-02	3.21	149	NM_020299	150	NP_064695.2
73	Sensitive		RAB38, member RAS oncogene family	RAB38	219412_at	4.70E-03	2.54E-02	3.2	151	NM_022337	152	NP_071732.1

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74	Sensitive		guanine nucleotide binding protein	GNA15	205349_at	2.34E-03	1.96E-02	3.2	153	NM_002068	154	NP_002059.1
75	Sensitive		transforming growth factor, beta receptor II	TGFB2	208944_at	2.13E-03	1.35E-02	3.19	155	NM_001024847	156	NP_001020018.1
76	Sensitive		peptidylglycine alpha-amidating monooxygenase	PAM	214620_x_at	3.97E-03	1.76E-02	3.17	157	NM_000919	158	NP_000910.2
77	Sensitive		cathepsin L2	CTSL2	210074_at	3.17E-05	1.39E-03	3.17	159	NM_001333	160	NP_001324.2
78	Sensitive		PDZ domain containing 3	PDZK3	209493_at	3.19E-03	1.67E-02	3.16	161	NM_178140	162	NP_835260.2
79	Sensitive		integrin, beta 4	ITGB4	204989_s_at	1.76E-04	3.28E-03	3.16	163	NM_000213	164	NP_000204.3
80	Sensitive		pleckstrin homology-like domain, family A, member 1	PHLDA1	217996_at	9.17E-03	1.62E-02	3.15	165	NM_007350	166	NP_031376.3
81	Sensitive		interferon, alpha-inducible protein	G1P3	204415_at	5.98E-04	1.95E-03	3.13	167	NM_002038	168	NP_002029.3
82	Sensitive		fibroblast growth factor receptor 2	FGFR2	203638_s_at	7.62E-03	4.20E-02	3.13	169	NM_000141	170	NP_000132.1
83	Sensitive		periplakin	PPL	203407_at	2.49E-04	4.47E-03	3.12	171	NM_002705	172	NP_002696.3
84	Sensitive		heparanase	HPSE	219403_s_at	5.48E-04	3.67E-03	3.12	173	NM_006665	174	NP_006656.2
85	Sensitive		N-myc (and STAT) interactor	NMI	203964_at	3.12E-03	1.20E-02	3.12	175	NM_004688	176	NP_004679.1
86	Sensitive		phospholipid scramblase 1	PLSCR1	202430_s_at	8.22E-03	2.41E-02	3.1	177	NM_021105	178	NP_066928.1
87	Sensitive		proteoglycan 1, secretory granule	PRG1	201858_s_at	4.77E-02	4.19E-02	3.1	179	NM_002727	180	NP_002718.2
88	Sensitive		trophoblast glycoprotein	TPBG	203476_at	9.40E-03	2.96E-02	3.09	181	NM_006670	182	NP_006661.1
89	Sensitive		DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	DDX58	218943_s_at	1.32E-03	5.71E-03	3.09	183	NM_014314	184	NP_055129.2
90	Sensitive		5'-nucleotidase, ecto (CD73)	NT5E	203939_at	3.82E-03	7.69E-03	3.09	185	NM_002526	186	NP_002517.1
91	Sensitive		suppressor of cytokine signaling 2	SOCS2	203372_s_at	7.70E-05	1.00E-04	3.08	187	NM_003877	188	NP_003868.1

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92	Sensitive		regulator of G-protein signalling 20	RGS20	210138_at	1.81E-03	1.20E-02	3.08	189	NM_003702	190	NP_003693.2
93	Sensitive		cathepsin C	CTSC	201487_at	6.81E-03	2.15E-02	3.07	191	NM_001814	192	NP_001805.1
94	Sensitive		v-myb myeloblastosis viral oncogene homolog (avian)-like 1	MYBL1	213906_at	7.99E-03	1.89E-02	3.07	193	XM_034274	194	XP_034274.7
95	Sensitive		interleukin 1, beta	IL1B	39402_at	2.77E-02	3.45E-02	3.07	195	NM_000576	196	NP_000567.1
96	Sensitive		insulin-like growth factor binding protein 6	IGFBP6	203851_at	1.34E-02	2.21E-02	3.07	197	NM_002178	198	NP_002169.1
97	Sensitive		gap junction protein, beta 5 (connexin 31.1)	GJB5	206156_at	3.68E-03	3.61E-02	3.07	199	NM_005268	200	NP_005259.1
98	Sensitive		keratin, hair, basic, 1	KRTHB1	213711_at	6.73E-03	1.08E-02	3.07	201	NM_002281	202	NP_002272.1
99	Sensitive		FYVE, RhoGEF and PH domain containing 6	FGD6	219901_at	3.06E-04	1.83E-03	3.06	203	NM_018351	204	NP_060821.2
100	Sensitive	decreased	aryl hydrocarbon receptor nuclear translocator-like 2	ARNTL2	220658_s_at	4.15E-03	4.71E-03	3.06	205	NM_020183	206	NP_064568.3
101	Sensitive		2'-5'-oligoadenylate synthetase 2, 69/71kDa	OAS2	204972_at	3.75E-02	3.95E-02	3.06	207	NM_001032731	208	NP_001027903.1
102	Sensitive		SH3 domain binding glutamic acid-rich protein like	SH3BGRL	201312_s_at	8.44E-03	7.12E-03	3.06	209	NM_003022	210	NP_003013.1
103	Sensitive		opsin 3 (encephalopsin, panopsin)	OPN3	219032_x_at	4.03E-03	5.85E-03	3.06	211	NM_001030011	212	NP_001025182.1
104	Sensitive		vesicle-associated membrane protein 8	VAMP8	202546_at	1.09E-03	2.74E-03	3.06	213	NM_003761	214	NP_003752.2
105	Sensitive		annexin A1	ANXA1	201012_at	7.84E-03	2.98E-02	3.05	215	NM_000700	216	NP_000691.1
106	Sensitive		deafness, autosomal dominant 5	DFNA5	203695_s_at	7.57E-04	4.90E-03	3.05	217	NM_004403	218	NP_004394.1
107	Sensitive		secretory leukocyte peptidase inhibitor	SLPI	203021_at	4.01E-03	1.32E-02	3.05	219	NM_003064	220	NP_003055.1

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108	Sensitive		serpin peptidase inhibitor, clade A, member 1	SERPINA1	202833_s_at	4.93E-02	3.16E-02	3.04	221	NM_000295	222	NP_000286.3
109	Sensitive		zinc finger protein 185 (LIM domain)	ZNF185	203585_at	2.33E-04	8.85E-04	3.04	223	NM_007150	224	NP_009081.1
110	Sensitive		Rho family GTPase 3	RND3	212724_at	1.07E-02	3.81E-02	3.04	225	NM_005168	226	NP_005159.1
111	Sensitive		IGF-II mRNA-binding protein 3	IMP-3	203820_s_at	7.38E-03	2.08E-02	3.04	227	NM_006547	228	NP_006538.2
112	Sensitive		epithelial membrane protein 1	EMP1	201325_s_at	1.14E-02	3.97E-02	3.03	229	NM_001423	230	NP_001414.1
113	Sensitive		tumor-associated calcium signal transducer 2	TACSTD2	202286_s_at	4.98E-03	2.63E-02	3.02	231	NM_002353	232	NP_002344.1
114	Sensitive		ADAM metallopeptidase domain 19	ADAM19	209765_at	9.42E-03	3.24E-02	3.02	233	NM_023038	234	NP_075525.2
115	Sensitive		lectin, galactoside-binding, soluble, 3 binding protein	LGALS3BP	200923_at	5.54E-04	5.87E-03	3.02	235	NM_005567	236	NP_005558.1
116	Sensitive		cadherin 3, type 1, P-cadherin	CDH3	203256_at	1.46E-03	1.05E-02	3.02	237	NM_001793	238	NP_001784.2
117	Sensitive		keratin 15	KRT15	204734_at	4.20E-03	2.37E-02	3.02	239	NM_002275	240	NP_002266.2
118	Sensitive		ATP-binding cassette, sub-family A (ABC1), member 12	ABCA12	215465_at	1.52E-02	2.79E-02	3	241	NM_015657	242	NP_056472.2
119	Sensitive		tumor necrosis factor, alpha-induced protein 3	TNFAIP3	202644_s_at	2.71E-03	1.35E-02	3	243	NM_006290	244	NP_006281.1
120	Sensitive		vascular endothelial growth factor C	VEGFC	209946_at	6.89E-03	1.48E-02	3	245	NM_005429	246	NP_005420.1
121	Sensitive		interferon-stimulated transcription factor 3, gamma	ISGF3G	203882_at	3.43E-04	2.19E-03	3	247	NM_006084	248	NP_006075.3

Gene No.	Expressed In	Modulated by BMS-A (p<0.05)	Unigene Title	Gene Symbol	Probe ID	p-value (1-way ANOVA)	p-value (IC50 correlation)	Fold Change*	DNA SEQ ID NO:	GENBANK® Accession No.	Amino Acid SEQ ID NO:	GENBANK® Accession No.
122	Sensitive		squamous cell carcinoma antigen recognized by T cells 2	SART2	218854_at	6.21E-03	3.33E-02	2.99	249	NM_013352	250	NP_037484.1
123	Sensitive		potassium channel tetramerisation domain containing 12	KCTD12	212192_at	1.69E-02	4.51E-02	2.99	251	NM_138444	252	NP_612453.1
124	Sensitive		chemokine (C-X-C motif) ligand 1	CXCL1	204470_at	3.53E-02	3.92E-02	2.99	253	NM_001511	254	NP_001502.1
125	Resistant		tetraspanin 8	TSPAN8	203824_at	2.73E-05	5.27E-04	5.41	255	NM_004616	256	NP_004607.1
126	Resistant		trinucleotide repeat containing 9	TNRC9	214774_x_at	7.44E-05	4.01E-04	5.1	257	XM_049037	258	XP_049037.9
127	Resistant		kallikrein 2, prostatic	KLK2	209854_s_at	1.86E-03	5.88E-03	4.84	259	NM_001002231	260	NP_001002231.1
128	Resistant		T cell receptor gamma constant 2	TRGC2	211144_x_at	1.91E-03	6.58E-03	4.6	261	NM_001003799	262	NP_001003799.1
129	Resistant		albumin	ALB	211298_s_at	3.38E-03	8.64E-03	4.52	263	NM_000477	264	NP_000468.1
130	Resistant		cysteine-rich secretory protein 3	CRISP3	207802_at	1.58E-02	2.15E-02	4.35	265	NM_006061	266	NP_006052.1
131	Resistant		Rho GTPase activating protein 6	ARHGAP6	206167_s_at	9.90E-04	6.09E-03	4.25	267	NM_001174	268	NP_001165.2
132	Resistant		lymphoid enhancer-binding factor 1	LEF1	221558_s_at	4.71E-06	3.74E-05	3.93	269	NM_016269	270	NP_057353.1
133	Resistant		collectin sub-family member 12	COLEC12	221019_s_at	1.06E-05	1.61E-04	3.88	271	NM_030781	272	NP_110408.2
134	Resistant		folate hydrolase (prostate-specific membrane antigen) 1	FOLH1	215363_x_at	1.29E-02	3.81E-02	3.76	273	NM_001014986	274	NP_001014986.1
135	Resistant		neuropeptide Y	NPY	206001_at	1.93E-02	2.60E-02	3.74	275	NM_000905	276	NP_000896.1
136	Resistant		cytochrome P450, family 4, subfamily F, polypeptide 8	CYP4F8	210576_at	2.80E-03	3.59E-03	3.7	277	NM_007253	278	NP_009184.1
137	Resistant		androgen receptor	AR	211621_at	1.31E-03	4.66E-03	3.68	5	NM_000044	6	NP_000035.2

Gene No.	Expressed In	Modulated by BMS-A (p<0.05)	Unigene Title	Gene Symbol	Probe ID	p-value (1-way ANOVA)	p-value (IC50 correlation)	Fold Change*	DNA SEQ ID NO:	GENBANK® Accession No.	Amino Acid SEQ ID NO:	GENBANK® Accession No.
138	Resistant		fibroblast growth factor 13	FGF13	205110_s_at	3.08E-03	1.34E-02	3.61	279	NM_004114	280	NP_004105.1
139	Resistant		hepatocyte growth factor	HGF	210755_at	2.24E-04	6.10E-04	3.57	281	NM_000601	282	NP_000592.3
140	Resistant		phospholipase A2, group VII	PLA2G7	206214_at	6.02E-04	5.21E-03	3.56	283	NM_005084	284	NP_005075.2
141	Resistant		kallikrein 3 (prostate specific antigen)	KLK3 (PSA)	204583_x_at	1.33E-02	2.70E-02	3.51	7	NM_001030047	8	NP_001025218.1
142	Resistant		protein kinase, cAMP-dependent, regulatory, type II, beta	PRKAR2B	203680_at	3.56E-04	3.75E-03	3.46	285	NM_002736	286	NP_002727.2
143	Resistant		acid phosphatase, prostate	ACPP	204393_s_at	6.94E-03	1.68E-02	3.45	287	NM_001099	288	NP_001090.2
144	Resistant		signal peptide, CUB domain, EGF-like 2	SCUBE2	219197_s_at	1.86E-03	4.07E-03	3.43	289	NM_020974	290	NP_066025.1
145	Resistant		phospholipase A1 member A	PLA1A	219584_at	2.71E-02	4.47E-02	3.42	291	NM_015900	292	NP_056984.1
146	Resistant		integral membrane protein 2A	ITM2A	202747_s_at	1.88E-02	2.70E-02	3.42	293	NM_004867	294	NP_004858.1
147	Resistant		secretogranin III	SCG3	219196_at	2.24E-03	4.82E-03	3.39	295	NM_013243	296	NP_037375.2
148	Resistant		phospholipase A2, group IVC	PLA2G4C	209785_s_at	3.30E-03	5.78E-03	3.37	297	NM_003706	298	NP_003697.1
149	Resistant		v-ets erythroblastosis virus E26 oncogene like	ERG	213541_s_at	2.09E-02	3.31E-02	3.37	299	NM_004449	300	NP_004440.1
150	Resistant		tudor domain containing 1	TDRD1	221018_s_at	2.31E-03	4.25E-03	3.36	301	NM_198795	302	NP_942090.1
151	Resistant		acyl-CoA synthetase medium-chain family member 3	ACSM3	205942_s_at	3.62E-03	1.99E-02	3.35	305	NM_005622	306	NP_005613.2
152	Resistant		aldehyde dehydrogenase 1 family, member A1	ALDH1A1	212224_at	8.50E-03	1.94E-02	3.26	307	NM_000689	308	NP_000680.2
153	Resistant		protein phosphatase 1E	PPM1E	205938_at	2.86E-04	2.14E-03	3.24	309	NM_014906	310	NP_055721.3
154	Resistant		Ras-related GTP binding D	RRA6D	221523_s_at	1.43E-03	6.19E-03	3.24	311	NM_021244	312	NP_067067.1

Gene No.	Expressed In	Modulated by BMS-A (p<0.05)	Unigene Title	Gene Symbol	Probe ID	p-value (1-way ANOVA)	p-value (IC50 correlation)	Fold Change*	DNA SEQ ID NO:	GENBANK® Accession No.	Amino Acid SEQ ID NO:	GENBANK® Accession No.
155	Resistant		regulator of G-protein signalling 4	RGS4	204337_at	1.01E-02	2.63E-02	3.23	313	NM_005613	314	NP_005604.1
156	Resistant		neurofilament, heavy polypeptide	NEFH	33767_at	7.68E-04	1.32E-03	3.22	315	NM_021076	316	NP_066554.2
157	Resistant		sucrase-isomaltase	SI	206664_at	1.58E-02	2.51E-02	3.21	317	NM_001041	318	NP_001032.1
158	Resistant		UDP glucuronosyltransferase 2 family, polypeptide B4	UGT2B4	206505_at	5.51E-03	1.02E-02	3.18	319	NM_021139	320	NP_066962.1
159	Resistant		myosin VIIA and Rab interacting protein	MYRIP	214156_at	7.82E-03	2.40E-02	3.17	321	NM_015460	322	NP_056275.2
160	Resistant		leucine-rich, glioma inactivated 1	LGI1	206349_at	2.42E-02	3.40E-02	3.15	323	NM_005097	324	NP_005088.1
161	Resistant		Phosphodiesterase 3B, cGMP-inhibited	PDE3B	222317_at	2.79E-03	1.34E-02	3.15	325	NM_000922	326	NP_000913.2
162	Resistant		elongation of very long chain fatty acids-like 2	ELOVL2	213712_at	3.11E-04	2.76E-03	3.13	327	NM_017770	328	NP_060240.2
163	Resistant		gamma-aminobutyric acid (GABA) A receptor, beta 3	GABRB3	205850_s_at	2.80E-03	1.24E-02	3.12	329	NM_000814	330	NP_000805.1
164	Resistant		adrenergic, alpha-2A-, receptor	ADRA2A	209869_at	9.22E-03	2.54E-02	3.11	331	NM_000681	332	NP_000672.2
165	Resistant		zinc finger and BTB domain containing 10	ZBTB10	219312_s_at	5.94E-03	4.05E-02	3.1	333	NM_023929	334	NP_076418.2
166	Resistant		myosin binding protein C, slow type	MYBPC1	214087_s_at	6.78E-04	2.10E-03	3.09	335	NM_002465	336	NP_002456.2
167	Resistant		angiotensin II receptor, type 1	AGTR1	205357_s_at	3.01E-03	5.83E-03	3.08	337	NM_000685	338	NP_000676.1
168	Resistant		homogentisate 1,2-dioxygenase	HGD	205221_at	1.55E-03	4.28E-03	3.08	339	NM_000187	340	NP_000178.1
169	Resistant		reticulon 1	RTN1	203485_at	2.51E-02	4.68E-02	3.07	341	NM_021136	342	NP_066959.1
170	Resistant		kinesin family member 5C	KIF5C	203130_s_at	5.35E-03	9.48E-03	3.06	343	XM_377774	344	XP_377774.3

Gene No.	Expressed In	Modulated by BMS-A (p<0.05)	Unigene Title	Gene Symbol	Probe ID	p-value (1-way ANOVA)	p-value (IC50 correlation)	Fold Change*	DNA SEQ ID NO:	GENBANK® Accession No.	Amino Acid SEQ ID NO:	GENBANK® Accession No.
171	Resistant		ADAM metallopeptidase with thrombospondin type 1 motif, 3	ADAMTS3	214913_at	1.31E-03	5.82E-03	3.03	345	NM_014243	346	NP_055058.1
172	Resistant		coagulation factor V (proaccelerin, labile factor)	F5	204714_s_at	1.46E-02	3.10E-02	3.02	347	NM_000130	348	NP_000121.2
173	Resistant		solute carrier family 16 (monocarboxylic acid transporters), member 10	SLC16A10	219915_s_at	1.63E-03	4.08E-03	3.01	349	NM_018593	350	NP_061063.2
174	Resistant		secretoglobin, family 2A, member 1	SCGB2A1	205979_at	2.84E-03	5.38E-03	2.99	351	NM_002407	352	NP_002398.1

TABLE 3**Common predictive markers identified in prostate and breast preclinical models.**

Gene No.	Probe ID	Gene symbol	Gene description	p-value (1-way ANOVA)	p-value (IC50 correlation)	Fold change
17	203324_s_at	CAV2	caveolin 2	1.21E-03	5.74E-03	4.63
41	212097_at	CAV1	caveolin 1	2.74E-03	1.00E-02	4.19
63	211668_s_at	PLAU	plasminogen activator, urokinase	1.02E-03	1.50E-03	3.80
40	213139_at	SNAI2	snail homolog 2	9.26E-04	6.16E-03	3.62
46	200600_at	MSN	moesin	4.02E-04	1.04E-03	3.48
64	203510_at	MET	met proto-oncogene	4.93E-03	1.97E-02	3.30
38	208966_x_at	IFI16	interferon, gamma-inducible protein 16	1.64E-02	4.34E-02	3.27
65	209270_at	LAMB3	laminin, beta 3	4.23E-05	5.06E-04	3.26
75	208944_at	TGFB2	transforming growth factor, beta receptor II	2.13E-03	1.35E-02	3.19
105	201012_at	ANXA1	annexin A1	7.84E-03	2.98E-02	3.05
123	212192_at	KCTD12	potassium channel tetramerisation domain containing 12	1.69E-02	4.51E-02	2.99
175	203499_at	EPHA2	EPH receptor A2	5.35E-03	2.02E-02	2.69
176	219926_at	POPDC3	popeye domain containing 3	3.25E-02	2.72E-02	2.53
177	212510_at	GPD1L	glycerol-3-phosphate dehydrogenase 1-like	1.21E-02	4.41E-02	0.42

***The SEQ ID NOs for the polynucleotide and polypeptide sequences for Gene No. 175 (EPHA2), Gene No. 176 (POPDC3), and Gene No. 177 (GPD1L) are SEQ ID NO:9 and 10, SEQ ID NO:353 and 354, and SEQ ID NO:355 and 356, respectively.

[0072] Table 4 lists the predictor set of 10 polynucleotides. These 10 polynucleotides were selected from the 174 polynucleotides shown in Table 2 based upon data derived from three analysis methods and from drug treatment studies.

TABLE 4

Gene No.	Gene description	Gene symbol	Modulated by BMS-A	Highly Expressed in
3	sciellin	SCEL	Decreased	Sensitive
8	annexin A3	ANXA3	Decreased	Sensitive
15	cystatin E/M	CST6	Decreased	Sensitive
33	laminin, gamma 2	LAMC2	Decreased	Sensitive

Gene No.	Gene description	Gene symbol	Modulated by BMS-A	Highly Expressed in
34	zinc finger, BED-type containing 2	ZBED2	Decreased	Sensitive
43	epiregulin	EREG	Decreased	Sensitive
52	AXL receptor tyrosine kinase	AXL	Decreased	Sensitive
54	four and a half LIM domains 2	FHL2	Decreased	Sensitive
63	plasminogen activator, urokinase	PLAU	Decreased	Sensitive
100	aryl hydrocarbon receptor nuclear translocator-like 2	ARNTL2	Decreased	Sensitive

[0073] Table 5 lists the predictor set of 5 polynucleotides..

TABLE 5

Gene No.	Gene description	Gene symbol	Modulated by BMS-A	Highly Expressed in	GENBANK [®] Accession DNA	SEQ ID NO:	GENBANK [®] Accession Protein	SEQ ID NO
11	keratin 5	KRT5 (CK5)		Sensitive	NM_000424	1	NP_000415	2
142	kallikrein 3 (prostate specific antigen)	KLK3 (PSA)		Resistant	NM_001030047	7	NP_001025218	8
137	androgen receptor	AR		Sensitive	NM_000044	5	NP_000035	6
63	plasminogen activator, urokinase	PLAU	Decreased	Sensitive	NM_002658	3	NP_002649	4
175	EPH receptor A2	EPHA2	Decreased	Sensitive	NM_004431	9	NP_004422	10

5

[0074] Table 6 lists the representative RT-PCR primer sets for each of the protein tyrosine kinase biomarker polynucleotides of the present invention. The SEQ ID NO: for each RT-PCR primer is provided (SEQ ID NO: 357 thru 887).

TABLE 6

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
NM_002639	Forward	CCAAGGGAGTGGCCCTATCAAAT	357
	Probe	CCATAGAGGTGCCAGGAGCACGGATCCT	358
	Reverse	GGATGGTCAGCATTCAATTCATCCT	359
NM_005554	Forward	CGCAAGCTGCTGGAGGGTG	360
	Probe	GGAGTGCAGGCTGAATGGCGAAGGC	361
	Reverse	CTGGAGACGGTGGACTGCACC	362
NM_003843	Forward	TCCCATTCTTTTCAGCCAGATCCT	363
	Probe	CCCAGGGAATCACTACAGGCTGGTTAGCCA	364
	Reverse	AAGGTAACATTGGACATGCTGCCTT	365
NM_000963	Forward	ACCATGGTAGAAGTTGGAGCACCAT	366
	Probe	TGTTCTCCTGCCTACTGGAAGCCAAGCACT	367
	Reverse	ATTTGAAAACCCACTTCTCCACCAA	368
NM_005978	Forward	CATGATGTGCAGTTCTCTGGAGCA	369
	Probe	GCTGGCTGTGCTGGTCACTACCTTCCACA	370
	Reverse	TCGCCCTCTTGGCAGGAGTACT	371
NM_005556	Forward	CAGCTGCGTGAGTACCAGGAACTC	372
	Probe	GCGTGAAGCTGGCCCTGGACATCG	373
	Reverse	AGCAGCTTGCGGTAGGTGGC	374
NM_001562	Forward	TTGCTGAGCCCTTGTCTCC	375
	Probe	CGACTGCCTGGACAGTCAGCAAGGAATTGT	376
	Reverse	AGCCAGAGTTGGCAGCCAGG	377
NM_005139	Forward	GTGAGGAACACGCCGGCC	378
	Probe	TGCATCGAGCCTTGAAGGGTATTGGAAGTGT	379
	Reverse	TCTGGACACCATTATTCGGTTCAGA	380
NM_002638	Forward	AGAGGCAGCTGTACGGGAGTT	381
	Probe	GGCCGTGTTCCATTCAATGGACAAGATCC	382
	Reverse	CTGGACCTTTGACTGGCTCTTGC	383
NM_023915	Forward	TTGTGTTTGGGTGATCATGGCTG	384
	Probe	GCCAAACATCATCCTGACAAATGGTCAGCC	385
	Reverse	AGCTGTTACATAGGTGACTGCCG	386
NM_000424	Forward	TCCTCTGGATATGGCAGTGGCA	387
	Probe	TATGGCGGTGGCCTCGGTGGAGG	388
	Reverse	CCACCGAGGCCGCCG	389
NM_001657	Forward	GAGCACCTGGAAGCAGTAACATGC	390
	Probe	AGCAAGAATATTTGCGGTGAACGGTGTGGGG	391
	Reverse	GGCAGCTATGGCTGCTAATGCA	392
NM_002575	Forward	ATGGAGGACGCCTTCAACAAGG	393
	Probe	TGTTCCACCAAGCCATGGTGGATGTGAA	394
	Reverse	GCTGCTTCAGTGCCCTCCTCA	395
NM_000361	Forward	CACCGACTGTGACTCCGGCA	396
	Probe	ACGGTGGCGACAGCGGCTCTGG	397
	Reverse	CGTCGGGCTGGGCGG	398
NM_001323	Forward	CAGGCGGCCGTGGCC	399
	Probe	GAGACACGCACATCATCAAGGCGCAGAG	400
	Reverse	GGTCTTGCGGCAGTCTGTGCT	401
NM_001723	Forward	TGTCATTGACCCTGTGAGAGGCA	402
	Probe	TTCGTGTTCTCTCCAGAAATTGCTCTGCAGC	403
	Reverse	CTTGTGTTGCTGGATGGCTCATG	404
NM_001233	Forward	TGGATCTGCAGCCATGCCC	405
	Probe	AGTTCCTGACGGTGTTCCTGGCCATTC	406
	Reverse	TCCAGATGTGCAGACAGCTGAGG	407

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
NM_005564	Forward	TTGCCAGCCCGGCGAG	408
	Probe	GAGTTACCTCGTCCGAGTGGTGAGCACCAA	409
	Reverse	CTTGAAGAACACCATAGCATGCTGG	410
NM_004669	Forward	CGAGTCCCGCCGCCG	411
	Probe	ACGGCGACAGGCTCACGCTGGC	412
	Reverse	GCGCACACCGTGTCGACG	413
NM_003238	Forward	TAGGGTGGAATGGATACACGAACC	414
	Probe	ACAATGCCAACTTCTGTGCTGGAGCATGC	415
	Reverse	CCCTGCTGTGCTGAGTGTCTGAA	416
NM_001040084	Forward	GGACAGCATCAAGAGTGAGACCCA	417
	Probe	CACTGGAGGAGGCCATGCTCACTGTGG	418
	Reverse	GCGTCCCTGCTCCCTTCATG	419
NM_001548	Forward	TTCGGAGAAAGGCATTAGATCTGGA	420
	Probe	TGAAGCCCTGGAGTACTATGAGCGGGCC	421
	Reverse	TTCTCAAAGTCAGCAGCCAGTCTCA	422
NM_002820	Forward	TCCAAGCCCTCTCCCAACACA	423
	Probe	AACCACCCCGTCCGATTTGGGTCTGAT	424
	Reverse	GCTGCTCTTTGTACGTCTCCACCTT	425
NM_015892	Forward	AAGCAACAGTTTCTCATTCTTCGCC	426
	Probe	TCATGCATCCAACGTCAAGTACACCATGCA	427
	Reverse	CATCAAAGCCTCCTGCTTCTCACTT	428
NM_021101	Forward	AGAAGATGAGGATGGCTGTCATTGG	429
	Probe	GGGTGCGATATTTCTTCTTGCAAGGTCTGGC	430
	Reverse	TGCCATACCATGCTGTGGCAA	431
NM_005532	Forward	CCCTGGCCAGGATTGCTACAGT	432
	Probe	CTCAGTGCCATGGGCTTCACTGCGG	433
	Reverse	GAGGACGAGGCGATTCCCG	434
NM_005130	Forward	GGAACACAAAGCCCAGGAAGGA	435
	Probe	AGGGAGCACATCAAAGGCCAAAGAGACCACC	436
	Reverse	CGGGAGCTTTGGTGGCCA	437
NM_002965	Forward	GCTGGTGCGAAAAGATCTGCAA	438
	Probe	AAATGCAGACAAGCAGCTGAGCTTCGAGGA	439
	Reverse	CCTCGCCATCAGCATGATGAAC	440
NM_006820	Forward	ACTTCTCAAAGCCGGGTCATGAAT	441
	Probe	TGCTTCAGATTTGGAAGTGGACCCCATGAA	442
	Reverse	CCGCAGCATCTGCCTCAGTG	443
NM_000782	Forward	TAAATACCCAGGTGTTGGGATCCAG	444
	Probe	TTGCGCATCTTCCATTGGCGTTGG	445
	Reverse	TCGGCGACCAATGCACATTC	446
NM_001039348	Forward	CTGTAAGTGCAATGCTTGTGCTCG	447
	Probe	ATCGTGACCTGGAGATGCTGACAGTCAGC	448
	Reverse	TCTTAACACAGAGCTTGTGCGGAAG	449
NM_003641	Forward	ATCCACAGCGAGACCTCCGTG	450
	Probe	CCGACCATGTCGTCTGGTCCCTGTTCA	451
	Reverse	ATGAAGCCCAGACAGCACCAGTT	452
NM_005562	Forward	CCTGCATCTGATGGACCAGCC	453
	Probe	CTTACTGGAGCAGAAGCTTTCCCGAGCCAA	454
	Reverse	CCAGCTCTGACATCATGGGCC	455
NM_024508	Forward	GCTCCCCACAGGCATTGAGG	456
	Probe	GGTAGGCTCCTGGAGCAGGTGGGCAC	457
	Reverse	TGGCTGGCCCACAAAGCC	458
NM_001785	Forward	GGGCATCTGTGCTGAACGGAC	459
	Probe	CGCTATCCAGAAGGCCGTCTCAGAAGGGT	459

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
	Reverse	GATAGCAATTGCCCTGAAATCCTTG	460
NM_000067	Forward	AACAAAGGGCAAGAGTGCTGACTTC	461
	Probe	CTTCGATCCTCGTGGCCTCCTTCCTGAAT	462
	Reverse	TGGTCAGTGAGCCTGGGTAGGTC	463
NM_006417	Forward	AGCCTGTGAGGTCCAAGCTAGAGG	464
	Probe	CCTCTGAGTGGGAGCTGGACCCTGTAAAGG	465
	Reverse	CCCATAGCATTCTGCTCAGAGCAG	466
NM_005531	Forward	ATGGACGACTGACCACAATCAACTG	467
	Probe	CTGAAACTCACCTGCTTTGAATTGGCACCG	468
	Reverse	CAGATCTCAACTCCCCGGTATTCC	469
NM_002462	Forward	TCCAGTCCAGCTCGGCAACA	470
	Probe	ACCTGATGGCCTATCACCAGGAGGCCAG	471
	Reverse	GATGTGGCTGGAGATGCGCTT	472
NM_003068	Forward	TGGGCGCCCTGAAGATGC	473
	Probe	TTGTGTTTGCAAGATCTGCGGCAAGGC	474
	Reverse	CTTGAAGCAACCAGGGTCTGGAA	475
NM_001753	Forward	GGAAGGCCAGCTTCACCACCT	476
	Probe	CCGCTTGCTGTCTGCCCTCTTTGGC	477
	Reverse	ACAAGTCCCAGATGTGCAGGA	478
NM_002089	Forward	CCTGCGGGTGGCGCTG	479
	Probe	TGCTCCTGCTCCTGGTGGCCGC	480
	Reverse	GCACTGGCAGCGCAGTTCAG	481
NM_001432	Forward	CGCCCTGCCCCTGTCAC	482
	Probe	CGCAGCCGCCCTCCGCCA	483
	Reverse	CGCGGTCATCGGCGATG	484
NM_000577	Forward	AGGGAAGATGTGCCTGTCTGTG	485
	Probe	CTGGTGATGAGACCAGACTCCAGCTGGAGG	486
	Reverse	TCTGAGCGGATGAAGGCGAAG	487
NM_017654	Forward	TCACAAAGGAAAAATTGACCAGTGC	488
	Probe	TGGCAGATGGAGATGTGTGGAAGGAGGA	489
	Reverse	AGCTCGACCTTGTAACGAAGCAA	490
NM_002444	Forward	CTAGTGCTGACCTACGGGCTGATG	491
	Probe	GGCCAAGGACCGCAGTGAGGAGGAAC	492
	Reverse	GGTGCTTCTGCACACGCTCATT	493
NM_000433	Forward	GGTGACCAAGGCTTTCCAGATGA	494
	Probe	TCAGCTTAAGAAAGGCAGCCAAGTGGAGGC	495
	Reverse	TCCTGAAACTCCAGGTCCTCTGGT	496
NM_014398	Forward	ATTTACCAAGGAATCAAACATGCGG	497
	Probe	CCTTCAAGTGCGTGAGTGAACAGAGCCTCC	498
	Reverse	CACCTGCAGGTGGGCTGACA	499
NM_002306	Forward	CACGCTTCAATGAGAACAACAGGAG	500
	Probe	TCGGTTTTCCCATTTGAAAGTGGGAAACCA	501
	Reverse	CCTTGAAGTGGTCAGGTTCAACCA	502
NM_020672	Forward	CAACGCAGAGGATGCTCAGGAA	503
	Probe	TTCAGTGATGTGGAGAGGGCCATTGAGACC	504
	Reverse	GCTGCTGGGTGACCAGGTCC	505
NM_001040147	Forward	TCTGGGATTGCTTCGGGGG	506
	Probe	AGGTCAGTGAAGGGCACCAGGCTACT	507
	Reverse	GCGTGGACTGAGGGAGTTGCTT	508
NM_001699	Forward	GCTGCGGAAGATTTGGAGAAC	509
	Probe	GAAGGCCTTGCCTCCTGCCAGGAG	510
	Reverse	TCCACCCTCATCCATGTTGACATAG	511
NM_012427	Forward	GGTGCGAGGATGCTTACCCG	512

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
	Probe	CAGATAGATGACACCATGTTCTGCGCCGG	513
	Reverse	CCTGGCAGGAGTCTCTACCTGCTT	514
NM_001039492	Forward	CACCGCCTGCAGGAAGCAG	515
	Probe	GCAGCGCTTCACAGCTCGCGATGA	516
	Reverse	ACAAGTCACAGAAGCAGTTCAGGCA	517
NM_003088	Forward	CGGCTGCCGCAAGGTCA	518
	Probe	GGCACCTGGACGCCAACCGC	519
	Reverse	AACTCCAGCTGGAAGACGTCATAGC	520
NM_005522	Forward	AAGCAGCTCACGGAAGTGGAGAA	521
	Probe	GCGCGCCCGCAGGGTGA	522
	Reverse	GAGCTGCAGGGATGCAGCG	523
NM_001032409	Forward	TGGAGGCAGCTGGCACAAGA	524
	Probe	GCTGAGGCCTGGCTGAATTACCCATGCT	525
	Reverse	GAATCCAGGAGCTCACTGGGGA	526
NM_012242	Forward	CCCGGGCGGGAATAAGTACC	527
	Probe	AACTACCAGCCGTACCCGTGCGCAGAG	528
	Reverse	AGCGCAGTACTCATCAGTGCCG	529
NM_006528	Forward	TATGGGCTTCTGCGCACCAA	530
	Probe	GAGGGACTGTGCTCTGCCAATGTGACTCG	531
	Reverse	TCCACAGCCAGTATAGGTGAAAGCA	532
NM_002192	Forward	AAGGACATCGGCTGGAATGACTG	533
	Probe	CTGGCTATCATGCCAACTACTGCGAGGGTG	534
	Reverse	CCGGACGTGCCTGCTATATGG	535
NM_005864	Forward	CTCATCGCCTGGTGTGTTGTTGG	536
	Probe	CCTCTGAGAGCACAGGTCAGGGCTGCA	537
	Reverse	GCCTGGCCAGTGCTGTACC	538
NM_003897	Forward	AAGCGCAGCCGCAGGGT	539
	Probe	TCTCTACCCTCGAGTGGTCCGGCGC	540
	Reverse	CGGTTCTCTCGACTGGCAGCT	541
NM_002658	Forward	GGGAGTGTACGACGCCCCAC	542
	Probe	GTGTGCTGCTGACCCACAGTGGAACACAGA	543
	Reverse	TCATGCGGCCCTTGGAGGG	544
NM_000245	Forward	GGGAGAAGACTCCTACAACCCGAAT	545
	Probe	CACCCTAAAGCCGAAATGCGCCCATC	546
	Reverse	CGCTGATATCCGGGACACCAG	547
NM_000228	Forward	TATGCTGAGTTGAAGGACCGGTTG	548
	Probe	GGTCAGAGTTCCATGCTGGGTGAGCAGG	549
	Reverse	TCTGTCTTCACACTCTGGATCCGG	550
NM_000064	Forward	ATTGAGCAGACCATCAAGTCAGGCT	551
	Probe	GCAGGTTGGACAGCAGCGCACGTTT	552
	Reverse	GCTTCAGGGCTTCTCTGCACTTG	553
NM_004335	Forward	AGGCCAGGCCGCCAC	554
	Probe	CTGCAACCACACTGTGATGGCCCTAATGG	555
	Reverse	ATCTCTCCCTCAAGCTCCTCCACTT	556
NM_003236	Forward	AGGAGGACAAGCCAGCATGTGTC	557
	Probe	CATGCGGACCTCCTGGCCGTGG	558
	Reverse	AGGCGGTGATGGCCTGCTT	559
NM_001042454	Forward	CTGCAGGGAATGCTTCGCG	560
	Probe	GCGAGAACCATTCCACGCACGACG	561
	Reverse	GGCCACACGTGGCGCAC	562
NM_003174	Forward	TTCCAGCTGGGAGCACAGAG	563
	Probe	TCCAATCAGATCACCCCTCGTGGAAGACGTC	564
	Reverse	TGGCCAGGAGGTTCGGCC	565

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
NM_013451	Forward	GGAGGCCGACGAGAGGCC	566
	Probe	TGGTTCACCAACCCATGCAAGACCATGA	567
	Reverse	CGGCGCCACACGATGAACT	568
NM_020299	Forward	AAGGAGATTGCTGCAAAGCACAAA	569
	Probe	GCAGCCCAGGTTCTGATCCGTTTCCATATC	570
	Reverse	ACCTGAATGTTCTCAACAATGCGTG	571
NM_022337	Forward	AGTCTCCCTAATGGCAAACCGGT	572
	Probe	TGTGCTCATGAACAATGGCCTCAAGATGGA	573
	Reverse	ACGAAACCGTGCTCCTTGCAG	574
NM_002068	Forward	CCACCTCCCACCTGGCTACCTA	575
	Probe	TTTCCAGGGCCCTAAGCAGGATGCTGAG	576
	Reverse	ATGTCCAGGATGAACCTCTTGGCT	577
NM_001024847	Forward	GTTCCAAGGTGCGGGAGCAC	578
	Probe	ATTCCCAGCTTCTGGCTCAACCACCAGG	579
	Reverse	GGTCGTGGTCCCAGCACTCAG	580
NM_000919	Forward	TGCTGGCCATTGCCATATTTATTC	581
	Probe	CAAGGGCCTTTGGAGCAGATTCTGAACACA	582
	Reverse	AAGCCTCCACTTCCCTTTCCTCTAA	583
NM_001333	Forward	GCAGTCGCAACTGTGGGGC	584
	Probe	CTATGGATGCAGGCCATTTCGTCTTCCAG	585
	Reverse	CACCAGAACACCATGATCCAGGTT	586
NM_178140	Forward	AACGGCATGTCCGTGGCAG	587
	Probe	GCCGCGCTGGCCAGCCA	588
	Reverse	CGCCAGTCGGCCTGGGT	589
NM_000213	Forward	TTCGGGCCAGAGCGCG	590
	Probe	CCATAGAGTCCCAGGATGGAGGACCCTTCC	591
	Reverse	AGCGGGTGCTGGAAGAGCC	592
NM_007350	Forward	CGCAGCCGAGCTCCAG	593
	Probe	CCCAGCCTCAGCCTCAGCCGCA	594
	Reverse	GATGCGGATACGGGTGGAGC	595
NM_002038	Forward	CGGCTCCGGTTCTGGAAG	596
	Probe	ACTCGCAGTCGCCGGGCTGCC	597
	Reverse	GTTGGCCGCGATGCCG	598
NM_000141	Forward	TGCACCAACGAACTGTACATGATGA	599
	Probe	TTGGCATGCAGTGCCTTCCAGAGA	600
	Reverse	TGGCTGAGGTCCAAGTATTCCTCAT	601
NM_002705	Forward	TCCACCCTGACACAGGCCG	602
	Probe	CGGAGGAAGCCCACCGTGCCG	603
	Reverse	CTGGCTTCTGAGTTTACGAACATG	604
NM_006665	Forward	GGTGGATGATCAAACCTTGCCAC	605
	Probe	CTCTCCGGCCAGGAAGTTCACTGGGC	606
	Reverse	TCAGATGCAAGCAGCAACTTTGG	607
NM_004688	Forward	AAAGTTCCGAAATGGAGGCGG	608
	Probe	GAGGTGGACCGCTGGACTATGACAGACA	609
	Reverse	ATCTCCACAAACGTGATGACTGCAC	610
NM_021105	Forward	GTGCAGCTGTTGTGGAGATGTTGAT	611
	Probe	GTGTGGTTGGCAAATTTCCAAGCACTGGA	612
	Reverse	TCAGCGTGTAAATGCCTCTCTCA	613
NM_002727	Forward	CTTCCAGGTGAATCCAACAAGATCC	614
	Probe	GGACTACTCTGGATCAGGCTTCGGCTCCG	615
	Reverse	TCCTGATCCAGAGCCGGAGC	616
NM_006670	Forward	AATGAGGAATCGGGTCTCTTGG	617
	Probe	CAACAGTGCTGACCTGGACTGTGACCCG	618

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
	Reverse	ACATAAGAGGTTTGCAGGGATGGG	619
NM_014314	Forward	GGAATGCCATTACACTGTGCTTGG	620
	Probe	TGCTTTGTGAGTAGACCACATCCCAAGCCA	621
	Reverse	GTCATGGCTGCAGTTCTGTCTGG	622
	Reverse	GTCATGGCTGCAGTTCTGTCTGG	622
NM_002526	Forward	TCGAGTGCCCAAGTTATGACCCTC	623
	Probe	AAGGTGATCCTCCCAAACTTCCTGGCCAA	624
	Reverse	TTATCATCTGGAACCCATCTCCACC	625
NM_003877	Forward	GTTGAGATGTGCAAGGATAAGCGG	626
	Probe	CCCGGAACGGCACTGTTACCTTTATCTG	627
	Reverse	ATGGTGCTGACGTGTAGAGCGG	628
NM_003702	Forward	GGTGAGCTTAGACTCCCGGGTG	629
	Probe	ACATGGTGAGCCATCCCAACACATATTCG	630
	Reverse	GACAGCAGAGTTCATGAATCGAGGA	631
NM_001814	Forward	AGGGGATCTACCACCACACTGGTC	632
	Probe	AATCATGCTGTTCTGCTTGTGGGCTATGGC	633
	Reverse	ATCCATCCAGAGGCTGAGTCAGT	634
XM_034274	Forward	TTTAAGAATGCGCTTGCTGCTCA	635
	Probe	TTGTGTCCAGCCACTTGCTTTCTTGGAAG	636
	Reverse	GCAGGTTTCATCTTCCTCTTTGAGGA	637
NM_000576	Forward	ATACCTGTGGCCTTGGGCCTC	638
	Probe	TGTCCTGCGTGTTGAAAGATGATAAGCCCA	639
	Reverse	TTGTTGAAGACAAATCGCTTTTCCA	640
NM_002178	Forward	ACCTCTACCACGCCCTCCCAG	641
	Probe	ATTCTGCGGGTGTCGAAGACACTGAGATGG	642
	Reverse	GCAGCACTGAGTCCAGATGTCTACG	643
NM_005268	Forward	CCTCTTCATGGTGGCCACAGC	644
	Probe	ATCTGCATCCTGCTCAACCTCGTGGAGCT	645
	Reverse	CCTTGCTGCCAGGCACTCG	646
NM_002281	Forward	CCTGCTGGAGGGCGAGGAG	647
	Probe	GGGCTGTGAATGTCTGTGTCAGCAGCTCC	648
	Reverse	GGGAGCCTGACACGCAGAGG	649
NM_018351	Forward	CAGAGACACTCAAGATGCCCTTGC	650
	Probe	TAGCCAACCACGCCAATGACACCATGAA	651
	Reverse	ACCCGACCAGGCTGCACAAT	652
NM_020183	Forward	GGAGCTAGAGGCTACCAGGCAAAA	653
	Probe	ACTGTTGCTGTCCACAGCCATGAGCCACT	654
	Reverse	CCAACTGTGCACCATCACTGAGG	655
NM_001032731	Forward	TGATGGGTTCAACATCCAGGTG	656
	Probe	GAATCTCTTTTCGAGGTGCTGGCCGCC	657
	Reverse	CACCCAGCAATGCTTACTCAGAGC	658
NM_003022	Forward	ATGTGCTTGGTTTCCTAGAAGCCAA	659
	Probe	TTGCAGCCAATGAAGAGAATCGGAAGTGGA	660
	Reverse	GGGTAACCTGTGGCTGGTCGAC	661
NM_001030011	Forward	TGCTGGGCTCCATTGGGC	662
	Probe	CGTCGGCAACAACCTGCTGGTGCTC	663
	Reverse	GGAGGTGAGTGGGAGTGCGG	664
NM_003761	Forward	GGAAGCCAGTGAAGGTGGAGGA	665
	Probe	AATGATCGTGTGCGGAACCTGCAAAGTGA	666
	Reverse	CCCGGGCCAGGATCCG	667
NM_000700	Forward	GTTCTGGACCTGGAGTTGAAAGGTG	668
	Probe	CAGCTATCGTGAAGTGCGCCACAAGCAA	669
	Reverse	GAAGCTTCTCTGCAAAGAAAGCTGG	670
NM_004403	Forward	AGCGCAGCAGCTCTGCTGG	671

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
	Probe	TGCTGCAAACCTCCAGATCATTCCCACACTG	672
	Reverse	CATCATCAGACAGAGCACGAAGCA	673
NM_003064	Forward	TCTGTCCTCCTAAGAAATCTGCCCA	674
	Probe	CCTGAGTGCCAGAGTGACTGGCAGTGTCC	675
	Reverse	GGATCCAGGCATTTGATGCCA	676
NM_000295	Forward	ACCTATGATCTGAAGAGCGTCCTGG	677
	Probe	AAGCTCTCCAAGGCCGTGCATAAGGCTG	678
	Reverse	CCTTTCTCGTCGATGGTCAGCA	679
NM_007150	Forward	CTGCACGCTATAGCAACGTCAGC	680
	Probe	GCATTGAGGACTCATTGCCATGGAGAAGA	681
	Reverse	GAGTGTCTGCCACATGGAGGCT	682
NM_005168	Forward	AGCTCTCCAATCACAGGCAGACG	683
	Probe	GGGCAAATATGGCCAAACAGATTGGAGCA	684
	Reverse	TCCGACTGTAAAGCTGAGCATTCG	685
NM_006547	Forward	GCAGAGTTATTGGAAAAGGAGGCAA	686
	Probe	TTGTTGTCCCTCGTGACCAGACACCTGATG	687
	Reverse	ACCTGGCAAGCATAGAAGTGACCA	688
NM_001423	Forward	TCATCTTCTGTGTCATTGCCCTCC	689
	Probe	GGTCTTCGTGTTCCAGCTCTTCACCATGGA	690
	Reverse	CCTGAGAGGAAGAACCGGTTTCC	691
NM_002353	Forward	ACATCAAGGGCGAGTCTCTATTCCA	692
	Probe	GGCCGCGGCGGCCTGGA	693
	Reverse	CCGCGCACGCGCAAG	694
NM_023038	Forward	ACCCAGTGCCAGGCCGC	695
	Probe	CCTCCCCAGGCCAGGAGGTGCATC	696
	Reverse	CAAGTGCTGCCAGAGGCCG	697
NM_005567	Forward	CAGAGCTGCTGGAACTACGGCTT	698
	Probe	TCCTGCTCCTCGGACGAGCTCCCTG	699
	Reverse	AGCCGCCAGACTTGGTGAGG	700
NM_001793	Forward	ACGTGGCACCAACCATCATCC	701
	Probe	CCATGTACCGTCTCGGCCAGCCA	702
	Reverse	AGTCGAACACCAAGAGGGTGTCG	703
NM_002275	Forward	GGCTCATTGGTGGCCTGGAG	704
	Probe	GATGCGAGATGGAGGCTCAGAACCAGGAG	705
	Reverse	GTAGCGATCTCCTGCTCCAGCC	706
NM_015657	Forward	GTTTCATGCAGCTGCACTTTCCAA	707
	Probe	ACCAGTCACAGCAGGAGGAGTCGCAAACA	708
	Reverse	AAGCAGTCTTGTTGGTTTCCAGCA	709
NM_006290	Forward	ACAGAAGAGCAACTGAGATCGAGCC	710
	Probe	AGAGATGTGCCTCGAACCACACAAAGCACC	711
	Reverse	GTTCTTGCAGGAGGCCCGG	712
NM_005429	Forward	CCTGGAATAATGTGCCTGTGAATGTA	713
	Probe	CCACCACCAAACATGCAGCTGTTACAGACG	714
	Reverse	CAAGCCTTCTGGCGGTTTCGTA	715
NM_006084	Forward	CTGGAATGCACCCCAGGCTC	716
	Probe	CACCTGGGCCAGGCCCGCATC	717
	Reverse	GCACTCGTTGCTGGGCAGC	718
NM_013352	Forward	AATGGGGGCTTGATTAAAGGCC	719
	Probe	TTTGGACAGCACGGATGGTGACAACCTACA	720
	Reverse	AGGAAGCAGACAGTGATGGGGC	721
NM_138444	Forward	GACGCCAAGTCCGGCGA	722
	Probe	CGCATCACCGTTTGCGGAAAGACGTC	723
	Reverse	CCCAAACACCTCCTTGGCCA	724

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
NM_001511	Forward	CTGCGAGTGGCACTGCTGCT	725
	Probe	GCTCCTGGTAGCCGCTGGCCGG	726
	Reverse	GACGCTCCTGCTGCGCG	727
NM_004616	Forward	CGTTGCTGTGGACATATTGATTGCT	728
	Probe	TCATGATTCTGGGCTTCCTGGGATGCTG	729
	Reverse	AACAACAGAAGCATGCAGCGACTT	730
XM_049037	Forward	CCAGCAGCAAATTAATCAACAGCAG	731
	Probe	CTCCAGCTGCAGCAGCTGCAACACATG	732
	Reverse	GGAGAAGGCTGAGACTGGTGCTG	733
NM_001002231	Forward	GCCTGTGAGAGCCTGCCAAGA	734
	Probe	TCACAGATGTTGTGAAGGTCTGGGCCTG	735
	Reverse	TGCTGGCTCCTGGGTGGG	736
NM_001003799	Forward	TCAAGGTATTTGGTCCCGGAACA	737
	Probe	CAACTTGATGCAGATGTTTCCCCAAGCC	738
	Reverse	AGCCTTCTGGAGCTTTGTTTCAGC	739
NM_000477	Forward	CTGAAACATTACCTTCCATGCAGA	740
	Probe	TGCACTTGTTGAGCTCGTGAACACAAAGCC	741
	Reverse	AGCTTTCAGTTGCTCTTTGTTGCC	742
NM_006061	Forward	ATTGTCCTGCTGGTAATTGGGCTAA	743
	Probe	TCCCTTATGAACAAGGAGCACCTTGTGCCA	744
	Reverse	ATTGGTGCATAGTCCATCGTCACAG	745
NM_001174	Forward	GCTGCACAAGCAGAAATCATCAGAC	746
	Probe	CAGTTCAGAGTTCAGCCCGGGCTGAGG	747
	Reverse	ACAGCGATGATGGCCGTGC	748
NM_016269	Forward	TGAGAGCGAATGTCGTTGCTGAG	749
	Probe	AACCAGATTCTTGGCAGAAGGTGGCATGC	750
	Reverse	TTTAGCCTGCTCTTCACGGGAGA	751
NM_030781	Forward	CCCACCAGGCAAAGAGGGACT	752
	Probe	GGCCCTCAGGGCCCTCCTGGCT	753
	Reverse	CCAACGGTGCCCTGAAGTCC	754
NM_001014986	Forward	AAGCAAGCATGTCATCTATGCTCCA	755
	Probe	GCAGCCACAACAAGTATGCAGGGGAGTCA	756
	Reverse	CAGGCCTTGGAAGGGTCCACT	757
NM_000905	Forward	CACCATGCTAGGTAACAAGCGACTG	758
	Probe	CTGCTCGTGTGCCTGGGTGCGCT	759
	Reverse	CGGGTTGTCCGGCTTGGA	760
NM_007253	Forward	TCAACATCTTCGCAATCCATCACA	761
	Probe	CCCTCAGTCTGGCCAGACCCTGAGGTCT	762
	Reverse	TGCCCCGATGCAGTTCCTGG	763
NM_000044	Forward	CAAGGAACCTCGATCGTATCATTGCA	764
	Probe	CCACATCCTGCTCAAGACGCTTCTACCAGC	765
	Reverse	AATAGGCTGCACGGAGTCCAGG	766
NM_004114	Forward	GAGATCATGAAAGGCAACCATGTGA	767
	Probe	TACAAGGAGCCATCACTGCACGATCTCACG	768
	Reverse	GGTCCCCTTCCAGATCGG	769
NM_000601	Forward	AAGATTGGATCAGGACCATGTGAGG	770
	Probe	GGATTATGGTGGCCCACTTGTGTGAGCA	771
	Reverse	ACCAGGAACAATGACACCAAGAACC	772
NM_005084	Forward	TGGACATTCTTTGGTGGAGCAAC	773
	Probe	GATGTGGTATTGCCCTGGATGCATGGATGT	774
	Reverse	GAGGGGCTGAGGAATTCTGGAATAT	775
NM_001030047	Forward	GACCTGCCCAACCAGGAGC	776
	Probe	AGCACTGGGGACCACCTGCTACGCCT	777

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
	Reverse	AAGAACTCCTCTGGTTCAATGCTGC	778
NM_002736	Forward	GAATGGTGCAGTAGAAATCGCTCG	779
	Probe	GCTCGCGGGGACAGTACTTTGGAGAGCTT	780
	Reverse	GGCGTGGGCAGAAAGCTGC	781
NM_001099	Forward	GAGTGGCTACAGATGGCGCTAG	782
	Probe	ACGGACTCCTTCCCTCCCTATGCTTCTTGCC	783
	Reverse	ATACGGCTCGTGCTGCGTCTC	784
NM_020974	Forward	GTTCAAGTCCAATGAAGGGAACAGC	785
	Probe	GCTAGAGGGTTCCAGGTCCCATACGTGACA	786
	Reverse	AGAGCCTGCGATCTCGAACTATGTC	787
NM_015900	Forward	TCACCATACCTAAGCAGCAACGCT	788
	Probe	ATGGGAAAGGAATCATAGCCCATGCCACC	789
	Reverse	TCACTTGGTTTATCTGGCATTGTGG	790
NM_004867	Forward	AGTTGCTGTGGAGGAAATTCGTGAT	791
	Probe	ATAACAGAAAGTCCTTCCGCCTTCGTCGCA	792
	Reverse	GTTTGTGAAACCCAGCAAGAGGTC	793
NM_013243	Forward	ATGGAAGCTTGAAGGATTCCACAAA	794
	Probe	TCCAACCCAGGAGGAAAGACAGATGAACCC	795
	Reverse	TGATGGCTTCCAAATAGGCTTCTGT	796
NM_003706	Forward	CGCCAGCTGCTACATCCTGAAA	797
	Probe	TCCCCTGTTCAACATAGATGCCTGTGGAGG	798
	Reverse	TCGTATGTGTCACTCCATGCCTCA	799
NM_004449	Forward	GAAGCGCTACGCCTACAAGTTCG	800
	Probe	CTTCCACGGGATCGCCCAGGCC	801
	Reverse	CCATGTACGGGAGGTCTGAGGG	802
NM_198795	Forward	TGTTCTGAGAATGGGACTGTCGATG	803
	Probe	TCTGGCAAAAAACATCACACCTCAAAGGCA	804
	Reverse	TCTGTGCAGCAGCAATTCATCCTAT	805
NM_005622	Forward	CCATTTGAGGTAGAAAATGCCCTGA	806
	Probe	TCAGTTGCAGAGTCAGCTGTTGTGACGAGC	807
	Reverse	ACCTCTCCTCTGATGGGGTCTGG	808
NM_000689	Forward	GCCATAACAATCTCCTCTGCTCTGC	809
	Probe	GCAGGAACAGTGTGGGTGAATTGCTATGGC	810
	Reverse	GGGGCACTGGGCACTTACCA	811
NM_014906	Forward	TCCCCGGGAAACAGAGTTTCTAGA	812
	Probe	CGCCACCACTACTCAAAGAAGTGGCACAGA	813
	Reverse	GGACAGGCTAGTGCCTATTTTGTGG	814
NM_021244	Forward	TTCCTGGCTCTCGTTTGCTTTGT	815
	Probe	TTCATTGCTTCCGGAAGGCCATTTCATGA	816
	Reverse	CAGCCGATTCTGAACCTTTCGAG	817
NM_005613	Forward	TCCAGGCAACCAAAGAGGTGAAC	818
	Probe	TTGCACCAGGGAAGAGACAAGCCGGA	819
	Reverse	GCGGTAGGAATCCTTCTCCATCAG	820
NM_021076	Forward	CAGAGGTAGCCAAGAAGGAACCAGA	821
	Probe	AGCAGAGAAGAAGGAGGCAGCACCGGAG	822
	Reverse	AGGCTTCTTGCCCTTCTCCTCC	823
NM_001041	Forward	TGGCGTCAGAGGACAATTCAAAC	824
	Probe	TCCGTGGTGGTCACATCCTACCATGTCAAG	825
	Reverse	TCTGCAGCAACAATGAGCTTCATG	826
NM_021139	Forward	CATCATGATCAACCAGTGAAGCCC	827
	Probe	GAGCCAAGCACCTTCGGGTGTCAGC	828
	Reverse	GGTACTGGAACCAGGTGAGGTCGT	829
NM_015460	Forward	GCCCTGACCATTGCAGGATTAAA	830

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
	Probe	CGCTTCACAAGAAGACGGGATCAGAAGCAA	831
	Reverse	TTCTCCTTTGCTGCCTTGATGTA	832
NM_005097	Forward	TTCCAGGATATTCAGAGGATGCCAT	833
	Probe	GCGAGGATCCATGGTGTCCAGCCTCTT	834
	Reverse	TTGGCTTTCTCTGCATCCCAGTT	835
NM_000922	Forward	GTGGTGATGATGAAGACGGTGAAGA	836
	Probe	CCACCAAGAAGGAAAAGCAGACGGCGA	837
	Reverse	AGTGAGGTGGTGCATTAGCTGACAA	838
NM_017770	Forward	CACAGGCTCAGCTGGTGCAGTT	839
	Probe	TGCTCGCCATCACGCACACCATGA	840
	Reverse	ACACGGTTTCACGACGGCG	841
NM_000814	Forward	TGGCGATACCAGGAATTCAGCA	842
	Probe	CAGAGCATGCCTCGAGAAGGGCATGG	843
	Reverse	CTGTGAAGACCTCCTCCGTAGATGG	844
NM_000681	Forward	CGCGTCGGGGCTGCC	845
	Probe	AGGCGTCGCGCTGGCGCG	846
	Reverse	AAGCGCTTCTCGCGGTTCTG	847
NM_023929	Forward	TCATGTTAGCAGCCAGTGTTGGAAT	848
	Probe	TGTGCAGATAAATCACAGCCAGGAGGGCA	849
	Reverse	CCGAGGGAATTCTGTATCCTGTCC	850
NM_002465	Forward	GTTCAAGCAACCAGGGAGTCTGTACC	851
	Probe	CTGCTGCAAAGCAGTCAATGACCTTGGGA	852
	Reverse	CCTCCAGTTTGCATTCAATCTCCA	853
NM_000685	Forward	CCAAATCCCACTCAAACCTTTCAAC	854
	Probe	AATGAGCACGCTTTCTACCGCCCTC	855
	Reverse	TGCAGGCTTCTTGGTGGATGAG	856
NM_000187	Forward	CAAGGTGGGTTCTGCCAGG	857
	Probe	GATGCTGACTGCTTTGAGAAGGCCAGCAAG	858
	Reverse	AATCCTCTCAGGTGCCAGCTTGA	859
NM_021136	Forward	AGGAACTGAGGAGGCTCTTCCTTGT	860
	Probe	TGATGTGGCTCCTGACCTACGTTGGCG	861
	Reverse	CCACAGCCATGAGCAGCAGG	862
XM_377774	Forward	GAAGTGCCAAGCTGGAGAAGC	863
	Probe	TGCCACGGCGGAGCGCGTC	864
	Reverse	TCCCGCATGGCGTTCTCC	865
NM_014243	Forward	TCTTCAGTGGGAGGTCCAAATGC	866
	Probe	GCCAGAGGAGTGCTCAGCAAGCAGGAAGT	867
	Reverse	TGGTACGGTGACCAGTCTCACAGTC	868
NM_000130	Forward	GGCAATTATAACACAGGGCTGCAA	869
	Probe	AGCAGGGAGTGGAATGGAAACCATACAGGC	870
	Reverse	TCTTGTCACCATGGAGGATTTC	871
NM_018593	Forward	CAATTGGATTCTGCTCGGATTCA	872
	Probe	TGACTGTTGGCCACCCATTGCAGG	873
	Reverse	CACATCATAGGAGCCAGTTTGTCA	874
NM_002407	Forward	CCTCGCCATGAAGCTGCTGAT	875
	Probe	TGCTGGCGGCCCTCCTCCTGC	876
	Reverse	TCAACCATGTCCTCCAGGAGTTTG	877
NM_004431	Forward	CCTCAAGACCCTGGCTGACTTTG	878
	Probe	GTGCCCTTCCCGACGGTGTCCG	879
	Reverse	TGCTGCATCTTGATGGACTCCAG	880
NM_022361	Forward	GGCATTTTTAGGTAACCCTCACTG	881
	Probe	TGCTCTTTGCTCAGCATCGCTACATCTCCC	882
	Reverse	CTGCAATGTCACTGCCAATTAGCA	883

GENBANK® Accession No.	Primer / probe type	Sequence	SEQ ID NO:
NM_015141	Forward	GCTGCGGGGTGGCCG	884
	Probe	TGATCACCACTGTTACGGAGGGCGG	885
	Reverse	CTCGGCCACCCTGCGGT	886

DETAILED DESCRIPTION OF THE INVENTION

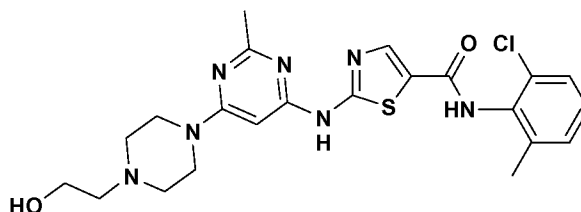
[0075] The present invention describes the identification of polynucleotides that correlate with drug sensitivity or resistance of untreated cell lines to determine or predict sensitivity of the cells to a drug, compound, or biological agent. These polynucleotides, called marker or predictor polynucleotides herein, can be employed for predicting drug response. The marker polynucleotides have been determined in an *in vitro* assay employing microarray technology to monitor simultaneously the expression pattern of thousands of discrete polynucleotides in untreated cells, whose sensitivity to compounds or drugs, in particular, compounds that modulate, e.g., inhibit, protein tyrosine kinase or protein tyrosine kinase activity is tested. The protein tyrosine kinases, or activities thereof, associated with response to a drug, compound, or biological agent include, for example, members of the Src family of protein tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as the Bcr-abl, Jak, PDGFR, c-kit and Eph receptors protein tyrosine kinases. (See, e.g., P. Blume-Jensen and T. Hunter, "Oncopolynucleotide Kinase Signaling", *Nature*, 411:355-365 (2001)).

[0076] The assay according to this invention has allowed the identification of the marker polynucleotides, called protein tyrosine kinase biomarkers herein, having expression levels in the cells that are highly correlated with drug sensitivity exhibited by the cells. Such marker polynucleotides encompass the above-listed protein tyrosine kinase-encoding polynucleotides, and serve as useful molecular tools for predicting a response to drugs, compounds, biological agents, chemotherapeutic agents, and the like, preferably those drugs and compounds, and the like, that affect protein tyrosine kinase activity via direct or indirect inhibition or antagonism of the protein tyrosine kinase function or activity.

[0077] In its preferred aspect, the present invention describes polynucleotides that correlate with sensitivity or resistance of prostate cell lines to treatment with a protein tyrosine kinase inhibitor compound, e.g., BMS-A, as described herein. (Fig. 1 and

Table 2). The protein tyrosine kinase inhibitor compound, BMS-A, utilized for identifying the polynucleotide predictor sets of this invention, was described in WO 00/62778, published October 26, 2000, and is hereby incorporated by reference in its entirety. BMS-A has potent inhibitory activity for a number of protein tyrosine kinases, for example, members of the Src family of protein tyrosine kinases, including Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as the Bcr-abl, Jak, PDGFR, c-kit and Eph receptors protein tyrosine kinases. Specifically, for the BMS-A protein tyrosine kinase inhibitor compound analyzed, the expression of 176 predictor polynucleotides was found to correlate with resistance/sensitivity of the prostate cell lines to the compound.

[0078] The term “BMS-A” as used herein refers to a compound having the following structure (I):



(I)

Compound (I) can also be referred to as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or as N-(2-chloro-6-methylphenyl)-2-(((6-(4-(2-hydroxyethyl)-1-piperazinyl)-2-methyl-4-pyrimidinyl)amino)-1,3-thiazole-5-carboxamide in accordance with IUPAC nomenclature. Use of the term “N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide” encompasses (unless otherwise indicated) solvates (including hydrates) and polymorphic forms of the compound (I) or its salts (such as the monohydrate form of (I) described in USSN 11/051,208, filed February 4, 2005, incorporated herein by reference in its entirety and for all purposes). Pharmaceutical compositions of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide include all pharmaceutically acceptable compositions comprising N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide and one or more diluents, vehicles and/or excipients, such as

those compositions described in USSN 11/402,502, filed April 12, 2006, incorporated herein by reference in its entirety and for all purposes. One example of a pharmaceutical composition comprising N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide is SPRYCEL[®] (Bristol-Myers Squibb Company). SPRYCEL[®] comprises N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide as the active ingredient, also referred to as dasatinib, and as inactive ingredients or excipients, lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, hydroxypropyl cellulose, and magnesium stearate in a tablet comprising hypromellose, titanium dioxide, and polyethylene glycol.

[0079] In accordance with the invention, an approach has been discovered in which polynucleotides and combinations of polynucleotides have been identified whose expression pattern, in a subset of cell lines, correlates to and can be used as an *in vitro* predictor of cellular response to treatment or therapy with one compound, or with a combination or series of compounds, that are known to inhibit or activate the function of a protein, enzyme, or molecule (e.g., a receptor) that is directly or indirectly involved in cell proliferation, cell responses to external stimuli, (such as ligand binding), or signal transduction, e.g., a protein tyrosine kinase. Preferred are antagonists or inhibitors of the function of a given protein, e.g., a tyrosine kinase.

[0080] In a preferred aspect, the BMS-A protein tyrosine kinase inhibitor was employed to determine drug sensitivity in a panel of prostate cell lines following exposure of the cells to this compound. Some of the cell lines were determined to be resistant to treatment with the inhibitor compound, while others were determined to be sensitive to the inhibitor. (Table 1). A subset of the cell lines examined provided an expression pattern or profile of polynucleotides, and combinations of polynucleotides, that correlated to, and thus serve as a predictor of, a response by the cells to the inhibitor compound, and to compounds having similar modes of action and/or structure. (Tables 2, 3, 4-5).

[0081] Such a predictor set of polynucleotide expression patterns correlating with sensitivity or resistance of cells following exposure of the cells to a drug, or a combination of drugs, provides a useful tool for screening a cancer, tumor, or patient

test sample before treatment with the drug or a drug combination. The screening technique allows a prediction of cells of a cancer, tumor, or test sample exposed to a drug, or a combination of drugs, based on the polynucleotide expression results of the predictor set, as to whether or not the cancer, tumor, or test sample, and hence a patient harboring the cancer and/or tumor, will or will not respond to treatment with the drug or drug combination. In addition, the predictor polynucleotides or predictor polynucleotide set can also be utilized as described herein for monitoring the progress of disease treatment or therapy in those patients undergoing treatment involving a protein tyrosine kinase, e.g., src tyrosine kinase, inhibitor compound or chemotherapeutic agent for a disease, e.g., prostate cancer.

[0082] According to a particular embodiment of the present invention, oligonucleotide microarrays were utilized to measure the expression levels of over 22,000 probe sets in a panel of 16 untreated prostate cell lines for which the drug sensitivity to the protein tyrosine kinase inhibitor compound was determined. This analysis was performed to determine whether the polynucleotide expression signatures of untreated cells were sufficient for the prediction of chemosensitivity. Data analysis allowed the identification of marker polynucleotides whose expression levels were found to be highly correlated with drug sensitivity. In addition, the treatment of cells with the BMS-A protein tyrosine kinase inhibitor compound also provided polynucleotide expression signatures predictive of sensitivity to the compound. Thus, in one of its embodiments, the present invention provides these polynucleotides, i.e., polynucleotide "markers" or "biomarkers" or "predictors", which show utility in predicting drug response upon treatment or exposure of cells to a drug. In particular, the marker or predictor polynucleotides are protein tyrosine kinase biomarkers polynucleotides encoding protein tyrosine kinase biomarker proteins/polypeptides, such as a src tyrosine kinase inhibitor biomarker.

[0083] The performance of the polynucleotide expression and marker polynucleotide identification analyses embraced by the present invention is described in further detail and without limitation herein below.

[0084] The present invention provides methods of determining responsiveness of an individual having a BCR-ABL associated disorder to a certain treatment regimen and methods of treating an individual having a BCR-ABL associated disorder.

[0085] The term “BCR-ABL” as used herein is inclusive of both wild-type and mutant BCR-ABL.

[0086] “BCR-ABL associated disorders” are those disorders which result from BCR-ABL activity, including mutant BCR-ABL activity, and/or which are alleviated
5 by the inhibition of BCR-ABL, including mutant BCR-ABL, expression and/or activity. A reciprocal translocation between chromosomes 9 and 22 produces the oncogenic BCR-ABL fusion protein. The phrase “BCR-ABL associated disorders” is inclusive of “mutant BCR-ABL associated disorders”.

[0087] Disorders included in the scope of the present invention include, for
10 example, leukemias, including, for example, chronic myeloid leukemia, acute lymphoblastic leukemia, and Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL), squamous cell carcinoma, small-cell lung cancer, non-small cell lung cancer, glioma, gastrointestinal cancer, renal cancer, ovarian cancer, liver cancer, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid
15 cancer, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer, gastric cancer, germ cell tumor, pediatric sarcoma, sinonasal natural killer, multiple myeloma, acute myelogenous leukemia, chronic lymphocytic leukemia, mastocytosis and any symptom associated with mastocytosis. In addition,
20 disorders include urticaria pigmentosa, mastocytoses such as diffuse cutaneous mastocytosis, solitary mastocytoma in human, as well as dog mastocytoma and some rare subtypes like bullous, erythrodermic and teleangiectatic mastocytosis, mastocytosis with an associated hematological disorder, such as a myeloproliferative or myelodysplastic syndrome, or acute leukemia, myeloproliferative disorder
25 associated with mastocytosis, and mast cell leukemia. Various additional cancers are also included within the scope of protein tyrosine kinase-associated disorders including, for example, the following: carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid, testis, particularly testicular seminomas, and skin; including squamous cell carcinoma;
30 gastrointestinal stromal tumors (“GIST”); hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma,

5 hairy cell lymphoma and Burkett's lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; other tumors, including melanoma, seminoma, 5 tetratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other tumors, including melanoma, xenoderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer, teratocarcinoma, 10 chemotherapy refractory non-seminomatous germ-cell tumors, and Kaposi's sarcoma. In certain preferred embodiments, the disorder is leukemia, breast cancer, prostate cancer, lung cancer, colon cancer, melanoma, or solid tumors. In certain preferred embodiments, the leukemia is chronic myeloid leukemia (CML), Ph⁺ ALL, AML, imatinib-resistant CML, imatinib-intolerant CML, accelerated CML, lymphoid blast 15 phase CML,

[0088] A "solid tumor" includes, for example, sarcoma, melanoma, carcinoma, or other solid tumor cancer.

[0089] The terms "cancer", "cancerous", or "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell 20 growth. Examples of cancer include, for example, leukemia, lymphoma, blastoma, carcinoma and sarcoma. More particular examples of such cancers include chronic myeloid leukemia, acute lymphoblastic leukemia, Philadelphia chromosome positive acute lymphoblastic leukemia (Ph⁺ ALL), squamous cell carcinoma, small-cell lung cancer, non-small cell lung cancer, glioma, gastrointestinal cancer, renal cancer, 25 ovarian cancer, liver cancer, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer, gastric cancer, germ cell tumor, pediatric sarcoma, sinonasal natural killer, multiple myeloma, acute myelogenous leukemia 30 (AML), and chronic lymphocytic leukemia (CML).

[0090] "Leukemia" refers to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of

leukocytes and their precursors in the blood and bone marrow. Leukemia is generally clinically classified on the basis of: (1) the duration and character of the disease-- acute or chronic; (2) the type of cell involved; myeloid (myelogenous), lymphoid (lymphogenous), or monocytic; and (3) the increase or non-increase in the number of abnormal cells in the blood – leukemic or aleukemic (subleukemic). Leukemia includes, for example, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophylic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, and undifferentiated cell leukemia. In certain aspects, the present invention provides treatment for chronic myeloid leukemia, acute lymphoblastic leukemia, and/or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL).

[0091] The invention encompasses treatment methods based upon the demonstration that patients harboring different BCR-ABL mutations have varying degrees of resistance and/or sensitivity to imatinib and N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, respectively. Thus the methods of the present invention can be used, for example, in determining whether or not to treat an individual with N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof; whether or not to treat an individual with a more aggressive dosage regimen of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-

hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof; or whether or not to treat an individual with combination therapy, i.e., a combination of tyrosine kinase inhibitors, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof and additional BCR-ABL inhibitors(s) (e.g., such as imatinib, AMN107, PD180970, GGP76030, AP23464, SKI 606, NS-187, and/or AZD0530); a combination of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof and a tubulin stabilizing agent (such as, for example, paclitaxol, epothilone, taxane, and the like); a combination of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof and a farnesyl transferase inhibitor; a combination of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide and another protein tyrosine kinase inhibitor; a combination of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide and ATP non-competitive inhibitors ONO12380; a combination of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide and Aurora kinase inhibitor VX-680; a combination of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide and p38 MAP kinase inhibitor BIRB-796; any other combination disclosed herein.

[0092] The terms “treating”, “treatment” and “therapy” as used herein refer to curative therapy, prophylactic therapy, preventative therapy, and mitigating disease therapy.

[0093] In certain embodiments, the present invention provides a method of identifying a whether a patient is predicted to be resistant to inhibition of a protein tryrosine kinase activity, such as BCR-ABL kinase activity by N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-

pyrimidinyl]amino]-5-thiazolecarboxamide, the method comprising determining the expression level of one or more predictor polynucleotides in a patient or tissue sample, or in a mammalian cell. The mammalian cell can be a human cancer cell. The human cancer cell can be one obtained from an individual treated having a BCR-ABL associated disorder.

[0094] For use herein, a BCR-ABL inhibitor refers to any molecule or compound that can partially inhibit BCR-ABL or mutant BCR-ABL activity or expression. These include inhibitors of the Src family kinases such as BCR/ABL, ABL, c-Src, SRC/ABL, and other forms including, but not limited to, JAK, FAK, FPS, CSK, SYK, and BTK. A series of inhibitors, based on the 2-phenylaminopyrimidine class of pharmacophores, has been identified that have exceptionally high affinity and specificity for Abl (see, e.g., Zimmerman et al., *Bioorg. Med. Chem. Lett.*, 7:187 (1997)). All of these inhibitors are encompassed within the term a BCR-ABL inhibitor. Imatinib, one of these inhibitors, also known as STI-571 (formerly referred to as Novartis test compound CGP 57148 and also known as GLEEVEC[®]), has been successfully tested in clinical trial a therapeutic agent for CML. AMN107 is another BCR-ABL kinase inhibitor that was designed to fit into the ATP-binding site of the BCR-ABL protein with higher affinity than imatinib. In addition to being more potent than imatinib (IC₅₀<30 nM) against wild-type BCR-ABL, AMN107 is also significantly active against 32/33 imatinib-resistant BCR-ABL mutants. In preclinical studies, AMN107 demonstrated activity *in vitro* and *in vivo* against wild-type and imatinib-resistant BCR-ABL-expressing cells. In phase I/II clinical trials, AMN107 has produced haematological and cytogenetic responses in CML patients, who either did not initially respond to imatinib or developed imatinib resistance (Weisberg et al., *British Journal of Cancer*, 94:1765-1769 (2006)), incorporated herein by reference in its entirety and for all purposes). SKI-606, NS-187, AZD0530, PD180970, CGP76030, and AP23464 are all examples of kinase inhibitors that can be used in the present invention. SKI-606 is a 4-anilino-3-quinolinecarbonitrile inhibitor of Abl that has demonstrated potent antiproliferative activity against CML cell (Golas et al., *Cancer Research*, 63:375-381 (2003)). AZD0530 is a dual Abl/Src kinase inhibitor that is in ongoing clinical trials for the treatment of solid tumors and leukemia (Green et al., Preclinical Activity of AZD0530, a novel, oral, potent, and

selective inhibitor of the Src family kinases. Poster 3161 presented at the EORTC-NCI-AACR, Geneva Switzerland, 28 September 2004). PD180970 is a pyrido[2,3-d]pyrimidine derivative that has been shown to inhibit BCR-ABL and induce apoptosis in BCR-ABL expressing leukemic cells (Rosee et al., *Cancer Research*, 5 62:7149-7153 (2002)). CGP76030 is dual-specific Src and Abl kinase inhibitor shown to inhibit the growth and survival of cells expressing imatinib-resistant BCR-ABL kinases (Warmuth et al., *Blood*, 101(2):664-672 (2003)). AP23464 is an ATP-based kinase inhibitor that has been shown to inhibit imatinib-resistant BCR-ABL mutants (O'Hare et al., *Clin. Cancer Res.*, 11(19):6987-6993 (2005)). NS-187 is a
10 selective dual Bcr-Abl/Lyn tyrosine kinase inhibitor that has been shown to inhibit imatinib-resistant BCR-ABL mutants (Kimura et al., *Blood*, 106(12):3948-3954 (2005)).

[0095] Treatment regimens can be established based upon the whether the patient or sample or cell is predicted to be resistant to a protein tyrosine kinase inhibitor, including BMS-A. For example, the invention encompasses screening cells from an
15 individual who may suffer from, or is suffering from, a disorder that is commonly treated with a kinase inhibitor, including a BCR-ABL associated disorder. Such a disorder can include myeloid leukemia or disorders associated therewith, or cancers described herein. The cells of an individual are screened, using methods known in
20 the art, for measuring expression levels of a polynucleotide or polypeptide.

[0096] The term "about" as used herein in referring to increased expression levels of a predictive biomarker polynucleotide and/or polypeptide is meant to refer to either a normal level of expression of the biomarker relative to a standard level, or an expression level that is elevated by at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9,
25 1.0, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10 fold, or more higher than a standard level.

[0097] The term "about" as used herein in referring to decreased expression levels of a predictive biomarker polynucleotide and/or polypeptide is meant to refer to an expression level that is decreased by at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9,
30 1.0, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10 fold, or more below a standard level.

[0098] If the patient, sample, or cell is predicted to be resistant to a protein tyrosine kinase inhibitor, treatment regimens can be developed appropriately. For example, a decreased expression level of CK5 (KRT5), PLAU and/or EphA2 or elevated expression levels of AR and/or PSA in a sample relative to a standard can indicate that the cells in an individual are or are expected to become at least partially resistant to treatment with a kinase inhibitor such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide. As disclosed herein, in such cases, treatment can include the use of an increased dosing frequency or increased dosage of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a salt, hydrate, or solvate thereof, a combination of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof and another kinase inhibitor drug such as imatinib, AMN107, PD180970, GGP76030, AP23464, SKI 606, and/or AZD0530; a combination of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide and a tubulin stabilizing agent (e.g., paclitaxol, epothilone, taxane, etc.); a combination of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide and a farnesyl transferase inhibitor; any other combination disclosed herein; and any other combination or dosing regimen comprising N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide disclosed herein. In one aspect, an increased level of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide would be about 10, 20, 30, 40, 50, 60, 70, 80, 90, or 95% more than the typical N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide dose for a particular indication or for individual, or about 1.5x, 2x, 2.5x, 3x, 3.5x, 4x, 4.5x, 5x, 6x, 7x, 8x, 9x, or 10x more N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide than the typical N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-

methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide dose for a particular indication or for individual.

[0099] A therapeutically effective amount of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof can be orally administered as an acid salt of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide. The actual dosage employed can be varied depending upon the requirements of the patient and the severity of the condition being treated. Determination of the proper dosage for a particular situation is within the skill of the art. The effective amount of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof (and Compound I salt) can be determined by one of ordinary skill in the art, and includes exemplary dosage amounts for an adult human of from about 0.05 to about 100 mg/kg of body weight of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof, per day, which can be administered in a single dose or in the form of individual divided doses, such as from 1, 2, 3, or 4 times per day. In certain embodiments, N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof is administered 2 times per day at 70 mg. Alternatively, it can be dosed at, for example, 50, 70, 90, 100, 110, or 120 BID, or 100, 140, or 180 once daily. It will be understood that the specific dose level and frequency of dosing for any particular subject can be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion, drug combination, and severity of the particular condition. Preferred subjects for treatment include animals, most preferably mammalian species such as humans, and domestic animals such as dogs, cats, and the like, subject to protein tyrosine kinase-associated disorders. The same also applies to

Compound II or any combination of Compound I and II, or any combination disclosed herein.

[00100] A method of determining the responsiveness of an individual suffering from a protein tyrosine kinase-associated disorder to a combination of kinase inhibitors, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide and imatinib, is disclosed herein. For example, an individual can be determined to be a positive responder (or cells from said individual would be expected to have a degree of sensitivity) to a certain kinase inhibitor based upon the presence of a mutant BCR-ABL kinase. As disclosed herein, cells exhibiting decreased expression level of CK5 (KRT5), PLAU and/or EphA2 or elevated expression levels of AR and/or PSA in a sample relative to a standard, can develop at least partial resistance to of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof. Therefore, individuals suffering from a protein tyrosine kinase-associated disorder whose cells exhibit such a profile, among others disclosed herein, are or would be expected to be partially negative responders to a particular treatment regimen with N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof but a positive responder to a more aggressive treatment regimen of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof or to combination therapy with N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof and imatinib or other therapy.

[00101] A treatment regimen is a course of therapy administered to an individual suffering from a protein kinase associated disorder that can include treatment with one or more kinase inhibitors, as well as other therapies such as radiation and/or other agents (*i.e.*, combination therapy). When more than one therapy is administered, the therapies can be administered concurrently or consecutively (for example, more than one kinase inhibitor can be administered together or at different times, on a different

schedule). Administration of more than one therapy can be at different times (i.e., consecutively) and still be part of the same treatment regimen. As disclosed herein, for example, cells from an individual suffering from a protein kinase associated disorder can be found to develop at least partial resistance to N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide. Based upon the present discovery that such cells can be sensitive to combination therapy or a more aggressive dosage or dosing regimen of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide or a pharmaceutically acceptable salt, hydrate, or solvate thereof, a treatment regimen can be established that includes treatment with the combination either as a monotherapy, or in combination with another kinase inhibitor, or in combination with another agent as disclosed herein. Additionally, the combination can be administered with radiation or other known treatments.

15 **[00102]** Therefore the present invention includes a method for establishing a treatment regimen for an individual suffering from a protein tyrosine kinase associated disorder or treating an individual suffering from a protein tyrosine kinase disorder comprising determining whether a biological sample obtained from an individual is predicted to be resistant to a protein tyrosine kinase inhibitor, including

20 BMS-A, and administering to the subject an appropriate treatment regimen based on whether the mutation is present. The determination can be made by any method known in the art, for example, by measuring the expression level of at least one predictor polynucleotide or polypeptide of the present invention.

[00103] In practicing the many aspects of the invention herein, biological samples

25 can be selected from many sources such as tissue biopsy (including cell sample or cells cultured therefrom; biopsy of bone marrow or solid tissue, for example cells from a solid tumor), blood, blood cells (red blood cells or white blood cells), serum, plasma, lymph, ascetic fluid, cystic fluid, urine, sputum, stool, saliva, bronchial aspirate, CSF or hair. Cells from a sample can be used, or a lysate of a cell sample

30 can be used. In certain embodiments, the biological sample is a tissue biopsy cell sample or cells cultured therefrom, for example, cells removed from a solid tumor or

a lysate of the cell sample. In certain embodiments, the biological sample comprises blood cells.

[00104] Pharmaceutical compositions for use in the present invention can include compositions comprising one or a combination of inhibitors of a BCR-ABL kinase in an effective amount to achieve the intended purpose. The determination of an effective dose of a pharmaceutical composition of the invention is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, for example the ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population).

[00105] Dosage regimens involving N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide useful in practicing the present invention are described in U.S. Serial No. 10/395,503, filed March 24, 2003; and *Blood* (ASH Annual Meeting Abstracts) (2004) Volume 104: Abstract 20, "Hematologic and Cytogenetic Responses in imatinib-Resistant Accelerated and Blast Phase Chronic Myeloid Leukemia (CML) Patients Treated with the Dual SRC/ABL Kinase Inhibitor N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide: Results from a Phase I Dose Escalation Study.", by Moshe Talpaz, et al; which are hereby incorporated herein by reference in their entirety and for all purposes.

[00106] A "therapeutically effective amount" of an inhibitor of a BCR-ABL kinase can be a function of whether the patient, sample, or cell is predicted to be resistant to a protein tyrosine kinase inhibitor. For example, if a patient, sample, or cell is predicted to be resistant to a protein tyrosine kinase inhibitor, then an elevated dosage of said protein tyrosine kinase inhibitor may be warranted, or a more aggressive dosing regimen such as increased dosing frequency, or administration of a combination of said protein tyrosine kinase inhibitor with another agent described herein, or any combination of an elevated dose, increased dosing frequency, and/or combination with another agent may be warranted. One skilled in the art will

appreciate the difference in sensitivity of the BCR-ABL kinase cells and determine a therapeutically effective dose accordingly.

[00107] Examples of predicted therapeutically effective doses of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-

5 pyrimidinyl]amino]-5-thiazolecarboxamide that may be warranted based upon the relative sensitivity of BCR-ABL kinase to N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide compared to wild-type BCR-ABL kinase can be determined using various *in vitro* biochemical assays including expression profiling, cellular proliferation, BCR-ABL
10 tyrosine phosphorylation, peptide substrate phosphorylation, and/or autophosphorylation assays. For example, approximate therapeutically effective doses of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide can be calculated based upon multiplying the typical dose with the fold change in sensitivity of the patient sample
15 with said protein tyrosine kinase inhibitor in anyone or more of these assays. For example, if a sample is found to be 2 fold more resistant than a sensitive cell or tissue sample, then the administration of an about 2 fold higher level of said protein tyrosine kinase may be warranted. Accordingly, therapeutically relevant doses of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-
20 pyrimidinyl]amino]-5-thiazolecarboxamide for any sample found to be at least partially resistant to a protein tyrosine kinase inhibitor can be, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, or 300 fold higher than the prescribed or typical dose. Alternatively, therapeutically relevant doses of N-(2-chloro-6-
25 methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide can be, for example, 0.9x, 0.8x, 0.7x, 0.6x, 0.5x, 0.4x, 0.3x, 0.2x, 0.1x, 0.09x, 0.08x, 0.07x, 0.06x, 0.05x, 0.04x, 0.03x, 0.02x, or 0.01x of the prescribed dose.

[00108] According to the present invention, dosage regimens are adjusted to
30 provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus can be administered, several divided doses can be administered over time or the dose can be proportionally reduced or increased as indicated by the exigencies

of the therapeutic situation. Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without
5 being toxic to the patient. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds
10 and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors. See, e.g., the latest Remington's (*Remington's Pharmaceutical Science*, Mack Publishing Company, Easton, PA)

15 **Identification of markers correlated with BMS-A sensitivity**

[00109] In this study we aimed to identify both predictive and surrogate biomarkers that could potentially assist clinical development of BMS-A in prostate cancer. As outlined in Fig. 1A, our strategy was first to identify genes whose baseline expression levels correlated with drug sensitivity and passed additional variation
20 requirements to obtain the candidate predictive marker list. Then genes whose expression were modulated by BMS-A in a drug treatment study were identified and compared to the candidate predictive biomarker list to obtain list of genes that were not only associated with drug sensitivity but also modulated by drug treatment.

[00110] The IC_{50} values of 16 prostate cell lines to BMS-A were determined and
25 shown in Fig. 1B. Based on the IC_{50} s, cell lines were classified into two groups: 11 cell lines with IC_{50} lower than 200 nM were designated as sensitive group, and 5 cell lines with IC_{50} at least greater than 2 μ M were considered as resistant group. The separation of these two groups was quite well, and the sensitive/resistant demarcation of 200 nM is within the peak range of BMS-A plasma concentrations in patients
30 treated with the doses near the maximum tolerated dose.

[00111] The baseline gene expression of the 16 cell lines were profiled using AFFYMETRIX[®] gene chips. Two statistical tests (1-way ANOVA and correlation to

log₂IC₅₀ values) were performed to identify genes that were differentially expressed between sensitive and resistant groups (5961 probe sets with $p < 0.05$) and those that were highly correlated with IC₅₀ (4575 probe sets with $p < 0.05$), respectively. A total of 4248 probe sets were overlapped in these two analyses suggesting that the categorization of sensitive and resistant groups reflected well the sensitivity of the cells (IC₅₀) to BMS-A. The list was further filtered with requirement of 10% CV across all samples and minimum 3-fold differential expression between the sensitive and resistant groups, resulting selection of 213 probe sets. Expressed sequence tags (EST) and duplicate probe sets were further removed to generate the candidate predictive marker list of 174 genes (Table 2).

[00112] The expression pattern of the 174 genes on the 16 cell lines was visualized by Cluster analysis. As shown in Fig. 1C, these 16 cell lines were separated into two major groups, with the BMS-A-sensitive cell lines highlighted. Interestingly, the cell lines in the left cluster were all sensitive to BMS-A; within the right cluster, albeit clustered relatively close to the resistant cell lines, DU145, PC3 and LNCaP cells were also highly sensitive to BMS-A. DU145 and PC3 may further represented a smaller subgroup that was distinct from the rest of cells in the right cluster. Of the 174 genes, ~70% (124/174) were expressed higher in sensitive cells lines than in resistant cell line, and ~30% (50/174) of genes were expressed higher in resistant cells.

[00113] There are a number of interesting genes in Table 2. For example, amphiregulin and epiregulin, are components in the EGF-EGFR pathway; TGF α , TGF β 2 and TGF β RII are transforming growth factor pathway genes; and other receptor tyrosine kinases such as met proto-oncogene and fibroblast growth factor receptor 2. These genes were expressed higher in sensitive cell lines. Most strikingly, several important prostatic cell markers such as kallikrein 3 (KLK3 or prostate specific antigen, PSA) and androgen receptor (AR) were over-expressed in the resistant cell lines, while cytokeratin 5 (CK5/KRT5) was highly expressed in the sensitive cell lines as marked in Fig. 1C.

[00114] The relative expression level of CK5, PSA and AR in these 16 cell lines were further shown in Fig. 1D. It is clear that resistant cell lines all expressed very low level of CK5 and sensitive cell lines all expressed high level of CK5 except for

DU145, PC3 and LNCaP cells (see Fig. 1C). As CK5 is a marker of basal cell type for the prostatic cell lineage, this data suggests that cells exhibiting the basal phenotype are sensitive to BMS-A and that cells expressing lower level of CK5 tend to be resistant. The expression pattern of PSA and AR, two luminal cell markers, 5 complimentarily reinforces the above observation. While higher expression of PSA and AR correlated with drug resistance, low expression of these two genes highly correlated with BMS-A sensitivity. LNCaP cell line, which was sensitive to BMS-A and expressed high levels of PSA and AR, is the only exception to this observation.

10 **Identification of markers that are also modulated by BMS-A treatment**

[00115] Five BMS-A-sensitive cell lines including two CK5-expressing, PWR1E and RWPE2, and three CK5-nonexpressing cell lines, PC3, DU145 and LNCaP were treated with BMS-A. Comparison of the gene expression profiles between BMS-A-treated and mock-treated same cell line by paired *t*-test revealed that 1628 probe sets 15 were significantly modulated by drug treatment ($p < 0.05$). Comparison of these 1628 probe sets with the list of candidate predictive markers (Table 2) indicated that 10 genes were common on both lists. These 10 genes, which may be potentially used to predict sensitivity to BMS-A and to serve as surrogates for drug response, are indicated as “decreased” in the third column of Table 2. Interestingly, all these 10 20 genes were highly expressed in sensitive cell lines and decreased in expression after BMS-A treatment. These genes include epiregulin, a component in the EGF-EGFR pathway, FHL2 and AXL kinases, and plasminogen activator urokinase (PLAU). Three of these 10 genes including LAMC2, EREG and PLAU encode proteins that are secreted to the extracellular matrix. This set of genes may represent genes whose 25 expression are under the regulation of genes targeted by BMS-A.

Common biomarkers identified in prostate and breast preclinical studies

[00116] To facilitate clinical development of BMS-A for breast cancer, a similar preclinical biomarker study was performed in this laboratory (5). Biomarkers 30 predictive of BMS-A sensitivity in breast cell lines were identified and are currently being assessed in clinical trials. Since the majority of breast tumors are also epithelial origin, we compared the biomarkers discovered in current prostate cancer study with

those identified in the breast cancer study. To this end, in addition to those 174 genes
aforementioned, we also included probe sets in the list of 1475 probe sets (after the
10% CV step, but before fold-change > 3 filter step) that were also significantly
modulated by BMS-A, i.e., present on the list of 1628 probes, as candidate prostate
5 biomarkers and compared them to the breast cancer biomarker list of 161 genes (5).
Fourteen genes were identified as common biomarkers for both tissue types (Table 3).
Notably, EphA2, one of BMS-A targets, was significantly correlated with BMS-A
sensitivity in both prostate and breast cancer cell lines. As shown in Table 3, the
mean expression of EphA2 was significantly higher in sensitive cell lines than in
10 resistant cell lines (2.69 fold with $p = 0.005$ in t -test).

[00117] As shown in Fig. 2A, the expression level of EphA2, as detected by
microarray baseline profiling, was correlated with the IC_{50} values of the prostate cell
lines (higher EphA2 expression with lower IC_{50} or high sensitivity, Pearson
correlation coefficient = -0.66). As a validation, we also performed Western blot
15 analysis to examine the expression of EphA2 protein in 5 sensitive and 3 resistant cell
lines (Fig. 2B). Overall, cell lines with high level of EphA2 mRNA expressed
relatively high levels of EphA2 protein, indicating good concordance between gene
and protein expression for EphA2. Our Western blot results on EphA2 protein in PC3,
DU145 and LNCaP cells are consistent with a previous report (15). With the
20 correlation of its expression with BMS-A sensitivity in cell lines, and being a target
of the drug, EphA2 appeared as a strong candidate biomarker for BMS-A in prostate
cancer.

Down-regulation of uPA expression by BMS-A

25 [00118] Since PLAU gene codes for a secreted protein uPA that is under the
regulation of Src (16), we further evaluated the expression of PLAU gene and its
modulation by BMS-A. As shown in Fig. 3A (and also Table 2), the expression level
of the PLAU gene in sensitive cell lines was significantly higher than in resistant cell
lines. A second probe set for the PLAU gene also showed a similar expression
30 pattern. Additionally, 3 probe sets of PLAU receptor, which partners with PLAU in
its function, all showed a similar expression pattern as PLAU in these cell lines (data
not shown). These, together with the correlation of PLAU expression with BMS-A

sensitivity in breast cancer cell lines (5), suggest strongly that PLAU expression, and potentially the regulation of its function correlate highly with the sensitivity of cells to BMS-A.

[00119] The down-regulation of PLAU mRNA expression by dasatinib was observed (Fig. 3B). Compared to the relatively mild reduction of PLAU expression in two CK5-expressing cells (PWR1E and RWPE2), the reduction in PC3 and DU145 cells were much stronger (approximately 50%). LNCaP cells, which expressed a much lower level of PLAU, also showed a reduction. When we extended the same drug treatment study in additional three resistant cell lines, 22Rv, VCaP and MDAPCa2b, interestingly as shown in Fig. 3C, the magnitude of PLAU reduction by BMS-A was correlated nicely with the sensitivity of cells to BMS-A ($r = 0.72$) with the highest reduction seen in the most sensitive cell line. This suggests PLAU as a potential surrogate biomarker for the biological effect of BMS-A. Furthermore, in a multiple-dose treatment study with PC3 cells, we found that the reduction of PLAU mRNA level by dasatinib at 4h was minimal for all doses compared to untreated control, but the changes were dramatic at 24h in a dose-dependent fashion (Fig. 3D).

[00120] The down-regulation of PLAU expression by BMS-A was also seen at the protein level. By an ELISA assay, we found in a time course experiment that amount of uPA protein secreted by PC3 cells into the growth medium was reduced by BMS-A treatment, and the extent of reduction in secreted uPA protein level is dose-dependent as shown in Fig. 3E using the data at 24h. As a control, when using a cytotoxic agent, TAXOL[®], we did not see a dose-dependent reduction in secreted uPA protein level, suggesting that BMS-A specifically down-regulates PLAU expression.

25

Rationale for patient stratification in BMS-A prostate cancer trials

[00121] Based on their differential expression, we reasoned that CK5, PSA, AR could serve as predictive biomarkers for identification of subtypes of prostate cancer cells or tumors that would respond to BMS-A. We also reasoned that PLAU and EphA2 could potentially be used as surrogate markers to monitor BMS-A response because of their correlation with drug sensitivity and link with the mechanisms of drug action. The expression pattern of these 5 genes in the 16 cell lines was shown in

Fig. 4A. Five BMS-A resistant cell lines WPMY1, MDAPCa2b, 22Rv, VCaP, and DUCaP all expressed high levels of AR, PSA and low levels of CK5 (KRT5), PLAU and EphA2. In contrast, sensitive cell lines expressed low levels of AR and PSA, with exception of LNCaP, and high levels of PLAU, EphA2 and/or CK5.

5 [00122] The dynamic range in expression of these 5 genes and the approximate patient population exhibiting BMS-A-responsive expression pattern were examined using a previously published prostate tumor data set consisting of 52 tumor samples (17). As shown by the cluster analysis in Fig. 4B, nearly 44% (23/52, sample ID labeled in red) of the patient population showed the BMS-A-responsive expression
10 patterns, i.e., low AR and PSA and high PLAU, EphA2 and/or CK5. In the remaining ~56% of patients, the expression of AR and PSA were concordantly relatively high and the expression of PLAU and EphA2 were relatively low. There was certain degrees of co-expression as well as mutual exclusive expression of AR and CK5 in this data set, reminiscent of the expression pattern of these two genes in basal,
15 intermediate and luminal cells of normal prostatic epithelium (18, 19). Co-expression of AR and CK5 may also be alternatively explained as a result of presence of both types of epithelial cells, normal or diseased, in the samples collected, reflecting the heterogeneity of prostate tumors and/or samples. Our data suggest that the five biomarkers identified from preclinical models could help identify patient populations
20 based on expression pattern. Such a gene expression pattern could potentially be used for patient stratification in clinical trials.

Discussion

[00123] The ideal scenario for identifying biomarkers for a drug under clinical
25 development is to use samples obtained from patients undergoing the particular therapy and to analyze gene expression data in the context of patient response data. Since BMS-A is a novel agent during early development, using preclinical models to identify potential biomarkers for assisting clinical development appears the best option. In this study, we used 16 prostate cell lines to identify biomarkers that were
30 correlated with the sensitivity of cells and with the mechanisms of drug action. These biomarkers could potentially be used for predicting and monitoring BMS-A response. In particular, we identified 5 genes including AR, PSA, CK5, PLAU and EphA2 that

were highly associated with drug sensitivity/ resistance and/or modulated by drug treatment. Consistent with breast cancer, it appeared in prostate cancer expressing low levels of AR and PSA and high level of CK5 or with a basal type of gene expression pattern is associated with BMS-A response. Higher expression levels of PLAU and EphA2 may also help to define the potential responders to BMS-A. In addition, PLAU expression was found specifically regulated by BMS-A and such regulation was highly correlated with the sensitivity of cells to BMS-A, suggesting that PLAU expression can potentially be used as a surrogate marker to follow drug action.

10 **[00124]** In our data analysis, we used an approach that emphasized both statistical significance and high-fold differential gene expression between subgroups. Since we have only 16 cell lines, a pool which may not be necessarily big enough for stringent *p* value cutoff in statistical analyses, we included other requirements including stringent filters of 10% coefficient of variation and greater than 3-fold changes in
15 expression between two groups. Notably, the approach we undertook was essentially consistent with the one reported by a recent publication from the MicroArray Quality Control initiative led by the Food and Drug Administration, which showed that gene lists ranked by fold change and filtered with non-stringent yet statistical significant tests were more reproducible across platforms than lists generated with other
20 analytical strategies (20). The result we obtained was biologically meaningful as we identified subtypes of cells sensitive to BMS-A that reflects normal and/or pathogenic prostatic biology and genes that reflect the function of BMS-A targets. In addition, a number of biomarkers identified were also observed from breast cancer study (7).

[00125] Inside normal prostatic epithelium, there exist two major types of
25 epithelial cells, basal and luminal epithelial cells. Studies from recent years suggest that there may be further divisions of epithelial cells into subtypes that are more specialized in function and may fall at different stages of cell differentiation process. Among these subtypes are prostatic stem cells, transit amplifying cells, intermediate cells and secretory luminal cells which can be differentiated based on their expression
30 pattern of certain cellular marker. In our study, we found that cells with low AR, PSA expression and high CK5 expression represent a sub-population that is sensitive to BMS-A. This expression pattern coincides with those possessed by epithelial cells

residing in the basal compartment which can potentially be prostatic stem cells and transit amplifying cells. Since these two types of cells are able to self-renew and quickly proliferate to give rise to new or more differentiated cells, such a coincidence may suggest an intrinsic relationship between the expression and function of BMS-A targets including Src and other Src family members such as LYN and the proliferation of these cells (8). Indeed, LYN is found expressed higher in basal layer than in luminal layer, and that in tumors LYN tends to be higher in less differentiated regions as well (21).

[00126] In contrast to the well established and validated classification of breast cancer subtypes that have been used in clinic to aid patient stratification (22, 23), molecular classification of prostate cancer using genome-wide profiling techniques has been explored (24) but lacks validation with independent tumor cohorts. This may be certainly caused in part by the heterogeneity of prostate tumors and difficulty to obtain biopsies from prostate cancer patients. It remained to be determined whether the molecular subtypes of prostate cancer identified mimic the epithelial subtypes seen in normal prostatic epithelium, to a degree like those seen in breast cancer (22, 23). From our analysis of the data set published by Singh et al. (17), subtypes that expressed lower level of AR, PSA and higher level of CK5, i.e., mimicking basal type, and that with opposite expression patterns, i.e., mimicking luminal type, apparently exist. The derivation of prostate cancer cell lines such as MDAPCa2b, VCaP, DUCaP and LNCaP also demonstrates clearly the existence of luminal type of prostate cancer. The low expression levels PSA/AR as well as CK5 in PC3 and DU145 may also suggest subtypes other than luminal and basal subtypes. It is noted that the subtype with high expression of CK5 and low expression of AR and PSA seen in our study are mainly based on immortalized prostatic cell lines. However a recent study showed that sub-cell populations with varying degree of differentiation co-exist in both AR-expressing LAPC-4/ LAPC-9 human cancer xenograft models and PC3 or DU145 cancer cell populations. The sub-population that expressed CD44, another basal cell marker (25), showed the highest tumorigenicity (26). These strongly suggest that a subtype of cells with the phenotypes of normal basal cells exists in prostate tumors and may contribute a major role in determining the malignancy of tumors. With more tumors samples being profiled, ideally on the same

platform, and advancement of techniques that could improve sample acquisition, processing and/or signal amplification, the molecular subtype classification for prostate tumors will become better understood and may eventually facilitate the delivery of personalized medicine to patients.

5 **[00127]** We found in our study that LNCaP cells, an androgen-sensitive prostate cancer cell line, exhibited a sensitivity to BMS-A distinct from other androgen-sensitive cell lines including 22Rv, MDAPCa2b, VCaP and DuCaP. Transcriptionally these 5 cell lines resemble each other in terms of expression of AR/PSA, those of 174 genes identified, as well as global gene expression (Fig. 1C and other data not
10 shown). It is not clear what mechanism developed in LNCaP cells causes BMS-A susceptibility. The AR gene mutation may be an appealing but not necessarily a straight-forward one as LNCaP cells possess one T877A mutation and other cells either have no mutation (VCaP and DuCaP) or have other (22Rv, H874Y) or additional types of mutations (MDAPCa2b, L701H and T877A) (27). Alteration in
15 expression or sequence of AR may affect the function of AR in terms of binding to androgen (28) or cross-talk with growth factor and receptor pathways such as phosphorylation of AR by signaling cascades (29). The inhibition of growth factor and receptor pathways by BMS-A may also induce the cells to re-establish the balance of signaling networks and modify the mode of action of AR. It is also
20 possible that the function of one or more targets of BMS-A is indispensable for LNCaP cell growth.

[00128] By identifying genes whose expression were altered by drug treatment, we found a set of genes that may correlate with the mechanisms of action of BMS-A. This is desirable in drug development in two ways. First, although concordance
25 among different gene expression-based predictors have been confirmed in the setting of breast cancer (30), markers correlated with mechanisms of action would enhance the level of confidence when biomarkers are used in clinical testing for the specific drug. Secondly, knowledge of drug efficacy through sensitive molecular testing can help to prevent premature discontinuation of clinical studies due to low pathological
30 responses in early stage trials with small number of patients. In particular, while BMS-A is potent in inhibiting cell adhesion, migration and invasion, it appears to be cytostatic rather than cytotoxic. Thus sensitive surrogate markers become critical for

evaluation of drug efficacy in early trials. In our study, we found that uPA, a downstream target of Src kinase, was modulated significantly by BMS-A, and such down-regulation was specifically caused by BMS-A and not by other cytotoxic agent such as TAXOL[®]. In addition, the magnitude of drug-induced uPA reduction correlated very well with the sensitivity of cell lines to BMS-A with higher reduction observed in sensitive cell lines and little or no change in resistant ones. These data suggest that uPA could potentially be used as a surrogate biomarker for monitoring the effect of BMS-A. More excitingly, uPA has been demonstrated to play an important role in prostatic tumorigenesis in numerous studies. For example, it is highly expressed in high grade prostate tumors and metastases (31), and when tumor progresses to androgen independence, the level of uPA expression was enhanced (32). In clinic, elevated uPA or uPA receptor densities are shown to be correlated with poorer survival in prostate cancer (33) and its value as a prognostic marker has been well established in breast cancer (34, 35).

[00129] BMS-A is a potent inhibitor against several cytosolic or receptor tyrosine kinases. Its role in inhibiting Src kinase in prostate cancer cell lines through inhibiting cell adhesion, migration and invasion via the focal adhesion kinase pathway has been reported (36). Interestingly, EphA2, an additional target of BMS-A with an enzymatic IC₅₀ at nanomolar range (5), has also been associated with prostate cancer progression (15). In addition to its correlation with sensitivity of cell lines to BMS-A, EphA2 is also down-regulated by BMS-A in prostate cells (data not shown) and in breast cancer cell lines (5). Furthermore, EphA2 may also act in down-stream of Src kinase, as siRNA knock-down of Src reduces EphA2 expression (5). The exact role of EphA2 in prostatic tumorigenesis and the contribution of inhibited EphA2 activity to BMS-A-induced cell growth inhibition remained to be further determined.

[00130] Among the candidate predictive biomarker gene list, a number of genes are components of signaling pathways important for cell survival and proliferation, such as EGF-EGFR, TGF-TGFR, FGFR and Met pathways. As an intracellular tyrosine kinase, Src can act as a signal transducer in down-stream of these receptor tyrosine kinases (8, 9, 37). Alternatively, Src kinase may function independently of one or more pathways. Although the mechanisms of either co-operation or cross-talk of these pathways with Src-mediated pathway in prostate cancer is not quite clear,

they may still represent candidate target pathway for combination therapies to achieve additive or synergistic effects. This hypothesis should be tested and may provide insight for future clinical development strategies.

5 Utility of highly correlated polynucleotides to make predictions

[00131] Polynucleotides that correlate to a specific property of a biological system can be used to make predictions about that biological system and other biological systems. The Genecluster software or other programs can be used to select polynucleotides and combinations of polynucleotides that can predict properties using
10 a “weighted-voting cross-validation algorithm” (T.R. Golub et al., *Science*, 286:531-537 (1999)). In particular, the Genecluster software was used to build predictors that demonstrate the utility of polynucleotides that correlate to drug sensitivity and resistance. As used herein, the terms “predictor” or “predictor sets” are used as follows: a predictor or a predictor set refers to a single gene, or combination of
15 polynucleotides, whose expression pattern or properties can be used to make predictions, with different error rates, about a property or characteristic of any given biological system.

[00132] The ability of polynucleotide expression patterns to predict a resistance/sensitive classification was further investigated using a “weighted-voting
20 cross-validation algorithm” which uses a leave one out cross-validation strategy as described by T.R. Golub et al., *Science*, 286:531-537 (1999). The program was formatted to select the optimal number of polynucleotides whose expression pattern could be used to predict, with optimal accuracy, the classification of a cell line based on resistance or sensitivity toward a given protein tyrosine kinase inhibitor
25 compound, e.g., BMS-A. A brief description of the cross-validation strategy of the program is described.

[00133] Thus, in accordance with the present invention, an approach has been developed in which polynucleotides and combinations of polynucleotides have been discovered whose expression pattern in a subset of cell lines correlates with, and can
30 be used as a predictor of, response to treatment with compounds that inhibit the function of protein tyrosine kinases.

Predictor sets, error rates and algorithms used to demonstrate utility

[00134] The number of polynucleotides in any given predictor or predictor set may influence the error rate of the predictor set in cross validation experiments and with other mathematical algorithms. The data show that the error rate of a predictor is
5 somewhat dependent on the number of polynucleotides in the predictor set and the contribution of each individual polynucleotide in the given predictor set and the number of cell lines that are tested in the cross validation experiment. For example, in a given predictor set, one polynucleotide may contribute more significantly than other polynucleotides to the prediction.

10 [00135] It is very likely that if a polynucleotide significantly contributes to a predictor set, then it can be used in different combinations with other polynucleotides to achieve different error rates in different predictor sets. For example, polynucleotide A alone gives an error rate of 30%. In combination with polynucleotides, B, C and D, the error rate becomes 10%; in combination with polynucleotides B, D and E, the
15 error rate becomes 12%; while a combination of polynucleotide A with polynucleotides E-X gives an error rate of 8%, and so on. As demonstrated in Fig. 5, different selection and combination of polynucleotides in a predictor set achieve different error rates in the cross-validation tests.

20 Utility of predictor sets to make predictions on testing data of same tissue type

[00136] As described herein, 176 polynucleotides (listed in Table 2) were demonstrated to have their expression levels correlated with sensitivity to a protein tyrosine kinase inhibitor, BMS-A in 16 prostate cancer cell lines. The ability of these 176 polynucleotides, either individually, in combination with one or more, or a
25 represented by polynucleotide subsets to make sensitivity and/or resistance predictions of cell lines to BMS-A was then evaluated in test samples that were different than the test samples used to identify these polynucleotides. Such test samples were utilized to further demonstrate the ability of these 176 polynucleotides, to any combination of one or more of these polynucleotides, and/or a predictor
30 polynucleotide subset, to accurately predict the sensitivity or resistance of a particular test line to BMS-A.

[00137] Based on biological function of the markers shown in Table 2 in relation to protein tyrosine kinases, especially src-family kinase related signaling pathways, and based on changes of their expression level modulated by BMS-A treatment, five polynucleotides as listed in Table 5 were selected to comprise a polynucleotide predictor set that was then utilized to make sensitivity predictions on prostate cell line test samples.

Utility of polynucleotide predictor sets to make predictions on testing data of different tissue type

10 [00138] As described herein, the polynucleotides listed in Table 2 were identified from prostate cancer cell lines. Furthermore, as the results described herein demonstrate, the predictor polynucleotides are able to accurately predict the sensitivity and/or resistance of prostate cancer cell line test samples to exposure to BMS-A. However, it would also be important to demonstrate that these predictor
15 polynucleotides are also able to predict the sensitivity and/or resistance of cancer cell line test samples to exposure to BMS-A derived from a tissue type other than prostate tissue.

[00139] Thus, in accordance with the present invention, an approach has been developed in which predictor polynucleotides, and/or combinations of predictor
20 polynucleotides, have been discovered whose expression pattern in a subset of prostate cell lines correlates with response to treatment with compounds that inhibit the function of protein tyrosine kinases, and can be used as in predicting additional samples of same tissue types (breast), as well as different tissue types (lung), among others. It is expected that these predictor polynucleotides will be useful in predicting
25 the sensitivity and/or resistance of other tissue types, including, but not limited to, colon, and prostate, tissue, and in particular, colon cancer and prostate cancer tissues.

Applications of predictor sets

[00140] Predictor sets with different error rates can be used in different
30 applications. Predictor sets can be built from any combination of the polynucleotides listed in Table 2, or the predictor polynucleotide subsets of 14, 10, and 5 polynucleotides, as presented in each of Tables 2, 3, 4, and 5 respectively, to make

predictions about the likely effect of protein tyrosine modulator compounds, e.g., BMS-A, protein tyrosine kinase inhibitors, or compounds that affect a protein tyrosine kinase signaling pathway in different biological systems. The various predictor sets described herein, or the combination of these predictor sets with other polynucleotides or other co-variants of these polynucleotides, can have broad utility. For example, the predictor sets can be used as diagnostic or prognostic indicators in disease management; they can be used to predict how patients with cancer might respond to therapeutic intervention with compounds that modulate the protein tyrosine kinase family (e.g., the src tyrosine kinase family); and they can be used to predict how patients might respond to therapeutic intervention that modulate signaling through an entire protein tyrosine kinase regulatory pathway, such as, for example, the src tyrosine kinase regulatory pathway.

[00141] While the data described herein were generated in cell lines that are routinely used to screen for and identify compounds that have potential utility for cancer therapy, the predictors can have both diagnostic and prognostic value in other diseases areas in which signaling through a protein tyrosine kinase or a protein tyrosine kinase pathway is of importance, e.g., in immunology, or in cancers or tumors in which cell signaling and/or proliferation controls have gone awry. Such protein tyrosine kinases and their pathways comprise, for example, members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors. Although the data described herein have been generated using the particularly exemplified protein tyrosine kinase inhibitor compound, BMS-A, three other protein tyrosine kinase inhibitor compounds were tested in addition to BMS-A and were found to have similar sensitivity and resistance classifications in the 23 breast cell lines evaluated. Thus, the predictors can have both diagnostic and prognostic value related to other inhibitor molecules, as well as any molecules or therapeutic interventions that affect protein tyrosine kinases, such as Src tyrosine kinase, or a protein tyrosine kinase signaling pathways, such as that of the Src tyrosine kinase.

[00142] Those having skill in the pertinent art will appreciate that protein tyrosine kinase pathways, e.g., the Src tyrosine kinase pathway, are present and functional in

cell types other than cell lines of breast tissue. Therefore, the described predictor set of polynucleotides, or combinations of polynucleotides within the predictor set, can show utility for predicting drug sensitivity or resistance to compounds that interact with, or inhibit, a protein tyrosine kinase activity in cells from other tissues or organs associated with a disease state, or cancers or tumors derived from other tissue or organ types. Non-limiting examples of such cells, tissues and organs include colon, breast, lung, heart, prostate, testes, ovaries, cervix, esophagus, pancreas, spleen, liver, kidney, intestine, stomach, lymphocytic and brain, thereby providing a broad and advantageous applicability to the predictor polynucleotide sets described herein. Cells for analysis can be obtained by conventional procedures as known in the art, for example, tissue or organ biopsy, aspiration, sloughed cells, e.g., colonocytes, clinical or medical tissue, or cell sampling procedures.

Functionality of polynucleotides that make up a predictor set

[00143] The use of a predictor, or predictor set, (e.g., predictor polynucleotides, or a predictor set of polynucleotides) allows for the prediction of an outcome prior to having any knowledge about a biological system. Essentially, a predictor can be considered to be a tool that is useful in predicting the phenotype that is used to classify the biological system. In the specific embodiment provided by the present invention, the classification as “resistant” or “sensitive” is based on the IC₅₀ value of each cell line to a compound (e.g., the protein tyrosine kinase inhibitor compound BMS-A as exemplified herein).

[00144] As a particular example, a number of the polynucleotides described herein (Table 2) are known to be substrates for the src tyrosine kinase family, e.g., caveolin-1 and caveolin-2 (M.T. Brown and J.A. Cooper, *Biochemica et Biophysica Acta*, 1287:121-149 (1996)). EphA2 is a tyrosine kinase receptor. The data presented herein demonstrated that EphA2 is highly expressed in the sensitive cell lines, and its expression level and activity are down regulated by treatment of the protein tyrosine kinase inhibitor compound BMS-A. This is expected, since polynucleotides that contribute to a high predictor accuracy are likely to play a functional role in the pathway that is being modulated. For example, HERCEPTIN[®] therapy (i.e., antibody that binds to the Her2 receptor and prevents function via internalization) is indicated

when the Her2 polynucleotide is overexpressed. It is unlikely, although not impossible, that a therapy will have a therapeutic effect if the target enzyme is not expressed.

[00145] However, although the complete function of some of the polynucleotides and their functional products (proteins and mRNAs) that make up a predictor set are not currently known, some of the polynucleotides are likely to be directly or indirectly involved in a protein tyrosine kinase signaling pathway, such as the Src tyrosine kinase signaling pathway. In addition, some of the polynucleotides in the predictor set may function in the metabolic or other resistance pathways specific to the compounds being tested. Notwithstanding, a knowledge about the function of the polynucleotides is not a requisite for determining the accuracy of a predictor according to the practice of the present invention.

[00146] As described herein, polynucleotides have been discovered that correlate to the relative intrinsic sensitivity or resistance of prostate cell lines to treatment with compounds that interact with and inhibit protein tyrosine kinases, e.g., Src tyrosine kinase. These polynucleotides have been shown, through a weighted voting, leave-one-out, cross validation program, to have utility in predicting the intrinsic resistance and sensitivity of prostate cell lines to these compounds.

[00147] An embodiment of the present invention relates to a method of determining or predicting if an individual requiring drug or chemotherapeutic treatment or therapy for a disease, for example, a prostate cancer or a prostate tumor, will be likely to successfully respond or not respond to the drug or chemotherapeutic agent prior to subjecting the individual to such treatment or chemotherapy. The drug or chemotherapeutic agent can be one that modulates a protein tyrosine kinase activity or signaling involving a protein tyrosine kinase. Nonlimiting examples of such protein tyrosine kinases include members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors. In accordance with the method of the invention, cells from a tissue or organ associated with disease, e.g., a patient biopsy of a tumor or cancer, preferably a prostate cancer or tumor biopsy, are subjected to an *in vitro* assay as described herein, to determine their marker polynucleotide expression pattern (polynucleotides from Table 2 and/or

the predictor polynucleotide subsets of Tables 3, 4, or 5) prior to their treatment with the compound or drug, preferably an inhibitor of a protein tyrosine kinase. The resulting polynucleotide expression profile of the cells before drug treatment is compared with the polynucleotide expression pattern of the same polynucleotides in
5 cells that are either resistant or sensitive to the drug or compound, as provided by the present invention.

[00148] In another related embodiment, the present invention includes a method of predicting, prognosing, diagnosing, and/or determining whether an individual requiring drug therapy for a disease state or chemotherapeutic for cancer (e.g.,
10 prostate cancer) will or will not respond to treatment prior to administration of treatment. The treatment or therapy preferably involves a protein tyrosine kinase modulating agent, compound, or drug, for example, an inhibitor of the protein tyrosine kinase activity. Protein tyrosine kinases include, without limitation, members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck
15 and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors. Preferred is src tyrosine kinase and inhibitors thereof. In accordance with this embodiment, cells from a patient's tissue sample, e.g., a prostate tumor or cancer biopsy, are assayed to determine their polynucleotide expression pattern prior to treatment with the protein tyrosine kinase modulating agent,
20 compound, or drug. The resulting polynucleotide expression profile of the test cells before exposure to the compound or drug is compared with that of one or more of the predictor subsets of polynucleotides comprising either 174, 14, 10, or 5 polynucleotides as described herein and shown in Table 2, Tables 3, 4, or 5, respectively.

[00149] Success or failure of treatment of a patient's cancer or tumor with the drug can be determined based on the polynucleotide expression pattern of the patient's cells being tested, compared with the polynucleotide expression pattern of the predictor polynucleotides in the resistant or sensitive panel of that have been exposed to the drug or compound and subjected to the predictor polynucleotide analysis
30 detailed herein. Thus, if following exposure to the drug, the test cells show a polynucleotide expression pattern corresponding to that of the predictor polynucleotide set of the control panel of cells that is sensitive to the drug or

compound, it is highly likely or predicted that the individual's cancer or tumor will respond favorably to treatment with the drug or compound. By contrast, if, after drug exposure, the test cells show a polynucleotide expression pattern corresponding to that of the predictor polynucleotide set of the control panel of cells that is resistant to the drug or compound, it is highly likely or predicted that the individual's cancer or tumor will not respond to treatment with the drug or compound.

[00150] In a related embodiment, screening assays are provided for determining if a patient's cancer or tumor is or will be susceptible or resistant to treatment with a drug or compound, particularly, a drug or compound directly or indirectly involved in protein tyrosine kinase activity or a protein tyrosine kinase pathway, e.g., the Src tyrosine kinase activity or pathway.

[00151] Also provided by the present invention are monitoring assays to monitor the progress of a drug treatment involving drugs or compounds that interact with or inhibit protein tyrosine kinase activity. Protein tyrosine kinases encompassed by these monitoring assays include members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors. Such *in vitro* assays are capable of monitoring the treatment of a patient having a disease treatable by a compound or agent that modulates or interacts with a protein tyrosine kinase by comparing the resistance or sensitivity polynucleotide expression pattern of cells from a patient tissue sample, e.g., a tumor or cancer biopsy, preferably a prostate cancer or tumor sample, prior to treatment with a drug or compound that inhibits the protein tyrosine kinase activity and again following treatment with the drug or compound with the expression pattern of one or more of the predictor polynucleotide sets described, or combinations thereof. Isolated cells from the patient are assayed to determine their polynucleotide expression pattern before and after exposure to a compound or drug, preferably a protein tyrosine kinase inhibitor, to determine if a change of the polynucleotide expression profile has occurred so as to warrant treatment with another drug or agent, or discontinuing current treatment. The resulting polynucleotide expression profile of the cells tested before and after treatment is compared with the polynucleotide expression pattern of the predictor set of polynucleotides that have been described and shown herein to be highly expressed

in cells that are either resistant or sensitive to the drug or compound. Alternatively, a patient's progress related to drug treatment or therapy can be monitored by obtaining a polynucleotide expression profile as described above, only after the patient has undergone treatment with a given drug or therapeutic compound. In this way, there is
5 no need to test a patient sample prior to treatment with the drug or compound.

[00152] Such a monitoring process can indicate success or failure of a patient's treatment with a drug or compound based on the polynucleotide expression pattern of the cells isolated from the patient's sample, e.g., a tumor or cancer biopsy, as being relatively the same as or different from the polynucleotide expression pattern of the
10 predictor polynucleotide set of the resistant or sensitive control panel of cells that have been exposed to the drug or compound and assessed for their polynucleotide expression profile following exposure. Thus, if, after treatment with a drug or compound, the test cells show a change in their polynucleotide expression profile from that seen prior to treatment to one which corresponds to that of the predictor
15 polynucleotide set of the control panel of cells that are resistant to the drug or compound, it can serve as an indicator that the current treatment should be modified, changed, or even discontinued. Also, should a patient's response be one that shows sensitivity to treatment by a protein tyrosine kinase inhibitor compound, e.g., a Src tyrosine kinase inhibitor, based on correlation of the expression profile of the
20 predictor polynucleotides of cells showing drug sensitivity with the polynucleotide expression profile from cells from a patient undergoing treatment, the patient's treatment prognosis can be qualified as favorable and treatment can continue. Further, if a patient has not been tested prior to drug treatment, the results obtained after treatment can be used to determine the resistance or sensitivity of the cells to the drug
25 based on the polynucleotide expression profile compared with the predictor polynucleotide set.

[00153] In a related embodiment, the present invention embraces a method of monitoring the treatment of a patient having a disease treatable by a compound or agent that modulates a protein tyrosine kinase, i.e., prostate cancer. Protein tyrosine
30 kinases encompassed by such treatment monitoring assays include members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit

and Eph receptors. For these assays, test cells from the patient are assayed to determine their polynucleotide expression pattern before and after exposure to a protein tyrosine kinase inhibitor compound or drug. The resulting polynucleotide expression profile of the cells tested before and after treatment is compared with the polynucleotide expression pattern of the predictor set of polynucleotides that have been described and shown herein to be highly expressed in cells that are either resistant or sensitive to the drug or compound. Thus, if a patient's response is or becomes one that is sensitive to treatment by a protein tyrosine kinase inhibitor compound, based on correlation of the expression profile of the predictor polynucleotides, the patient's treatment prognosis can be qualified as favorable and treatment can continue. Also, if after treatment with a drug or compound, the test cells do not exhibit a change in their polynucleotide expression profile to a profile that corresponds to that of the control panel of cells that are sensitive to the drug or compound, this serves as an indicator that the current treatment should be modified, changed, or even discontinued. Such monitoring processes can be repeated as necessary or desired and can indicate success or failure of a patient's treatment with a drug or compound, based on the polynucleotide expression pattern of the cells isolated from the patient's sample. The monitoring of a patient's response to a given drug treatment can also involve testing the patient's cells in the assay as described, only after treatment, rather than before and after treatment, with drug or active compound.

[00154] In a preferred embodiment, the present invention embraces a method of monitoring the treatment of a patient having a disease treatable by a compound or agent that modulates a src tyrosine kinase, i.e., prostate cancer. The test cells from the patient are assayed to determine their polynucleotide expression pattern before and after exposure to a src tyrosine kinase inhibitor compound or drug. The resulting polynucleotide expression profile of the cells tested before and after treatment is compared with the polynucleotide expression pattern of the predictor set of polynucleotides that have been described and shown herein to be highly expressed in cells that are either resistant or sensitive to the drug or compound. Thus, if a patient's response is or becomes one that is sensitive to treatment by a src tyrosine kinase inhibitor compound, based on correlation of the expression profile of the predictor

polynucleotides, the patient's treatment prognosis can be qualified as favorable and treatment can continue. Also, if after treatment with a drug or compound, the test cells do not exhibit a change in their polynucleotide expression profile to a profile that corresponds to that of the control panel of cells that are sensitive to the drug or compound, this serves as an indicator that the current treatment should be modified, changed, or even discontinued. Such monitoring processes can be repeated as necessary or desired and can indicate success or failure of a patient's treatment with a drug or compound, based on the polynucleotide expression pattern of the cells isolated from the patient's sample. The monitoring of a patient's response to a given drug treatment can also involve testing the patient's cells in the assay as described only after treatment, rather than before and after treatment, with drug or active compound.

[00155] In another embodiment, the present invention encompasses a method of classifying whether a biological system, preferably cells from a tissue, organ, tumor or cancer of an afflicted individual, will be resistant or sensitive to a compound that modulates the system. In a preferred aspect of this invention, the sensitivity or resistance of cells, e.g., those obtained from a tumor or cancer, to a protein tyrosine kinase inhibitor compound, or series of compounds, e.g., a Src tyrosine kinase inhibitor, is determined. Inhibitors can include those compounds, drugs, or biological agents that inhibit, either directly or indirectly, the protein tyrosine kinases as described previously hereinabove. According to the method, a resistance/sensitivity profile of the cells after exposure to the protein tyrosine kinase inhibitor drug or compound can be determined via polynucleotide expression profiling protocols set forth herein. Such resistance/sensitivity profile of the cells reflects an IC_{50} value of the cells to the compound(s) as determined using a suitable assay, such as an *in vitro* assay as described in Example 1. A procedure of this sort can be performed using a variety of cell types and compounds that interact with the protein tyrosine kinase, or affect its activity in the signaling pathway of the protein tyrosine kinase.

[00156] In another of its embodiments, the present invention includes the preparation of one or more specialized microarrays (e.g., oligonucleotide microarrays or cDNA microarrays) comprising all of the polynucleotides in Tables 2, 3, 4, or 5, or any combinations thereof, of the predictor polynucleotide sets described herein that

have been demonstrated to be most highly correlated with sensitivity (or resistance) to protein tyrosine kinase modulators, particularly inhibitors of src tyrosine kinase. Preferably, the predictor polynucleotide sets are common for predicting sensitivity among more than one protein tyrosine kinase modulator, e.g., a protein tyrosine kinase inhibitor such as a Src tyrosine kinase inhibitor, as demonstrated herein. In accordance with this aspect of the invention, the oligonucleotide sequences or cDNA sequences include any of the predictor polynucleotides or polynucleotide combinations as described herein, which are highly expressed in resistant or sensitive cells, and are contained on a microarray, e.g., a oligonucleotide microarray or cDNA microarray in association with, or introduced onto, any supporting material, such as glass slides, nylon membrane filters, glass or polymer beads, chips, plates, or other types of suitable substrate material.

[00157] Cellular nucleic acid, e.g., RNA, is isolated either from cells undergoing testing after exposure to a drug or compound that interacts with a protein tyrosine kinase as described herein, or its signaling pathway, or from cells being tested to obtain an initial determination or prediction of the cells' sensitivity to the drug or compound, and, ultimately, a prediction of treatment outcome with the drug or compound. The isolated nucleic acid is appropriately labeled and applied to one or more of the specialized microarrays. The resulting pattern of polynucleotide expression on the specialized microarray is analyzed as described herein and known in the art. A pattern of polynucleotide expression correlating with either sensitivity or resistance to the drug or compound is able to be determined, e.g., via comparison with the polynucleotide expression pattern as shown in Figure 1 for the panel of cells exposed to the protein tyrosine kinase inhibitor assayed herein.

[00158] In accordance with the specialized microarray embodiment of this invention, the microarray contains the polynucleotides of one or more of the predictor polynucleotide set(s) or subset(s), or a combination thereof, or all of the polynucleotides in Tables 2, 3, 4, or 5, that are highly correlated with drug sensitivity or resistance by a prostate cell type. If the nucleic acid target isolated from test cells, such as tumor or cancer cells, preferably prostate cancer or tumor cells, shows a high level of detectable binding to the polynucleotides of the predictor set for drug sensitivity relative to control, then it can be predicted that a patient's cells will

respond to the drug, or a series of drugs, and that the patient's response to the drug, or a series of drugs, will be favorable.

[00159] Such a result predicts that the cells of a tumor or cancer are good candidates for the successful treatment or therapy utilizing the drug, or series of drugs. Alternatively, if the nucleic acid target isolated from test cells shows a high level of detectable binding to the polynucleotides of the predictor set for drug resistance, relative to control, then it can be predicted that a patient is likely not to respond to the drug, or a series of drugs, and that the patient's response to the drug, or a series of drugs, is not likely to be favorable. Such a result predicts that the cells of a tumor or cancer are not good candidates for treatment or therapy utilizing the drug, or series of drugs.

[00160] The utilization of microarray technology is known and practiced in the art. Briefly, to determine polynucleotide expression using microarray technology, polynucleotides, e.g., RNA, DNA, cDNA, preferably RNA, are isolated from a biological sample, e.g., cells, as described herein for prostate cells, using procedures and techniques that are practiced in the art. The isolated nucleic acid is detectably labeled, e.g., fluorescent, enzyme, radionuclide, or chemiluminescent label, and applied to a microarray, e.g., the specialized microarrays provided by this invention. The array is then washed to remove unbound material and visualized by staining or fluorescence, or other means known in the art depending on the type of label utilized.

[00161] In another embodiment of this invention, the predictor polynucleotides (Table 2), or one or more subsets of polynucleotides comprising the predictor polynucleotide sets (e.g., Tables 3, 4, or 5) can be used as biomarkers for cells that are resistant or sensitive to protein tyrosine kinase inhibitor compounds, e.g., Src tyrosine kinase inhibitors. With the predictor polynucleotides in hand, screening and detection assays can be carried out to determine whether or not a given compound, preferably a protein tyrosine kinase inhibitor compound such as a Src tyrosine kinase inhibitor compound, elicits a sensitive or a resistant phenotype following exposure of cells, e.g., cells taken from a tumor or cancer biopsy sample, such as a prostate cancer cell sample, to the compound. Thus, methods of screening, monitoring, detecting, prognosing and/or diagnosing to determine the resistance or sensitivity of cells to a drug or compound that interacts with a protein tyrosine kinase, or a protein tyrosine

kinase pathway, preferably an inhibitor compound, and to which the cells are exposed, are encompassed by the present invention.

[00162] Such methods embrace a variety of procedures and assays to determine and assess the expression of polynucleotides, in particular, the predictor or src
5 biomarker polynucleotides and predictor polynucleotide subsets as described herein (Tables 2, 3, 4, or 5), in cells that have been exposed to drugs or compounds that interact with or effect a protein tyrosine kinase, or a protein tyrosine kinase pathway, wherein the protein tyrosine kinases include members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other
10 protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors. Suitable methods include detection and evaluation of polynucleotide activation or expression at the level of nucleic acid, e.g., DNA, RNA, mRNA, and detection and evaluation of encoded protein. For example, PCR assays as known and practiced in the art can be employed to quantify RNA or DNA in cells being assayed for
15 susceptibility to drug treatment, for example, protein tyrosine kinase inhibitors. (see Example 3, RT-PCR).

[00163] In another embodiment, the present invention is directed to a method of identifying cells, tissues, and/or patients that are predicted to be resistant to either protein tyrosine inhibitor compounds or compounds that affect protein tyrosine kinase
20 signaling pathways, e.g., Src tyrosine kinase, or that are resistant in different biological systems to those compounds. The method comprises the step(s) of: (i) analyzing the expression of only those polynucleotides listed in Tables 2, 3, 4, or 5, or any combination thereof, that have been shown to be correlative to predicting resistant responses to such compounds; (ii) comparing the observed expression levels
25 of those correlative resistant polynucleotides in the test cells, tissues, and/or patients to the expression levels of those same polynucleotides in a cell line that is known to be resistant to the compounds; and (iii) predicting whether the cells, tissues, and/or patients are resistant to the compounds based upon the overall similarity of the observed expression of those polynucleotides in step (ii).

[00164] In another embodiment, the present invention is directed to a method of identifying cells, tissues, and/or patients that are predicted to be sensitive to either
30 protein tyrosine inhibitor compounds or compounds that affect protein tyrosine kinase

signaling pathways, e.g., the Src tyrosine kinase, or that are sensitive in different biological systems to those compounds. The method involves the step(s) of: (i) analyzing the expression of only those polynucleotides listed in Tables 2, 3, 4, or 5, or any combination thereof, that have been shown to be correlative to predicting sensitive responses to such compounds; (ii) comparing the observed expression levels of those correlative sensitive polynucleotides in the test cells, tissues, and/or patients to the expression levels of those same polynucleotides in a cell line that is known to be sensitive to the compounds; and (iii) predicting whether the cells, tissues, and/or patients are sensitive to the compounds based upon the overall similarity of the observed expression of those polynucleotides in step (ii).

[00165] The present invention further encompasses the detection and/or quantification of one or more of the protein tyrosine kinase biomarker proteins of the present invention using antibody-based assays (e.g., immunoassays) and/or detection systems. As mentioned, protein tyrosine kinases encompass members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors. Such assays include the following non-limiting examples, ELISA, immunofluorescence, fluorescence activated cell sorting (FACS), Western Blots, etc., as further described herein.

[00166] In another embodiment, the human protein tyrosine kinase biomarker polypeptides and/or peptides of the present invention, or immunogenic fragments or oligopeptides thereof, can be used for screening therapeutic drugs or compounds in a variety of drug screening techniques. The fragment employed in such a screening assay can be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The reduction or abolition of activity of the formation of binding complexes between the biomarker protein and the agent being tested can be measured. Thus, the present invention provides a method for screening or assessing a plurality of compounds for their specific binding affinity with a protein kinase inhibitor biomarker polypeptide, or a bindable peptide fragment thereof, of this invention. The method comprises the steps of providing a plurality of compounds; combining the protein kinase inhibitor biomarker polypeptide, or a bindable peptide fragment thereof, with each of the plurality of compounds, for a time sufficient to

allow binding under suitable conditions; and detecting binding of the biomarker polypeptide or peptide to each of the plurality of test compounds, thereby identifying the compounds that specifically bind to the biomarker polypeptide or peptide. More specifically, the biomarker polypeptide or peptide is that of a Src tyrosine kinase inhibitor biomarkers.

5 [00167] Methods to identify compounds that modulate the activity of the human protein tyrosine kinase biomarker polypeptides and/or peptides provided in Table 2 by the present invention, comprise combining a candidate compound or drug modulator of protein kinases and measuring an effect of the candidate compound or drug modulator on the biological activity of the protein kinase inhibitor biomarker polypeptide or peptide. Such measurable effects include, for example, a physical binding interaction; the ability to cleave a suitable protein kinase substrate; effects on a native and cloned protein kinase biomarker-expressing cell line; and effects of modulators or other protein kinase-mediated physiological measures.

10 [00168] Another method of identifying compounds that modulate the biological activity of the protein tyrosine kinase biomarker polypeptides of the present invention comprises combining a potential or candidate compound or drug modulator of a protein tyrosine kinase biological activity, e.g., a Src tyrosine kinase, with a host cell that expresses the protein tyrosine kinase biomarker polypeptide and measuring an effect of the candidate compound or drug modulator on the biological activity of the protein tyrosine kinase biomarker polypeptides. The host cell can also be capable of being induced to express the protein tyrosine kinase biomarker polypeptide, e.g., via inducible expression. Physiological effects of a given modulator candidate on the protein tyrosine kinase biomarker polypeptide can also be measured. Thus, cellular assays for particular protein tyrosine kinase modulators, e.g., a src kinase modulator, can be either direct measurement or quantification of the physical biological activity of the protein tyrosine kinase biomarker polypeptide, or they may be measurement or quantification of a physiological effect. Such methods preferably employ a protein tyrosine kinase biomarker polypeptide as described herein, or an overexpressed recombinant protein tyrosine kinase biomarker polypeptide in suitable host cells containing an expression vector as described herein, wherein the protein tyrosine

kinase biomarker polypeptide is expressed, overexpressed, or undergoes up-regulated expression.

[00169] Another aspect of the present invention embraces a method of screening for a compound that is capable of modulating the biological activity of a protein tyrosine kinase biomarker polypeptide, e.g., a Src kinase biomarker polypeptide. The method comprises providing a host cell containing an expression vector harboring a nucleic acid sequence encoding a protein tyrosine kinase biomarker polypeptide, or a functional peptide or portion thereof (e.g., the src polypeptide, protein, peptide, or fragment sequences as set forth in Table 2, or the Sequence Listing herein); determining the biological activity of the expressed protein tyrosine kinase biomarker polypeptide in the absence of a modulator compound; contacting the cell with the modulator compound and determining the biological activity of the expressed protein tyrosine kinase biomarker polypeptide in the presence of the modulator compound. In such a method, a difference between the activity of the protein tyrosine kinase biomarker polypeptide in the presence of the modulator compound and in the absence of the modulator compound indicates a modulating effect of the compound.

[00170] Essentially any chemical compound can be employed as a potential modulator or ligand in the assays according to the present invention. Compounds tested as protein tyrosine kinase modulators can be any small chemical compound, or biological entity (e.g., protein, sugar, nucleic acid, or lipid). Test compounds are typically small chemical molecules and peptides. Generally, the compounds used as potential modulators can be dissolved in aqueous or organic (e.g., DMSO-based) solutions. The assays are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source. Assays are typically run in parallel, for example, in microtiter formats on microtiter plates in robotic assays. There are many suppliers of chemical compounds, including, for example, Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs, Switzerland). Also, compounds can be synthesized by methods known in the art.

[00171] High throughput screening methodologies are particularly envisioned for the detection of modulators of the novel protein tyrosine kinase biomarker, e.g., src biomarker, polynucleotides and polypeptides described herein. Such high throughput

screening methods typically involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds (e.g., ligand or modulator compounds). The combinatorial chemical libraries or ligand libraries are then screened in one or more assays to identify those library members (e.g., particular
5 chemical species or subclasses) that display a desired characteristic activity. The compounds so identified can serve as conventional lead compounds, or can themselves be used as potential or actual therapeutics.

[00172] A combinatorial chemical library is a collection of diverse chemical compounds generated either by chemical synthesis or biological synthesis, prepared
10 by combining a number of chemical building blocks (i.e., reagents such as amino acids). As an example, a linear combinatorial library, e.g., a polypeptide or peptide library, is formed by combining a set of chemical building blocks in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide or peptide compound). Millions of chemical compounds can be synthesized through
15 such combinatorial mixing of chemical building blocks.

[00173] The preparation and screening of combinatorial chemical libraries is well known to those having skill in the pertinent art. Combinatorial libraries include, without limitation, peptide libraries (e.g., U.S. Patent No. 5,010,175; Furka, *Int. J. Pept. Prot. Res.*, 37:487-493 (1991); and Houghton et al., *Nature*, 354:84-88 (1991)).
20 Other chemistries for generating chemical diversity libraries can also be used. Nonlimiting examples of chemical diversity library chemistries include, peptoids (PCT Publication No. WO 91/019735), encoded peptides (PCT Publication No. WO 93/20242), random bio-oligomers (PCT Publication No. WO 92/00091), benzodiazepines (U.S. Patent No. 5,288,514), diversomers such as hydantoins,
25 benzodiazepines and dipeptides (Hobbs et al., *Proc. Natl. Acad. Sci. USA*, 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara et al., *J. Amer. Chem. Soc.*, 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann et al., *J. Amer. Chem. Soc.*, 114:9217-9218 (1992)), analogous organic synthesis of small compound libraries (Chen et al., *J. Amer. Chem. Soc.*, 116:2661
30 (1994)), oligocarbamates (Cho et al., *Science*, 261:1303 (1993)), and/or peptidyl phosphonates (Campbell et al., *J. Org. Chem.*, 59:658 (1994)), nucleic acid libraries (see Ausubel, Berger and Sambrook, all supra), peptide nucleic acid libraries (U.S.

Patent No. 5,539,083), antibody libraries (e.g., Vaughn et al., *Nature Biotechnology*, 14(3):309-314 (1996) and PCT/US96/10287), carbohydrate libraries (e.g., Liang et al., *Science*, 274-1520-1522 (1996) and U.S. Patent No. 5,593,853), small organic molecule libraries (e.g., benzodiazepines, Baum, *C&EN*, p. 33 (Jan. 18, 1993) and
5 U.S. Patent No. 5,288,514); isoprenoids (U.S. Patent No. 5,569,588); thiazolidinones and metathiazanones (U.S. Patent No. 5,549,974); pyrrolidines (U.S. Patent Nos. 5,525,735 and 5,519,134); morpholino compounds (U.S. Patent No. 5,506,337); and the like.

[00174] Devices for the preparation of combinatorial libraries are commercially
10 available (e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY; Symphony, Rainin, Woburn, MA; 433A Applied Biosystems, Foster City, CA; 9050 Plus, Millipore, Bedford, MA). In addition, a large number of combinatorial libraries are commercially available (e.g., ComGenex, Princeton, NJ; Asinex, Moscow, Russia; Tripos, Inc., St. Louis, MO; ChemStar, Ltd., Moscow, Russia; 3D
15 Pharmaceuticals, Exton, PA; Martek Biosciences, Columbia, MD, and the like).

[00175] In one aspect, the invention provides solid phase-based *in vitro* assays in a high throughput format, where the cell or tissue expressing a tyrosine kinase protein/polypeptide/peptide is attached to a solid phase substrate. In such high throughput assays, it is possible to screen up to several thousand different modulators
20 or ligands in a single day. In particular, each well of a microtiter plate can be used to perform a separate assay against a selected potential modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can be used to test a single modulator. Thus, a single standard microtiter plate can be used in to assay about 96 modulators. If 1536 well plates are used, then a single plate can easily assay
25 from about 100 to about 1500 different compounds. It is possible to assay several different plates per day; thus, for example, assay screens for up to about 6,000-20,000 different compounds are possible using the described integrated systems.

[00176] In another of its aspects, the present invention encompasses screening and small molecule (e.g., drug) detection assays which involve the detection or
30 identification of small molecules that can bind to a given protein, i.e., a tyrosine kinase biomarker polypeptide or peptide, such as a Src tyrosine kinase biomarker

polypeptide or peptide. Particularly preferred are assays suitable for high throughput screening methodologies.

[00177] In such binding-based detection, identification, or screening assays, a functional assay is not typically required. All that is needed, in general, is a target protein, preferably substantially purified, and a library or panel of compounds (e.g., ligands, drugs, or small molecules), or biological entities to be screened or assayed for binding to the protein target. Preferably, most small molecules that bind to the target protein modulate the target's activity in some manner due to preferential, higher affinity binding to functional areas or sites on the protein.

10 [00178] An example of such an assay is the fluorescence based thermal shift assay (3-Dimensional Pharmaceuticals, Inc., 3DP, Exton, PA) as described in U.S. Patent Nos. 6,020,141 and 6,036,920 to Pantoliano et al. (See also, J. Zimmerman, *Gen. Eng. News*, 20(8) (2000)). The assay allows the detection of small molecules (e.g., drugs, ligands) that bind to expressed, and preferably purified, tyrosine kinase biomarker proteins/polypeptides/peptides, such as the Src tyrosine kinase, based on affinity of binding determinations by analyzing thermal unfolding curves of protein-drug or ligand complexes. The drugs or binding molecules determined by this technique can be further assayed, if desired, by methods such as those described herein to determine if the molecules affect or modulate function or activity of the target protein.

20 [00179] To purify a tyrosine kinase biomarker polypeptide or peptide, e.g., Src tyrosine kinase, to measure a biological binding or ligand binding activity, the source may be a whole cell lysate that can be prepared by successive freeze-thaw cycles (e.g., one to three) in the presence of standard protease inhibitors. The tyrosine kinase biomarker polypeptide can be partially or completely purified by standard protein purification methods, e.g., affinity chromatography using specific antibody(ies) described herein, or by ligands specific for an epitope tag engineered into the recombinant tyrosine kinase biomarker polypeptide molecule, also as described herein. Binding activity can then be measured as described.

25 [00180] Compounds which are identified according to the methods provided herein, and which modulate or regulate the biological activity or physiology of the tyrosine kinase biomarker polypeptides according to the present invention, are a preferred embodiment of this invention. It is contemplated that such modulatory

compounds can be employed in treatment and therapeutic methods for treating a condition that is mediated by the tyrosine kinase biomarker polypeptides, e.g., Src tyrosine kinase biomarker polypeptides, by administering to an individual in need of such treatment a therapeutically effective amount of the compound identified by the methods described herein.

[00181] In addition, the present invention provides methods for treating an individual in need of such treatment for a disease, disorder, or condition that is mediated by the tyrosine kinase biomarker polypeptides of the invention, comprising administering to the individual a therapeutically effective amount of the tyrosine kinase biomarker-modulating compound identified by a method provided herein. In accordance with this invention, the tyrosine kinase biomarker polypeptides or proteins encompassed by the methods include members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors.

[00182] The present invention particularly provides methods for treating an individual in need of such treatment for a disease, disorder, or condition that is mediated by Src biomarker polypeptides of the invention, comprising administering to the individual a therapeutically effective amount of the Src biomarker-modulating compound identified by a method provided herein.

[00183] The present invention further encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of one or more of the protein tyrosine kinase biomarkers, preferably the Src biomarker amino acid sequences as set forth in Table 2. The present invention also encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the protein tyrosine kinase biomarkers of the invention.

[00184] The term "epitopes" as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope" as used herein, refers to a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies

described *infra*. (See, for example, Geysen et al., *Proc. Natl. Acad. Sci. USA*, 81:3998-4002 (1983)). The term "antigenic epitope" as used herein refers to a portion of a protein to which an antibody can immunospecifically bind to its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding, but does not necessarily exclude cross-reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic. Either the full-length protein or an antigenic peptide fragment can be used. Antibodies are preferably prepared from these regions or from discrete fragments in regions of the tyrosine kinase biomarker nucleic acid and protein sequences comprising an epitope. Polypeptide or peptide fragments that function as epitopes may be produced by any conventional means. (See, e.g., Houghten, *Proc. Natl. Acad. Sci. USA*, 82:5131-5135 (1985); and as described in U.S. Patent No. 4,631,211).

[00185] Moreover, antibodies can also be prepared from any region of the polypeptides and peptides of the protein tyrosine kinase biomarkers, including Src kinase biomarkers as described herein. In addition, if a polypeptide is a receptor protein, antibodies can be developed against an entire receptor or portions of the receptor, for example, the intracellular carboxy terminal domain, the amino terminal extracellular domain, the entire transmembrane domain, specific transmembrane segments, any of the intracellular or extracellular loops, or any portions of these regions. Antibodies can also be developed against specific functional sites, such as the site of ligand binding, or sites that are glycosylated, phosphorylated, myristylated, or amidated, for example.

[00186] In the present invention, antigenic epitopes for generating antibodies preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acid residues. Combinations of the foregoing epitopes are included. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions

thereof, as well as any combination of two, three, four, five or more of these antigenic epitopes. Such antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., *Cell*, 37:767-778 (1984); and Sutcliffe et al., *Science*, 219:660-666 (1983)). The fragments as described herein are
5 not to be construed, however, as encompassing any fragments which may be disclosed prior to the invention.

[00187] Protein tyrosine kinase biomarker polypeptides comprising one or more immunogenic epitopes which elicit an antibody response can be introduced together with a carrier protein, such as an albumin, to an animal system (such as rabbit or
10 mouse). Alternatively, if the polypeptide is of sufficient length (e.g., at least about 15-25 amino acids), the polypeptide can be presented without a carrier. However, immunogenic epitopes comprising as few as 5 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

[00188] Epitope-bearing polypeptides of the present invention can be used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, *in vitro* immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*; and Bittle et al., *supra*). If *in vivo* immunization is used, animals can be immunized with free peptide of
20 appropriate size; however, the anti-peptide antibody titer can be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH), or tetanus toxoid (TT). For instance, peptides containing cysteine residues can be coupled to a carrier using a linker such as maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general
25 linking agent, such as glutaraldehyde.

[00189] Peptides containing epitopes can also be synthesized as multiple antigen peptides (MAPs), first described by J.P. Tam et al. (*Biomed. Pept., Proteins, Nucleic Acids*, 1(3):123-132 (1995)) and Calvo et al. (*J. Immunol.*, 150(4):1403-1412 (1993)), which are hereby incorporated by reference in their entirety herein. MAPs contain
30 multiple copies of a specific peptide attached to a non-immunogenic lysine core. MAP peptides usually contain four or eight copies of the peptide, which are often referred to as MAP4 or MAP8 peptides. By way of non-limiting example, MAPs can

be synthesized onto a lysine core matrix attached to a polyethylene glycol-polystyrene (PEG-PS) support. The peptide of interest is synthesized onto the lysine residues using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry. For example, Applied Biosystems (Foster City, CA) offers commercially available MAP resins, such as, for example, the Fmoc Resin 4 Branch and the Fmoc Resin 8 Branch which can be used to synthesize MAPs. Cleavage of MAPs from the resin is performed with standard trifluoroacetic acid (TFA)-based cocktails known in the art. Purification of MAPs, except for desalting, is not generally necessary. MAP peptides can be used in immunizing vaccines which elicit antibodies that recognize both the MAP and the native protein from which the peptide was derived.

[00190] Epitope-bearing peptides of the invention can also be incorporated into a coat protein of a virus, which can then be used as an immunogen or a vaccine with which to immunize animals, including humans, in order stimulate the production of anti-epitope antibodies. For example, the V3 loop of the gp120 glycoprotein of the human immunodeficiency virus type 1 (HIV-1) has been engineered to be expressed on the surface of rhinovirus. Immunization with rhinovirus displaying the V3 loop peptide yielded apparently effective mimics of the HIV-1 immunogens (as measured by their ability to be neutralized by anti-HIV-1 antibodies as well as by their ability to elicit the production of antibodies capable of neutralizing HIV-1 in cell culture). This techniques of using engineered viral particles as immunogens is described in more detail in Smith et al., *Behring Inst Mitt Feb*, (98):229-239 (1997); Smith et al., *J. Virol.*, 72:651-659 (1998); and Zhang et al., *Biol. Chem.*, 380:365-374 (1999)), which are hereby incorporated by reference herein in their entirety.

[00191] Moreover, polypeptides or peptides containing epitopes according to the present invention can be modified, for example, by the addition of amino acids at the amino- and/or carboxy-terminus of the peptide. Such modifications are performed, for example, to alter the conformation of the epitope bearing polypeptide such that the epitope will have a conformation more closely related to the structure of the epitope in the native protein. An example of a modified epitope-bearing polypeptide of the invention is a polypeptide in which one or more cysteine residues have been added to the polypeptide to allow for the formation of a disulfide bond between two cysteines, thus resulting in a stable loop structure of the epitope-bearing polypeptide under non-

reducing conditions. Disulfide bonds can form between a cysteine residue added to the polypeptide and a cysteine residue of the naturally-occurring epitope, or between two cysteines which have both been added to the naturally-occurring epitope-bearing polypeptide.

5 **[00192]** In addition, it is possible to modify one or more amino acid residues of the naturally-occurring epitope-bearing polypeptide by substitution with cysteines to promote the formation of disulfide bonded loop structures. Cyclic thioether molecules of synthetic peptides can be routinely generated using techniques known in the art, e.g., as described in PCT publication WO 97/46251, incorporated in its entirety by
10 reference herein. Other modifications of epitope-bearing polypeptides contemplated by this invention include biotinylation.

[00193] For the production of antibodies *in vivo*, host animals, such as rabbits, rats, mice, sheep, or goats, are immunized with either free or carrier-coupled peptides or MAP peptides, for example, by intraperitoneal and/or intradermal injection. Injection
15 material is typically an emulsion containing about 100 µg of peptide or carrier protein and Freund's adjuvant, or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer
20 of anti-peptide antibodies in serum from an immunized animal can be increased by selection of anti-peptide antibodies, e.g., by adsorption of the peptide onto a solid support and elution of the selected antibodies according to methods well known in the art.

[00194] As one having skill in the art will appreciate, and as discussed above, the
25 tyrosine kinase biomarker polypeptides of the present invention, which include the following: e.g., members of the Src family of tyrosine kinases, such as Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors, and which comprise an immunogenic or antigenic epitope, can be fused to other polypeptide sequences. For example, the
30 polypeptides of the present invention can be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgD, or IgM), or portions thereof, e.g., CH1, CH2, CH3, or any combination thereof, and portions thereof, or with albumin (including,

but not limited to, recombinant human albumin, or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969; EP Patent No. 0413622; and U.S. Patent No. 5,766,883, incorporated by reference in their entirety herein), thereby resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may
5 increase half-life *in vivo*. This has been shown for chimeric proteins containing the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (see, e.g., Traunecker et al., *Nature*, 331:84-86 (1988)).

[00195] Enhanced delivery of an antigen across the epithelial barrier to the
10 immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner, such as IgG or Fc fragments (see, e.g., WO 96/22024 and WO 99/04813). IgG fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than are monomeric polypeptides, or fragments
15 thereof, alone. (See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995)).

[00196] Nucleic acids encoding epitopes can also be recombined with a polynucleotide of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system for the ready purification of non-denatured fusion proteins expressed in
20 human cell lines has been described by Janknecht et al., (*Proc. Natl. Acad. Sci. USA*, 88:8972- 8976 (1991)). In this system, the polynucleotide of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the polynucleotide is translationally fused to an amino-terminal tag having six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts
25 from cells infected with the recombinant vaccinia virus are loaded onto an Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

[00197] Additional fusion proteins of the invention can be generated by employing the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling can be
30 employed to modulate the activities of polypeptides of the invention; such methods can be used to generate polypeptides with altered activity, as well as agonists and

antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., *Curr. Opinion Biotechnol.*, 8:724-733 (1997); Harayama, *Trends Biotechnol.*, 16(2):76-82 (1998); Hansson et al., *J. Mol. Biol.*, 287:265-276 (1999); and Lorenzo and Blasco, *Biotechniques*, 24(2):308-313 (1998), the contents of each of which are hereby incorporated by reference in its entirety.

[00198] In an embodiment of the invention, alteration of polynucleotides corresponding to one or more of the src biomarker polynucleotide sequences as set forth in Table 2, and the polypeptides encoded by these polynucleotides, can be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or their encoded polypeptides, may be altered by being subjected to random mutapolynucleotidesis by error-prone PCR, random nucleotide insertion, or other methods, prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of this invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[00199] Another aspect of the present invention relates to antibodies and T-cell antigen receptors (TCRs), which immunospecifically bind to a polypeptide, polypeptide fragment, or variant one or more of the src biomarker amino acid sequences as set forth in Table 2, and/or an epitope thereof, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding).

[00200] A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods, including fusion of hybridomas or linking of Fab' fragments. (See, e.g., Songsivilai & Lachmann, *Clin. Exp. Immunol.*, 79:315-321 (1990); Kostelny et al., *J. Immunol.*, 148:1547-1553 (1992)). In addition, bispecific antibodies can be formed as "diabodies" (See, Holliger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)), or "Janusins" (See,

Traunecker et al., *EMBO J.*, 10:3655-3659 (1991) and Traunecker et al., *Int. J. Cancer Suppl.* 7:51-52 (1992)).

[00201] Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain
5 antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), intracellularly made antibodies (i.e., intrabodies), and epitope-binding fragments of any of the above. The term "antibody", as used herein, refers to immunoglobulin molecules and immunologically active portions or
10 fragments of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class or subclass (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) of immunoglobulin molecule. Preferably, immunoglobulin is an IgG1, an IgG2, or an
15 IgG4 isotype.

[00202] Immunoglobulins may have both a heavy and a light chain. An array of IgG, IgE, IgM, IgD, IgA, and IgY heavy chains can be paired with a light chain of the kappa or lambda types. Most preferably, the antibodies of the present invention are human antigen-binding antibodies and antibody fragments and include, but are not
20 limited to, Fab, Fab' F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Antigen-binding antibody fragments, including single-chain antibodies, can comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, and CH1, CH2, and CH3 domains. Also included in
25 connection with the invention are antigen-binding fragments comprising any combination of variable region(s) with a hinge region, and CH1, CH2, and CH3 domains. The antibodies of the invention can be from any animal origin including birds and mammals. Preferably, the antibodies are of human, murine (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken origin. As used
30 herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that

do not express endogenous immunoglobulins, as described *infra* and, for example, in U.S. Patent No. 5,939,598.

[00203] The antibodies of the present invention can be monospecific, bispecific, trispecific, or of greater multispecificity. Multispecific antibodies can be specific for
5 different epitopes of a polypeptide of the present invention, or can be specific for both a polypeptide of the present invention, and a heterologous epitope, such as a heterologous polypeptide or solid support material. (See, e.g., WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt et al., *J. Immunol.*, 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; and
10 Kostelny et al., *J. Immunol.*, 148:1547-1553 (1992)).

[00204] Antibodies of the present invention can be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) can be specified, e.g., by N-terminal and C-terminal positions, by size in contiguous amino
15 acid residues, or as presented in the sequences defined in Table 2 herein. Further included in accordance with the present invention are antibodies which bind to polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent, or moderately stringent, hybridization conditions as described herein.

20 [00205] The antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) can bind immunospecifically to a polypeptide or polypeptide fragment or to a variant human protein tyrosine kinase biomarker of the invention, e.g., the Src biomarker proteins as set forth in Table 2, and/or monkey src biomarker protein.

25 [00206] By way of non-limiting example, an antibody can be considered to bind to a first antigen preferentially if it binds to the first antigen with a dissociation constant (Kd) that is less than the antibody's Kd for the second antigen. In another non-limiting embodiment, an antibody can be considered to bind to a first antigen preferentially if it binds to the first antigen with an affinity that is at least one order of magnitude less
30 than the antibody's Ka for the second antigen. In another non-limiting embodiment, an antibody can be considered to bind to a first antigen preferentially if it binds to the

first antigen with an affinity that is at least two orders of magnitude less than the antibody's K_d for the second antigen.

[00207] In another nonlimiting embodiment, an antibody may be considered to bind to a first antigen preferentially if it binds to the first antigen with an off rate (koff) that is less than the antibody's koff for the second antigen. In another nonlimiting embodiment, an antibody can be considered to bind to a first antigen preferentially if it binds to the first antigen with an affinity that is at least one order of magnitude less than the antibody's koff for the second antigen. In another nonlimiting embodiment, an antibody can be considered to bind to a first antigen preferentially if it binds to the first antigen with an affinity that is at least two orders of magnitude less than the antibody's koff for the second antigen.

[00208] Antibodies of the present invention can also be described or specified in terms of their binding affinity to a tyrosine kinase biomarker polypeptide of the present invention, e.g., members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors. Preferred binding affinities include those with a dissociation constant or K_d of less than 5×10^{-2} M, 1×10^{-2} M, 5×10^{-3} M, 1×10^{-3} M, 5×10^{-4} M, or 1×10^{-4} M. More preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-5} M, 1×10^{-5} M, 5×10^{-6} M, 1×10^{-6} M, 5×10^{-7} M, 1×10^{-7} M, 5×10^{-8} M, or 1×10^{-8} M. Even more preferred antibody binding affinities include those with a dissociation constant or K_d of less than 5×10^{-9} M, 1×10^{-9} M, 5×10^{-10} M, 1×10^{-10} M, 5×10^{-11} M, 1×10^{-11} M, 5×10^{-12} M, 1×10^{-12} M, 5×10^{-13} M, 1×10^{-13} M, 5×10^{-14} M, 1×10^{-14} M, 5×10^{-15} M, or 1×10^{-15} M.

[00209] In specific embodiments, antibodies of the invention bind to the protein tyrosine kinase biomarker polypeptides, or fragments, or variants thereof, with an off rate (koff) of less than or equal to about $5 \times 10^{-2} \text{ sec}^{-1}$, $1 \times 10^{-2} \text{ sec}^{-1}$, $5 \times 10^{-3} \text{ sec}^{-1}$, or $1 \times 10^{-3} \text{ sec}^{-1}$. More preferably, antibodies of the invention bind to src biomarker protein polypeptides or fragments or variants thereof with an off rate (koff) of less than or equal to about $5 \times 10^{-4} \text{ sec}^{-1}$, $1 \times 10^{-4} \text{ sec}^{-1}$, $5 \times 10^{-5} \text{ sec}^{-1}$, $1 \times 10^{-5} \text{ sec}^{-1}$, $5 \times 10^{-6} \text{ sec}^{-1}$, $1 \times 10^{-6} \text{ sec}^{-1}$, $5 \times 10^{-7} \text{ sec}^{-1}$, or $1 \times 10^{-7} \text{ sec}^{-1}$.

[00210] In other embodiments, antibodies of the invention bind to protein tyrosine kinase biomarker polypeptides or fragments or variants thereof with an on rate (k_{on}) of greater than or equal to $1 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$, $1 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$, or $5 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$. More preferably, antibodies of the invention bind to protein tyrosine kinase biomarker polypeptides or fragments or variants thereof with an on rate greater than or equal to $1 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$, $1 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$, or $1 \times 10^7 \text{ M}^{-1} \text{ sec}^{-1}$.

[00211] The present invention also provides antibodies that competitively inhibit the binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays as described herein. In preferred embodiments, the antibody competitively inhibits binding to an epitope by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

[00212] Antibodies of the present invention can act as agonists or antagonists of the protein tyrosine kinase biomarker polypeptides of the present invention. For example, the present invention includes antibodies which disrupt receptor/ligand interactions with polypeptides of the invention either partially or fully. The invention includes both receptor-specific antibodies and ligand-specific antibodies. The invention also includes receptor-specific antibodies which do not prevent ligand binding, but do prevent receptor activation. Receptor activation (i.e., signaling) can be determined by techniques described herein or as otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., on tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by Western Blot analysis. In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in the absence of the antibody.

[00213] In another embodiment of the present invention, antibodies that immunospecifically bind to a protein tyrosine kinase biomarker, or a fragment or variant thereof, comprise a polypeptide having the amino acid sequence of any one of the heavy chains expressed by an anti-protein tyrosine kinase biomarker antibody-expressing cell line of the invention, and/or any one of the light chains expressed by

an anti-protein tyrosine kinase biomarker antibody-expressing cell line of the invention.

[00214] In another embodiment of the present invention, antibodies that immunospecifically bind to a tyrosine kinase biomarker protein or a fragment or variant thereof, comprise a polypeptide having the amino acid sequence of any one of the V_H domains of a heavy chain expressed by an anti-protein tyrosine kinase biomarker antibody-expressing cell line, and/or any one of the V_L domains of a light chain expressed by an anti-protein tyrosine kinase biomarker antibody-expressing cell line. In preferred embodiments, antibodies of the present invention comprise the amino acid sequence of a V_H domain and V_L domain expressed by a single anti-protein tyrosine kinase biomarker protein antibody-expressing cell line. In alternative embodiments, antibodies of the present invention comprise the amino acid sequence of a V_H domain and a V_L domain expressed by two different anti-protein tyrosine kinase biomarker antibody-expressing cell lines.

[00215] Molecules comprising, or alternatively consisting of, antibody fragments or variants of the V_H and/or V_L domains expressed by an anti-protein tyrosine kinase biomarker antibody-expressing cell line that immunospecifically bind to a tyrosine kinase biomarker protein, e.g., Src tyrosine kinase, are also encompassed by the invention, as are nucleic acid molecules encoding these V_H and V_L domains, molecules, fragments and/or variants.

[00216] The present invention also provides antibodies that immunospecifically bind to a polypeptide, or polypeptide fragment or variant of a tyrosine kinase biomarker protein, e.g., a Src kinase biomarker protein, wherein such antibodies comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one, two, three, or more of the V_H CDRs contained in a heavy chain expressed by one or more anti-tyrosine kinase biomarker protein antibody expressing cell lines. In particular, the invention provides antibodies that immunospecifically bind to a tyrosine kinase biomarker protein, comprising, or alternatively consisting of, a polypeptide having the amino acid sequence of a V_H CDR1 contained in a heavy chain expressed by one or more anti-tyrosine kinase biomarker protein antibody expressing cell lines. In another embodiment, antibodies that immunospecifically bind to a tyrosine kinase biomarker protein, comprise, or alternatively consist of, a

polypeptide having the amino acid sequence of a V_H CDR2 contained in a heavy chain expressed by one or more anti-tyrosine kinase biomarker protein antibody expressing cell lines. In a preferred embodiment, antibodies that immunospecifically bind to a tyrosine kinase biomarker protein, comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a V_H CDR3 contained in a heavy chain expressed by one or more anti-tyrosine kinase biomarker protein antibody expressing cell line of the invention. Molecules comprising, or alternatively consisting of, these antibodies or antibody fragments or variants thereof that immunospecifically bind to a tyrosine kinase biomarker protein or a tyrosine kinase biomarker protein fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments and/or variants.

[00217] The present invention also provides antibodies that immunospecifically bind to a polypeptide, or polypeptide fragment or variant of a tyrosine kinase biomarker protein, e.g., a Src kinase biomarker protein, wherein the antibodies comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one, two, three, or more of the V_L CDRs contained in a heavy chain expressed by one or more anti-tyrosine kinase biomarker protein antibody expressing cell lines of the invention. In particular, the invention provides antibodies that immunospecifically bind to a tyrosine kinase biomarker protein, comprising, or alternatively consisting of, a polypeptide having the amino acid sequence of a V_L CDR1 contained in a heavy chain expressed by one or more anti-tyrosine kinase biomarker protein antibody-expressing cell lines of the invention. In another embodiment, antibodies that immunospecifically bind to a src biomarker protein, comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a V_L CDR2 contained in a heavy chain expressed by one or more anti-tyrosine kinase biomarker protein antibody-expressing cell lines of the invention. In a preferred embodiment, antibodies that immunospecifically bind to a tyrosine kinase biomarker protein, comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a V_L CDR3 contained in a heavy chain expressed by one or more anti-tyrosine kinase biomarker protein antibody-expressing cell lines of the invention. Molecules comprising, or alternatively consisting of, these antibodies, or antibody fragments or variants thereof,

that immunospecifically bind to a tyrosine kinase biomarker protein or a tyrosine kinase biomarker protein fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments and/or variants.

- 5 [00218] The present invention also provides antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants) that immunospecifically bind to a tyrosine kinase biomarker protein, polypeptide or polypeptide fragment or variant of a tyrosine kinase biomarker protein, e.g., Src tyrosine kinase, wherein the antibodies comprise, or alternatively consist of, one, two, 10 three, or more V_H CDRs, and one, two, three or more V_L CDRs, as contained in a heavy chain or light chain expressed by one or more anti-tyrosine kinase biomarker protein antibody-expressing cell lines of the invention. In particular, the invention provides antibodies that immunospecifically bind to a polypeptide or polypeptide fragment or variant of a tyrosine kinase biomarker protein, wherein the antibodies 15 comprise, or alternatively consist of, a V_H CDR1 and a V_L CDR1, a V_H CDR1 and a V_L CDR2, a V_H CDR1 and a V_L CDR3, a V_H CDR2 and a V_L CDR1, V_H CDR2 and V_L CDR2, a V_H CDR2 and a V_L CDR3, a V_H CDR3 and a V_H CDR1, a V_H CDR3 and a V_L CDR2, a V_H CDR3 and a V_L CDR3, or any combination thereof, of the V_H CDRs and V_L CDRs contained in a heavy chain or light chain immunoglobulin 20 molecule expressed by one or more anti-tyrosine kinase biomarker protein antibody-expressing cell lines of the invention. In a preferred embodiment, one or more of these combinations are from a single anti-tyrosine kinase biomarker protein antibody-expressing cell line of the invention. Molecules comprising, or alternatively consisting of, fragments or variants of these antibodies that immunospecifically bind 25 to the tyrosine kinase biomarker proteins are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments or variants.

- [00219] The present invention also provides nucleic acid molecules, generally isolated, encoding an antibody of the invention (including molecules comprising, or 30 alternatively consisting of, antibody fragments or variants thereof). In a specific embodiment, a nucleic acid molecule of the invention encodes an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants

thereof), comprising, or alternatively consisting of, a V_H domain having an amino acid sequence of any one of the V_H domains of a heavy chain expressed by an anti-tyrosine kinase biomarker protein antibody-expressing cell line of the invention and a V_L domain having an amino acid sequence of a light chain expressed by an anti-tyrosine kinase biomarker protein antibody-expressing cell line of the invention. In another embodiment, a nucleic acid molecule of the invention encodes an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), comprising, or alternatively consisting of, a V_H domain having an amino acid sequence of any one of the V_H domains of a heavy chain expressed by an anti-tyrosine kinase biomarker protein antibody-expressing cell line of the invention, or a V_L domain having an amino acid sequence of a light chain expressed by an anti-tyrosine kinase biomarker protein antibody-expressing cell line of the invention.

[00220] The present invention also provides antibodies that comprise, or alternatively consist of, variants (including derivatives) of the antibody molecules (e.g., the V_H domains and/or V_L domains) described herein, which antibodies immunospecifically bind to a tyrosine kinase biomarker protein or fragment or variant thereof, e.g., a Src tyrosine kinase polypeptide.

[00221] Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule of the invention, including, for example, site-directed mutapolynucleotidesis and PCR-mediated mutapolynucleotidesis which result in amino acid substitutions. Preferably the molecules are immunoglobulin molecules. Also preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions, relative to the reference V_H domain, V_H CDR1, V_H CDR2, V_H CDR3, V_L domain, V_L CDR1, V_L CDR2, or V_L CDR3.

[00222] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar

charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, 5 tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutapolynucleotidesis. The 10 resultant mutants can be screened for biological activity to identify mutants that retain activity.

[00223] For example, it is possible to introduce mutations only in framework regions or only in CDR regions of an antibody molecule. Introduced mutations can be silent or neutral missense mutations, i.e., have no, or little, effect on an antibody's 15 ability to bind antigen. These types of mutations can be useful to optimize codon usage, or to improve hybridoma antibody production. Alternatively, non-neutral missense mutations can alter an antibody's ability to bind antigen. The location of most silent and neutral missense mutations is likely to be in the framework regions, while the location of most non-neutral missense mutations is likely to be in the CDRs, 20 although this is not an absolute requirement. One of skill in the art is able to design and test mutant molecules with desired properties, such as no alteration in antigen binding activity or alteration in binding activity (e.g., improvements in antigen binding activity or change in antibody specificity). Following mutapolynucleotidesis, the encoded protein may routinely be expressed and the functional and/or biological 25 activity of the encoded protein can be determined using techniques described herein or by routinely modifying techniques known and practiced in the art.

[00224] In a specific embodiment, an antibody of the invention (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds to protein tyrosine kinase biomarker 30 polypeptides or fragments or variants thereof, comprises, or alternatively consists of, an amino acid sequence encoded by a nucleotide sequence that hybridizes to a nucleotide sequence that is complementary to that encoding one of the V_H or V_L

domains expressed by one or more anti-tyrosine kinase biomarker protein antibody-expressing cell lines of the invention, preferably under stringent conditions, e.g., hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45°C followed by one or more washes in 0.2 x SSC/0.1% SDS at about 50°C-
5 65°C, preferably under highly stringent conditions, e.g., hybridization to filter-bound nucleic acid in 6xSSC at about 45°C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68°C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., *Current Protocols in Molecular Biology*, Vol. 1, Green Publishing Associates,
10 Inc. and John Wiley & Sons, Inc., New York, pp. 6.3.1-6.3.6 and 2.10.3 (1989)). Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[00225] It is well known within the art that polypeptides, or fragments or variants thereof, with similar amino acid sequences often have similar structure and many of
15 the same biological activities. Thus, in one embodiment, an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds to a protein tyrosine kinase biomarker polypeptide or fragments or variants of a tyrosine kinase biomarker polypeptide, comprises, or alternatively consists of, a V_H domain having an amino acid sequence
20 that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of a V_H domain of a heavy chain expressed by an anti-tyrosine kinase biomarker protein antibody-expressing cell line of the invention.

[00226] In another embodiment, an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds to a tyrosine kinase biomarker polypeptide, or fragments or
25 variants of a tyrosine kinase biomarker protein polypeptide, comprises, or alternatively consists of, a V_L domain having an amino acid sequence that is at least
30 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of a V_L domain of a light chain

expressed by an anti-tyrosine kinase biomarker protein antibody-expressing cell line of the invention.

[00227] The present invention also provides antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof),
5 that down-regulate the cell-surface expression of a tyrosine kinase biomarker protein, as determined by any method known in the art such as, for example, FACS analysis or immunofluorescence assays. By way of a non-limiting hypothesis, such down-regulation can be the result of antibody-induced internalization of a tyrosine kinase biomarker protein. Such antibodies can comprise, or alternatively consist of, a portion
10 (e.g., V_H CDR1, V_H CDR2, V_H CDR3, V_L CDR1, V_L CDR2, or V_L CDR3) of a V_H or V_L domain having an amino acid sequence of an antibody of the invention, or a fragment or variant thereof.

[00228] In another embodiment, an antibody that down-regulates the cell-surface expression of a tyrosine kinase biomarker protein comprises, or alternatively consists
15 of, a polypeptide having the amino acid sequence of a V_H domain of an antibody of the invention, or a fragment or variant thereof and a V_L domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that down-regulates the cell-surface expression of a tyrosine kinase biomarker protein comprises, or alternatively consists of, a polypeptide having the amino acid sequence
20 of a V_H domain and a V_L domain from a single antibody (or scFv or Fab fragment) of the invention, or fragments or variants thereof. In another embodiment, an antibody that down-regulates the cell-surface expression of a tyrosine kinase biomarker protein comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a V_H domain of an antibody of the invention, or a fragment or variant thereof. In
25 another embodiment, an antibody that down-regulates the cell-surface expression of a tyrosine kinase biomarker protein comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a V_L domain of an antibody of the invention, or a fragment or variant thereof.

[00229] In a preferred embodiment, an antibody that down-regulates the cell-
30 surface expression of a tyrosine kinase biomarker protein comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a V_H CDR3 of an antibody of the invention, or a fragment or variant thereof. In another preferred

embodiment, an antibody that down-regulates the cell-surface expression of a tyrosine kinase biomarker protein comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a V_L CDR3 of an antibody of the invention, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[00230] In another preferred embodiment, an antibody that enhances the activity of a tyrosine kinase biomarker protein, or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a V_L CDR3 of an antibody of the invention, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[00231] As nonlimiting examples, antibodies of the present invention can be used to purify, detect, and target the protein tyrosine kinase polypeptides of the present invention, including both *in vitro* and *in vivo* diagnostic, detection, screening, and/or therapeutic methods. For example, the antibodies have been used in immunoassays for qualitatively and quantitatively measuring levels of src biomarker polypeptides in biological samples. (See, e.g., Harlow et al., *Antibodies : A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press (1988), which is incorporated by reference herein in its entirety).

[00232] By way of another nonlimiting example, antibodies of the invention can be administered to individuals as a form of passive immunization. Alternatively, antibodies of the present invention can be used for epitope mapping to identify the epitope(s) that are bound by the antibody. Epitopes identified in this way can, in turn, for example, be used as vaccine candidates, i.e., to immunize an individual to elicit antibodies against the naturally-occurring forms of one or more tyrosine kinase biomarker proteins.

[00233] As discussed in more detail below, the antibodies of the present invention can be used either alone or in combination with other compositions. The antibodies can further be recombinantly fused to a heterologous polypeptide at the N-or C-terminus, or chemically conjugated (including covalent and non-covalent conjugations) to polypeptides or other compositions. For example, antibodies of the present invention can be recombinantly fused or conjugated to molecules that are useful as labels in detection assays and to effector molecules such as heterologous

polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995 and EP 396,387.

[00234] The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody. For example, without limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, and the like. In addition, the antibody derivative can contain one or more non-classical amino acids.

[00235] The antibodies of the present invention can be generated by any suitable method known in the art. Polyclonal antibodies directed against an antigen or immunogen of interest can be produced by various procedures well known in the art. For example, a tyrosine kinase biomarker polypeptide or peptide of the invention can be administered to various host animals as elucidated above to induce the production of sera containing polyclonal antibodies specific for the biomarker antigen. Various adjuvants can also be used to increase the immunological response, depending on the host species; adjuvants include, but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

[00236] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art, including the use of hybridoma, recombinant and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques as known and practiced in the art and as taught, for example, in Kohler and Milstein, *Nature*, 256:495-497 (1975); Harlow et al., *Antibodies : A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press (1988); and Hammerling, et al., *Monoclonal Antibodies and T-Cell Hybridomas*,

Elsevier, N.Y., pp. 563-681 (1981), the contents of which are incorporated herein by reference in their entirety. The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any
5 eukaryotic, prokaryotic, or phage clone, and does not necessarily refer to the method by which it is produced. Techniques involving continuous cell line cultures can also be used. In addition to the hybridoma technique, other techniques include the trioma technique, the human B-cell hybridoma technique (Kozbor et al., *Immunol. Today*, 4:72 (1983)), and the EBV-hybridoma technique (Cole et al., *Monoclonal Antibodies
10 and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)).

[00237] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. As a nonlimiting example, mice can be immunized with a tyrosine kinase polypeptide or peptide of the invention, or variant thereof, or with a cell expressing the polypeptide or peptide or
15 variant thereof. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the sera of immunized mice, the spleen is harvested and splenocytes are isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP2/0 or P3X63-AG8.653 available from the ATCC[®]. Hybridomas are selected and cloned by limiting
20 dilution techniques. The hybridoma clones are then assayed by methods known in the art to determine and select those cells that secrete antibodies capable of binding to a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[00238] Another well known method for producing both polyclonal and
25 monoclonal human B cell lines is transformation using Epstein Barr Virus (EBV). Protocols for generating EBV-transformed B cell lines are commonly known in the art, such as, for example, the protocol outlined in Chapter 7.22 of *Current Protocols in Immunology*, Coligan et al., eds., John Wiley & Sons, NY (1994), which is hereby incorporated by reference herein in its entirety. The source of B cells for
30 transformation is commonly human peripheral blood, but B cells for transformation can also be obtained from other sources including, but not limited to, lymph node, tonsil, spleen, tumor tissue, and infected tissues. Tissues are generally prepared as

single cell suspensions prior to EBV transformation. In addition, T cells that may be present in the B cell samples can be either physically removed or inactivated (e.g., by treatment with cyclosporin A). The removal of T cells is often advantageous, because T cells from individuals seropositive for anti-EBV antibodies can suppress B cell
5 immortalization by EBV. In general, a sample containing human B cells is inoculated with EBV and cultured for 3-4 weeks. A typical source of EBV is the culture supernatant of the B95-8 cell line (ATCC; VR-1492). Physical signs of EBV transformation can generally be seen toward the end of the 3-4 week culture period.

[00239] By phase-contrast microscopy, transformed cells appear large, clear and
10 "hairy"; they tend to aggregate in tight clusters of cells. Initially, EBV lines are generally polyclonal. However, over prolonged periods of cell culture, EBV lines can become monoclonal as a result of the selective outgrowth of particular B cell clones. Alternatively, polyclonal EBV transformed lines can be subcloned (e.g., by limiting dilution) or fused with a suitable fusion partner and plated at limiting dilution to
15 obtain monoclonal B cell lines. Suitable fusion partners for EBV transformed cell lines include mouse myeloma cell lines (e.g., SP2/0, X63-Ag8.653), heteromyeloma cell lines (human x mouse ; e.g., SPAM-8, SBC-H20, and CB-F7), and human cell lines (e.g., GM 1500, SKO-007, RPMI 8226, and KR-4). Thus, the present invention also includes a method of generating polyclonal or monoclonal human antibodies
20 against protein tyrosine kinase polypeptides and peptides of the invention, or fragments thereof, comprising EBV-transformation of human B cells.

[00240] Antibody fragments that recognize specific epitopes can be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention can be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such
25 as papain (to produce Fab fragments) or pepsin (to produce F (ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

[00241] Antibodies encompassed by the present invention can also be generated using various phage display methods known in the art. In phage display methods,
30 functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire

or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds to the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured onto a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv, or disulfide stabilized antibody domains recombinantly fused to either the phage polynucleotide III or polynucleotide VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods*, 182:41-50 (1995); Ames et al., *J. Immunol. Methods*, 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.*, 24:952-958 (1994); Persic et al., *Gene*, 187:9-18 (1997); Burton et al., *Advances in Immunology*, 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108, each of which is incorporated herein by reference in its entirety.

[00242] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below.

[00243] Examples of techniques that can be used to produce single-chain Fvs and antibodies include those described in U.S. Patent Nos. 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology*, 203:46-88 (1991); Shu et al., *Proc. Natl. Acad. Sci. USA*, 90:7995-7999 (1993); and Skerra et al., *Science*, 240:1038-1040 (1988). For some uses, including the *in vivo* use of antibodies in humans and in *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal immunoglobulin and a human immunoglobulin constant region. Methods for producing chimeric antibodies are

known in the art. (See, e.g., Morrison, *Science*, 229:1202 (1985); Oi et al., *BioTechniques*, 4:214 (1986); Gillies et al., *J. Immunol. Methods*, 125:191-202 (1989); and U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety).

5 **[00244]** Humanized antibodies are antibody molecules from non-human species that bind to the desired antigen and have one or more complementarity determining regions (CDRs) from the nonhuman species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions are substituted with corresponding residues from the CDR and framework
10 regions of the donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding, and by sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al.,
15 U.S. Patent Nos. 5,693,762 and 5,585,089; and Riechmann et al., *Nature*, 332:323 (1988), which are incorporated herein by reference in their entireties). Antibodies can be humanized using a variety of techniques known in the art, including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089); veneering or resurfacing (EP 592,106; EP
20 519,596; Padlan, *Molecular Immunology*, 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering*, 7(6):805-814 (1994); Roguska et al., *Proc. Natl. Acad. Sci. USA*, 91:969-973 (1994); and chain shuffling (U.S. Patent No. 5,565,332).

[00245] Completely human antibodies can be made by a variety of methods known in the art, including the phage display methods described above, using antibody
25 libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[00246] Completely human antibodies are particularly desirable for therapeutic
30 treatment of human patients, so as to avoid or alleviate immune reaction to foreign protein. Human antibodies can be made by a variety of methods known in the art, including the phage display methods described above, using antibody libraries

derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

5 **[00247]** Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin polynucleotides. For example, the human heavy and light chain immunoglobulin polynucleotide complexes can be introduced randomly, or by homologous recombination, into mouse embryonic stem cells. Alternatively, the
10 human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells, in addition to the human heavy and light chain polynucleotides. The mouse heavy and light chain immunoglobulin polynucleotides can be rendered nonfunctional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular,
15 homozygous deletion of the J_H region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the
20 invention.

[00248] Thus, using such a technique, it is possible to produce useful human IgG, IgA, IgM, IgD and IgE antibodies. For an overview of the technology for producing human antibodies, see Lonberg and Huszar, *Intl. Rev. Immunol.*, 13:65-93 (1995). For a detailed discussion of the technology for producing human antibodies and human
25 monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; 5,939,598; 6,075,181; and 6,114,598, which are incorporated by reference herein in their entirety. In addition,
30 companies such as Abgenix, Inc. (Fremont, CA), Protein Design Labs, Inc. (Mountain View, CA) and Genpharm (San Jose, CA) can be engaged to provide human

antibodies directed against a selected antigen using technology similar to the above described technologies.

[00249] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection". In this approach, a
5 selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *BioTechnology*, 12:899-903 (1988)).

[00250] Further, antibodies to the protein tyrosine kinase polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic"
10 protein tyrosine kinase biomarker polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan and Bona, *FASEB J.*, 7(5):437-444 (1989) and Nissinoff, *J. Immunol.*, 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be
15 used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize the polypeptide and/or its ligand, e.g., in therapeutic regimens. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the
20 invention and/or to bind its ligands/receptors, and thereby activate or block its biological activity.

[00251] Intrabodies are antibodies, often scFvs, that are expressed from a recombinant nucleic acid molecule and are engineered to be retained intracellularly (e.g., retained in the cytoplasm, endoplasmic reticulum, or periplasm of the host
25 cells). Intrabodies can be used, for example, to ablate the function of a protein to which the intrabody binds. The expression of intrabodies can also be regulated through the use of inducible promoters in the nucleic acid expression vector comprising nucleic acid encoding the intrabody. Intrabodies of the invention can be produced using methods known in the art, such as those disclosed and reviewed in
30 Chen et al., *Hum. Polynucleotide Ther.*, 5:595-601 (1994); Marasco, W.A., *Polynucleotide Ther.*, 4:11-15 (1997); Rondon and Marasco, *Annu. Rev. Microbiol.*, 51:257-283 (1997); Proba et al., *J. Mol. Biol.*, 275:245-253 (1998); Cohen et al.,

Oncogene, 17:2445-2456 (1998); Ohage and Steipe, *J. Mol. Biol.*, 291:1119-1128 (1999); Ohage et al., *J. Mol. Biol.*, 291:1129-1134 (1999); Wirtz and Steipe, *Protein Sci.*, 8:2245-2250 (1999); and Zhu et al., *J. Immunol. Methods*, 231:207-222 (1999).

[00252] XENOMOUSE[®] Technology Antibodies in accordance with the invention
5 are preferably prepared by the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted into its genome, but that is rendered deficient in the production of endogenous murine antibodies (e.g., XENOMOUSE[®] strains available from Abgenix Inc., Fremont, CA). Such mice are capable of producing human immunoglobulin molecules and are virtually deficient in
10 the production of murine immunoglobulin molecules. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed herein.

[00253] The ability to clone and reconstruct megabase-sized human loci in YACs and to introduce them into the mouse germline provides a powerful approach to
15 elucidating the functional components of very large or crudely mapped loci, as well as generating useful models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human polynucleotide products during development, their communication with other systems, and their involvement
20 in disease induction and progression. An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig polynucleotides have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in
25 B cell development. Furthermore, such a strategy could provide an ideal source for the production of fully human monoclonal antibodies (Mabs), which is an important milestone toward fulfilling the promise of antibody therapy in human disease.

[00254] Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized monoclonal antibodies and
30 thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the

treatment of chronic and recurring human diseases, such as cancer, which require repeated antibody administrations.

[00255] One approach toward this goal was to engineer mouse strains deficient in mouse antibody production to harbor large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable polynucleotide diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human monoclonal antibodies with the desired specificity could be readily produced and selected.

[00256] This general strategy was demonstrated in connection with the generation of the first "XENOMOUSE[®] T" strains as published in 1994. See Green et al., *Nature Genetics*, 7:13-21 (1994). The XENOMOUSE[®] strains were engineered with yeast artificial chromosomes (YACS) containing 245 kb and 10, 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. *Id.* The human Ig-containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig polynucleotides. This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human monoclonal antibodies. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V polynucleotides, additional regulatory elements, and human Ig constant regions might recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through the use of megabase-sized, germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively, to produce XENOMOUSE[®] mice. See Mendez et

al., *Nature Genetics*, 15:146-156 (1997); Green and Jakobovits, *J. Exp. Med.*, 188:483-495 (1998); and Green, *Journal of Immunological Methods*, 231:11-23 (1999), the disclosures of which are hereby incorporated herein by reference.

[00257] Human anti-mouse antibody (HAMA) responses have led the industry to
5 prepare chimeric or otherwise humanized antibodies. While chimeric antibodies typically are comprised of a human constant region and a murine variable region, it is expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in treatments involving chronic or multi-dose utilizations of the antibody. Thus, it is desirable to provide fully human antibodies against protein
10 tyrosine kinase biomarker polypeptides in order to vitiate concerns and/or effects of HAMA or HACA responses.

[00258] Antibodies of the invention can be chemically synthesized or produced through the use of recombinant expression systems. Accordingly, the invention further embraces polynucleotides comprising a nucleotide sequence encoding an
15 antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, an antibody that specifically binds to a protein tyrosine kinase polypeptide of this invention, and more preferably, an antibody that binds to a polypeptide having
20 the amino acid sequence of one or more of the protein tyrosine kinase biomarker sequences, e.g., Src tyrosine kinase biomarkers, as set forth in Table 2.

[00259] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the
25 antibody can be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., *BioTechniques*, 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, the annealing and ligating of those oligonucleotides, and then the amplification of the ligated oligonucleotides by PCR.

30 [00260] Alternatively, a polynucleotide encoding an antibody can be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is

known, a nucleic acid encoding the immunoglobulin can be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, (or a nucleic acid, preferably poly A+ RNA, isolated from), any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence. Alternatively, cloning using an oligonucleotide probe specific for the particular polynucleotide sequence to be identified, e.g., a cDNA clone from a cDNA library that encodes the desired antibody can be employed. Amplified nucleic acids generated by PCR can then be cloned into replicable cloning vectors using any method well known in the art.

[00261] Once the nucleotide sequence and corresponding encoded amino acid sequence of the antibody are determined, the nucleotide sequence of the antibody can be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutapolynucleotidesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., *Molecular Cloning, A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1990); and F.M. Ausubel et al., eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1998), which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example, to create amino acid substitutions, deletions, and/or insertions.

[00262] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains can be inspected to identify the sequences of the CDRs by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions, to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs can be inserted within framework regions, e.g., into human framework regions, to humanize a non-human antibody, as described *supra*. The framework regions can be naturally occurring or consensus framework regions, and preferably, are human framework regions (see, e.g., Chothia et al., *J. Mol. Biol.*, 278:457-479 (1998), for a listing of human framework regions).

[00263] Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds to a protein tyrosine kinase biomarker polypeptide of the invention. Also preferably, as discussed *supra*, one or more amino acid substitutions can be made within the framework regions; such amino acid substitutions are performed with the goal of improving binding of the antibody to its antigen, e.g., greater antibody binding affinity. In addition, such methods can be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds.

10 Other alterations that can be made to the polynucleotide are encompassed by the present invention and are within the skill of the art.

[00264] For some uses, such as for *in vitro* affinity maturation of an antibody of the invention, it is useful to express the V_H and V_L domains of the heavy and light chains of one or more antibodies of the invention as single chain antibodies, or Fab fragments, in a phage display library using phage display methods as described *supra*. For example, the cDNAs encoding the V_H and V_L domains of one or more antibodies of the invention can be expressed in all possible combinations using a phage display library, thereby allowing for the selection of V_H/V_L combinations that bind to the protein tyrosine kinase biomarker polypeptides according to the present invention

20 with preferred binding characteristics such as improved affinity or improved off rates. In addition, V_H and V_L segments, particularly, the CDR regions of the V_H and V_L domains of one or more antibodies of the invention, can be mutated *in vitro*. Expression of V_H and V_L domains with "mutant" CDRs in a phage display library allows for the selection of V_H/V_L combinations that bind to protein tyrosine kinase

25 biomarkers, e.g., Src tyrosine kinase biomarker proteins, which are receptor polypeptides with preferred binding characteristics such as improved affinity or improved off rates.

[00265] Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it can be purified by any method known in the art for the purification of an immunoglobulin or polypeptide molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen, Protein A, and sizing column chromatography),

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centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

5 **[00266]** The present invention encompasses antibodies that are recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugated) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but can
10 occur through linker sequences. The antibodies can be specific for antigens other than polypeptides (or portions thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies can be used to target the polypeptides of the present invention to particular cell types, either *in vitro* or *in vivo*, by fusing or conjugating the polypeptides of the
15 present invention to antibodies specific for particular cell surface receptors.

[00267] The present invention further includes compositions comprising the protein tyrosine kinase biomarker polypeptides of the present invention fused or conjugated to antibody domains other than the variable region domain. For example, the polypeptides of the present invention can be fused or conjugated to an antibody Fc
20 region, or portion thereof. The antibody portion fused to a polypeptide of the present invention can comprise the constant region, hinge region, CH1 domain, CH2 domain, CH3 domain, or any combination of whole domains or portions thereof. The polypeptides can also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions of immunoglobulin molecules fused to the
25 polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. (See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851;
30 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., *Proc. Natl. Acad. Sci. USA*, 88:10535-10539 (1991); Zheng et al., *J. Immunol.*, 154:5590-5600 (1995); and Vil et al., *Proc. Natl. Acad. Sci. USA*,

89:11337-11341, which are hereby incorporated by reference herein in their entireties).

[00268] As discussed *supra*, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of one or more of the protein tyrosine kinase biomarker amino acid sequences as set forth in Table 2 can be fused or conjugated to the above antibody portions to increase the *in vivo* half life of the polypeptides, or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to one or more of the protein tyrosine kinase biomarker, e.g., src biomarker, sequences as set forth in Table 2 can be fused or conjugated to the above antibody portions to facilitate purification. For guidance, chimeric proteins having the first two domains of the human CD4 polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins have been described. (EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988)). The polypeptides of the present invention fused or conjugated to an antibody, or portion thereof, having disulfide-linked dimeric structures (due to the IgG), for example, can also be more efficient in binding and neutralizing other molecules than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995)). In many cases, the Fc portion in a fusion protein is beneficial in therapy, diagnosis, and/or screening methods, and thus can result in, for example, improved pharmacokinetic properties. (EP 232,262 A). In drug discovery, for example, human proteins, such as huIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of huIL-5. (See, Bennett et al., *J. Molecular Recognition*, 8:52-58 (1995); and Johanson et al., *J. Biol. Chem.*, 270:9459-9471 (1995)). Alternatively, deleting the Fc portion after the fusion protein has been expressed, detected, and purified may be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations.

[00269] Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide, to facilitate their purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, CA), among others, many of which are commercially available. As described in Gentz et al., *Proc.*

Natl. Acad. Sci. USA, 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin (HA) protein (Wilson et al., *Cell*, 5 37:767 (1984)) and the "flag" tag.

[00270] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure, for example, to determine the efficacy of a given 10 treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Nonlimiting examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. 15 The detectable substance can be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. (See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention).

20 [00271] Nonlimiting examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase. Nonlimiting examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; nonlimiting examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, 25 dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; a nonlimiting example of a luminescent material includes luminol; nonlimiting examples of bioluminescent materials include luciferase, luciferin, and aequorin; and nonlimiting examples of suitable radioactive material include iodine (^{125}I , ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{111}In and other radioactive isotopes of indium), 30 technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{19}F), ^{153}Sm , ^{177}Lu , Gd, radioactive Pm,

radioactive La, radioactive Yb, ^{166}Ho , ^{90}Y , radioactive Sc, radioactive Re, radioactive Re, ^{142}Pr , ^{105}Rh , and ^{97}Ru .

[00272] In specific embodiments, the protein tyrosine kinase biomarker polypeptides of the invention are attached to macrocyclic chelators useful for conjugating radiometal ions, including, but not limited to, ^{111}In , ^{177}Lu , ^{90}Y , ^{166}Ho , and ^{153}Sm , to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators attached to the protein tyrosine kinase biomarker polypeptides of the invention is ^{111}In . In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator attached to the protein tyrosine kinase biomarker polypeptides of the invention is ^{90}Y . In specific embodiments, the macrocyclic chelator is 1, 4, 7, 10-tetraazacyclododecane-N, N', N'', N'''-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the protein tyrosine kinase biomarker polypeptides of the invention via a linker molecule.

[00273] Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art. (See, for example, DeNardo et al., *Clin. Cancer Res.*, 4(10):2483-2490 (1998); Peterson et al., *Bioconjug. Chem.*, 10(4):553-557 (1999); and Zimmerman et al., *Nucl. Med. Biol.*, 26(8):943-950 (1999), which are hereby incorporated by reference in their entirety). In addition, U.S. Patent Nos. 5,652,361 and 5,756,065, which disclose chelating agents that can be conjugated to antibodies and methods for making and using them, are hereby incorporated by reference in their entirety. Though U.S. Patent Nos. 5,652,361 and 5,756,065 focus on conjugating chelating agents to antibodies, one skilled in the art can readily adapt the methods disclosed therein in order to conjugate chelating agents to other polypeptides.

[00274] Antibodies can also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. Techniques for conjugating therapeutic moieties to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting of Drugs in Cancer Therapy", *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al., eds., Alan R. Liss, Inc., pp. 243-256 (1985); Hellstrom et al.,

"Antibodies For Drug Delivery", *Controlled Drug Delivery*, 2nd Ed., Robinson et al., eds., Marcel Dekker, Inc., pp. 623-653 (1987); Thorpe, "Antibody Carriers of Cytotoxic Agents in Cancer Therapy: A Review", *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al., eds., pp. 475-506 (1985);

5 "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody In Cancer Therapy", *Monoclonal Antibodies for Cancer Detection and Therapy*, Baldwin et al., eds., Academic Press, pp. 303-316 (1985); and Thorpe et al., "The Preparation and Cytotoxic Properties of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-158 (1982). Alternatively, an antibody can be conjugated to a second

10 antibody to form an antibody heteroconjugate, e.g., as described in U.S. Patent No. 4,676,980 to Segal, which is incorporated herein by reference in its entirety. An antibody, i.e., an antibody specific for a protein tyrosine kinase biomarker polypeptide of this invention, with or without a therapeutic moiety conjugated to it, and administered alone or in combination with cytotoxic factor(s) and/or cytokine(s),

15 can be used as a therapeutic.

[00275] The antibodies of the invention can further be utilized for immunophenotyping of cell lines and biological samples. The translation product of the protein tyrosine kinase biomarker-encoding polynucleotides of the present invention can be useful as cell specific marker(s), or more specifically, as cellular

20 marker(s) that are differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, allow for the screening of cellular populations expressing the marker. Various techniques utilizing monoclonal antibodies can be employed to screen for cellular populations expressing the marker(s), including

25 magnetic separation using antibody-coated magnetic beads, "panning" with antibody(ies) attached to a solid matrix (i.e., tissue culture plate), and flow cytometry (See, e.g., U.S. Patent No. 5,985,660; Morrison et al., *Cell*, 96:737-749 (1999); and L.J. Wysocki and V.L. Sato, *Proc. Natl. Acad. Sci. USA*, 75(6):2844-2848 (1978)).

[00276] These techniques allow for the screening of particular populations of cells,

30 such as might be found with hematological malignancies (i.e., minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for

the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

[00277] Antibodies according to this invention can be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used
5 include, but are not limited to, competitive and non-competitive assay systems using techniques such as BIAcore analysis, FACS (Fluorescence Activated Cell Sorter) analysis, immunofluorescence, immunocytochemistry, Western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assays), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion
10 precipitin reactions, immunodiffusion assays, agglutination assays, complement fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known and practiced in the art (see, e.g., Ausubel et al., eds., *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York (1994), which is incorporated by
15 reference herein in its entirety). Nonlimiting, exemplary immunoassays are described briefly below.

[00278] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (i.e., 1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2,
20 1% TRASYLOL[®]) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate); adding the antibody of interest to the cell lysate; incubating for a period of time (e.g., 1 to 4 hours) at 4°C; adding protein A and/or protein G sepharose beads to the cell lysate; incubating for about 60 minutes or more at 4°C; washing the beads in lysis buffer; and resuspending the beads
25 in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, for example, Western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding
30 immunoprecipitation protocols, see, e.g., Ausubel et al., eds., *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York, at 10.16.1 (1994).

[00279] Western blot analysis generally comprises preparing protein samples; electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%-20% SDS PAGE depending on the molecular weight of the antigen); transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon; blocking the membrane in blocking solution (e.g., PBS with 3% BSA or nonfat milk); washing the membrane in washing buffer (e.g., PBS-Tween 20); blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer; washing the membrane in washing buffer; blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ^{32}P or ^{125}I) diluted in blocking buffer; washing the membrane in wash buffer; and detecting the presence of the bound antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding Western blot protocols, see, e.g., Ausubel et al., eds., *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York, at 10.8.1 (1994).

[00280] ELISAs comprise preparing antigen; coating the wells of a 96-well microtiter plate with antigen; adding to the wells the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase); incubating for a period of time; and detecting the presence of the antigen. In ELISAs, the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest bound to antigen) conjugated to a detectable compound can be added to the wells. Further, instead of coating the wells with antigen, the antibodies can be first coated onto the well. In this case, a second antibody conjugated to a detectable compound can be added to the antibody-coated wells following the addition of the antigen of interest. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected, as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs, see, e.g., Ausubel et al., eds., *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York, at 11.2.1 (1994).

[00281] The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay involving the incubation of labeled antigen (e.g., ^3H or ^{125}I), or a fragment or variant thereof, with the antibody of interest in the presence of increasing amounts of labeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a protein tyrosine kinase biomarker and the binding off rates can be determined from the data by Scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the tyrosine kinase biomarker protein is incubated with an antibody of interest conjugated to a labeled compound (e.g., a compound labeled with ^3H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody. This kind of competitive assay between two antibodies can also be used to determine if two antibodies bind to the same or different epitopes on an antigen.

[00282] In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of antibodies (including antibody fragments or variants thereof) to a tyrosine kinase biomarker protein, or fragments of a tyrosine kinase biomarker protein. Kinetic analysis comprises analyzing the binding and dissociation of antibodies from chips with immobilized tyrosine kinase biomarker protein on the chip surface.

[00283] It is to be further understood that the above-described techniques for the production, expression, isolation, and manipulation of antibody molecules, for example, by recombinant techniques involving molecular biology, as well as by other techniques related to the analysis of polynucleotides and proteins, are applicable to other polypeptide or peptide molecules of the invention as described herein, in particular, the tyrosine kinase biomarker polypeptides or peptides themselves, as applicable or warranted, in accordance with the various embodiments of this invention.

[00284] The present invention also embraces a kit for determining, predicting, or prognosing drug susceptibility or resistance by a patient having a disease, particularly a cancer or tumor, preferably, a prostate cancer or tumor. Such kits are useful in a clinical setting for use in testing patient's biopsied tumor or cancer samples, for

example, to determine or predict if the patient's tumor or cancer will be resistant or sensitive to a given treatment or therapy with a drug, compound, chemotherapy agent, or biological treatment agent. Provided in the kit are the predictor set comprising those polynucleotides correlating with resistance and sensitivity to protein tyrosine kinase modulators in a particular biological system, particularly protein tyrosine kinase inhibitors, and preferably comprising a microarray; and, in suitable containers, the modulator compounds for use in testing cells from patient tissue or patient samples for resistance/sensitivity; and instructions for use. Such kits encompass predictor set comprising those polynucleotides correlating with resistance and sensitivity to modulators of protein tyrosine kinases including members of the Src family of tyrosine kinases, for example, Src, Fgr, Fyn, Yes, Blk, Hck, Lck and Lyn, as well as other protein tyrosine kinases, including, Bcr-abl, Jak, PDGFR, c-kit and Eph receptors,

[00285] Also, as explained above, the kit is not limited to microarrays, but can encompass a variety of methods and systems by which the expression of the predictor/marker polynucleotides can be assayed and/or monitored, both at the level of mRNA and of protein, for example, via PCR assays, e.g., RT-PCR and immunoassay, such as ELISA. In kits for performing PCR, or *in situ* hybridization, for example, nucleic acid primers or probes from the sequences of one or more of the predictor polynucleotides, such as those described herein, in addition to buffers and reagents as necessary for performing the method, and instructions for use. In kits for performing immunoassays, e.g., ELISAs, immunoblotting assays, and the like, antibodies, or bindable portions thereof, to the protein tyrosine kinase biomarker polypeptides of the invention, or to antigenic or immunogenic peptides thereof, are supplied, in addition to buffers and reagents as necessary for performing the method, and instructions for use. The kits according to the present invention can also comprise predictor polynucleotides as set forth in Table 2, and/or one or more of the specific predictor polynucleotide subsets as presented in Tables 3, 4, or 5 herein.

[00286] In another embodiment, the present invention embraces the use of one or more polynucleotides among those of the predictor polynucleotides identified herein that can serve as targets for the development of drug therapies for disease treatment. Such targets may be particularly applicable to treatment of prostate diseases, such as

prostate cancers or tumors. Indeed, because these predictor polynucleotides are differently expressed in sensitive and resistant cells, their expression pattern is correlated with relative intrinsic sensitivity of cells to treatment with compounds that interact with and inhibit protein tyrosine kinases. Accordingly, the polynucleotides highly expressed in resistant cells can serve as targets for the development of drug therapies for the tumors which are resistant to protein tyrosine kinase inhibitor compounds, for example, Src tyrosine kinase inhibitors.

[00287] In another embodiment, the present invention embraces antisense and/or siRNAi reagents as specific modulators of the predictor polynucleotides of the present invention. In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in one or more of the sequences provided as SEQ ID NO:1 thru 176, or the complementary strand thereof. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, *Neurochem.*, 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, *Neurochem.*, 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., *Nucleic Acids Research*, 6:3073 (1979); Cooney et al., *Science*, 241:456 (1988); and Dervan et al., *Science*, 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

[00288] For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed *in vitro* by incubating cells with the oligoribonucleotide. A similar procedure for *in vivo* use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5' end and a HindIII site on the 3' end. Next, the pair of

oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl₂, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoRI/Hind III site of the retroviral vector PMV7 (WO 91/15580).

5 **[00289]** For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense
10 RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into receptor polypeptide. Antisense oligonucleotides may be single or double stranded. Double stranded RNA's may be designed based upon the teachings of Paddison et al., *Proc. Nat. Acad. Sci.*, 99:1443-1448 (2002); and International Publication Nos. WO 01/29058, and WO 99/32619; which are hereby
15 incorporated herein by reference.

[00290] In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic
20 acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a
25 polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature*, 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al.,
30 *Cell*, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.*, 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature*, 296:39-42 (1982)), etc.

[00291] The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence “complementary to at least a portion of an RNA” referred to herein, means a sequence
5 having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing
10 nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[00292] Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon,
15 should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5' -
20 or 3' - non- translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but
25 could be used in accordance with the invention. Whether designed to hybridize to the 5' -, 3' - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

30 [00293] The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or

phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide 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., *Proc. Natl. Acad. Sci. U.S.A.*, 86:6553-6556 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci.*, 84:648-652 (1987); PCT Publication NO: WO 88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO 89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., *BioTechniques*, 6:958-976 (1988)) or intercalating agents (see, e.g., Zon, *Pharm. Res.*, 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[00294] Double stranded RNA's may be designed based upon the teachings of Paddison et al., *Proc. Nat. Acad. Sci.*, 99:1443-1448 (2002); and International Publication Nos. WO 01/29058, and WO 99/32619; which are hereby incorporated herein by reference.

[00295] siRNA reagents are specifically contemplated by the present invention. Such reagents are useful for inhibiting expression of the polynucleotides of the present invention and may have therapeutic efficacy. Several methods are known in the art for the therapeutic treatment of disorders by the administration of siRNA reagents. One such method is described by Tiscornia et al (*Proc. Nat. Acad. Sci.*, 100(4):1844-1848 (2003)), which is incorporated by reference herein in its entirety.

[00296] The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid, wybutoxosine,

pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

- 5 **[00297]** The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

[00298] In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not
10 limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

[00299] In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded
15 hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., *Nucl. Acids Res.*, 15:6625-6641 (1987)). The oligonucleotide is a 2-0-methylribonucleotide (Inoue et al., *Nucl. Acids Res.*, 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., *FEBS Lett.*, 215:327-330 (1987)).

20 **[00300]** Polynucleotides of the invention may 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 may be synthesized by the method of Stein et al. (*Nucl. Acids Res.*, 16:3209 (1988)), methylphosphonate oligonucleotides can be
25 prepared by use of controlled pore glass polymer supports (Sarin et al., *Proc. Natl. Acad. Sci. U.S.A.*, 85:7448-7451 (1988)), etc.

[00301] While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

30 **[00302]** Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, *Science*, 247:1222-1225 (1990). While

ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5' -UG-3' . The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, *Nature*, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA corresponding to the polynucleotides of the invention; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

[00303] As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

[00304] Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e., stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

[00305] The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the

present invention may also be desirous in cases such as restenosis after balloon angioplasty.

[00306] The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

5 [00307] The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

[00308] Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of
10 a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

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15

EXAMPLES

[00309] The Examples herein are meant to exemplify the various aspects of carrying out the invention and are not intended to limit the scope of the invention in any way. The Examples do not include detailed descriptions for conventional
20 methods employed, such as in the construction of vectors, the insertion of cDNA into such vectors, or the introduction of the resulting vectors into the appropriate host. Such methods are well known to those skilled in the art and are described in numerous publications, for example, Sambrook, Fritsch, and Maniatis, *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, USA,
25 (1989).

EXAMPLE 1 – METHODS

Cell lines and cell culture

[00310] All cell lines were obtained from the American Type Culture Collection
30 (Manassas, VA), except DUCaP, which was obtained from Dr. Kenneth Pienta at University of Michigan. PWR1E and MDAPCa2b cells were grown in BRFF-HPC1 serum-free medium (AthenaES, Baltimore, MD), and all other cells were cultured in

RPMI 1640 supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 mg/ml streptomycin (Invitrogen, Carlsbad, CA). DUCaP cells were maintained in the presence of an immortalized mouse fibroblast cell line, which formed a layer beneath DUCaP cells that easily dislodged. Cells were incubated at 37°C with
5 circulation of 5% CO₂.

***In vitro* cell proliferation assay**

[00311] The effect of BMS-A (dasatinib) treatment on cell proliferation was measured using a MTS colorimetric method (Promega, Madison, WI). The optimum
10 number of cells to seed in 96-well plates to achieve linearity were determined in pilot experiments. Cells were plated at density of 2000-5000 cells/well into 96-well plates and cultured overnight. Cells were then treated with dasatinib at serially diluted concentrations. Three days later, MTS was added to the medium and quantified on a SPECTRAMAX[®] photometric plate reader (Molecular Devices, Sunnyvale, CA) at
15 490 nm. The results were plotted against drug concentrations and IC₅₀s were calculated using Prism4 software (GraphPad, San Diego, CA). The IC₅₀ was the concentration of dasatinib that would reduce cell proliferation by 50% compared to control. Minimum three independent assays were performed for each cell line. The mean ± standard deviation (SD) were calculated except for cell lines that were highly
20 resistant to the compound where accurate IC₅₀s were hard to obtain. In the latter case, the concentration of dasatinib that was able to consistently reduce cell proliferation was used as IC₅₀. The inhibition of dasatinib on cell growth was also visually confirmed under microscope. Dasatinib stock, dissolved in DMSO, was 10 mg/ml.

25 Microarray analysis

[00312] AFFYMETRIX[®] HG-U133v2 gene chips containing ~22,000 probe sets were used for gene expression profiling (Affymetrix, Santa Clara, CA). Total RNA was isolated from the cells using the RNeasy kits (Qiagen, Valencia, CA). The quality of the RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara,
30 CA). Ten µg of total RNA was used for the preparation of biotin-labeled cRNA. Chip hybridization, scanning and data acquisition were performed according to the Expression Analysis Technical Manual provided by the manufacturer.

Data analysis

[00313] The raw expression data were normalized using an RMA algorithm and analyzed in PARTEK[®] Discovery Suite software (Partek, St Louis, MO). Two
5 statistical analyses including 1-way ANOVA for comparison of gene expression between sensitive and resistant cell line groups, and Pearson correlation between gene expression level and log₂IC₅₀ values were performed to identify genes whose baseline expression levels correlated with the sensitivity to dasatinib in 16 prostate cell lines (*p* value < 0.05 in both analyses). The gene list was further narrowed down by variation
10 filters (10% coefficient of variation of gene expression values across all samples and minimum 3-fold differential expression between sensitive and resistant cell groups as defined by IC₅₀). ESTs and gene duplicates were eliminated from the final list. Gene expression profiles of drug treated (100 nM for 2 days) cell lines were compared with those of DMSO control using paired *t*-test (*p* value < 0.05). Clustering analysis was
15 performed using GeneCluster software and heatmaps were generated with red and green indicating high or low expression respectively.

Western blot analysis

[00314] Cell lysates were prepared from asynchronously growing cells using the
20 RIPA buffer supplemented with protease (Roche Diagnostics, Indianapolis, IN) and phosphatase inhibitor (Sigma, St. Louis, MO) cocktails. Protein concentration was determined using the BCA kit (Pierce, Rockford, IL). Thirty µg of lysate was loaded and resolved on NUPAGE[®] Novex 4-12% Bis-Tris gel (Invitrogen, Carlsbad, CA). The blots were probed with mouse monoclonal anti-EphA2 (Upstate Biotechnology,
25 Lake Placid, NY) and anti-tubulin antibodies (Abcam, Cambridge, MA) and developed with chemiluminescence reagent ECL PLUS[®] (GE Healthcare, Piscataway, NJ).

uPA protein ELISA assay

30 [00315] Cell were seeded in 24-well plates at density of 25,000 cells/well. Two days later, the cells were washed twice with PBS and the medium was changed to RPMI 1640 containing 0.1% FBS and different concentrations of dasatinib or

TAXOL[®]. Medium was sampled at 0, 2, 4, 8, 24, and 48 hours and immediately centrifuged at 10,000 g for 5 min. The supernatants were frozen at -80°C until analysis. Total number of cells were also quantitated using a cell counter, and the number of viable cells were assessed with Trypan Blue. The amount of uPA protein in the supernatant was determined using the uPA ELISA kit (America Diagnostica, Stamford, CT), and the concentrations of uPA secreted by 50,000 viable cells into the medium were calculated.

EXAMPLE 2 –DOWN-REGULATION OF UPA PROTEIN LEVEL IN

PLASMA CORRELATED WITH REDUCED GROWTH OF PC3 XENOGRAFT TUMORS INDUCED BY DASATINIB

[00316] In an effort to confirm that uPA can be used as a surrogate marker for reduced tumor growth, the uPA protein level in the plasma of mice bearing PC3 xenograft tumors in the absence or presence of dasatinib was examined. Xenograft tumors were established subcutaneously in nu/nu athymic mice. The mice were divided into three groups each with 8 mice after tumors had reached approximately 200mg. Two groups were treated with dasatinib at 15 or 30mg/kg respectively, and the third group as control did not receive dasatinib. Dasatinib was administered orally twice a day on a 5-day-on 2-day-off schedule for 20 doses. The tumor size measurement and blood sampling were performed prior to treatment and weekly for two weeks after treatment initiation. Tumor weight was determined using the following equation: tumor weight = (length*width²)/2. It is noted the ELISA kit used only detects human but not mouse uPA protein (data not shown). As expected, PC3 xenograft tumor growth was inhibited by dasatinib at the two dose levels tested, although 30mg/kg did not show higher tumor growth inhibition activity than the 15mg/kg. The plasma uPA level was markedly reduced following first week of dasatinib treatment especially at 30mg/kg (~30%). Although the magnitude of such down-regulation decreased as tumor progressed into second week, the trend remained. Since dasatinib in combination with paclitaxel demonstrated higher antitumor activity in preclinical models than single agent, down-regulation of uPA may occur with greater magnitude and thus may better be used as a surrogate marker

in combination studies. This finding is of particular importance when tumor measurement is unpractical such as in metastatic tumors.

[00317] These findings represent a second use for uPA expression profiles. As outlined herein, low levels of uPA expression in a sample correlate with an increased likelihood the sample will be at least partially resistant to a protein tyrosine kinase inhibitor. But, when uPA expression levels are elevated or at least normal, there is an increased likelihood the sample will be sensitive to protein tyrosine kinase inhibitor. In this instance, the cell line tested using xenograft transplantation was a sensitive cell line which responded to treatment with a protein tyrosine kinase inhibitor by showing decreased uPA expression. Such decreased expression further confirms that uPA represents a sensitive marker to protein tyrosine kinase inhibitor and may be used as a surrogate to measure efficacious treatment.

EXAMPLE 3 – PCR EXPRESSION PROFILING

[00318] RNA quantification is performed using the TAQMAN[®] real-time-PCR fluorogenic assay. The TAQMAN[®] assay is one of the most precise methods for assaying the concentration of nucleic acid templates.

[00319] RNA is prepared using standard methods, preferably, employing the RNeasy Maxi Kit commercially available from Qiagen (Valencia, CA). A cDNA template for real-time PCR can be generated using the SUPERScript[®] First Strand Synthesis system for RT-PCR. Representative forward and reverse RT-PCR primers for each of the protein tyrosine kinase biomarker polynucleotides of the present invention are provided in Table 6 (SEQ ID NO:357-887).

[00320] SYBR[®] Green real-time PCR reactions are prepared as follows: The reaction mix contains 20 ng first strand cDNA; 50 nM Forward Primer; 50 nM Reverse Primer; 0.75X SYBR[®] Green I (Sigma); 1X SYBR[®] Green PCR Buffer (50 mM Tris-HCl pH 8.3, 75 mM KCl); 10% DMSO; 3 mM MgCl₂; 300 μM each dATP, dGTP, dTTP, dCTP; 1 U PLATINUM[®] Taq DNA Polymerase High Fidelity (Cat# 11304-029; Life Technologies; Rockville, MD). Real-time PCR is performed using an Applied Biosystems 5700 Sequence Detection System. Conditions are 95°C for 10 minutes (denaturation and activation of PLATINUM[®] Taq DNA Polymerase), 40

cycles of PCR (95°C for 15 seconds, 60°C for 1 minute). PCR products are analyzed for uniform melting using an analysis algorithm built into the 5700 Sequence Detection System.

- [00321]** cDNA quantification used in the normalization of template quantity is performed using TAQMAN[®] technology. TAQMAN[®] reactions are prepared as follows: The reaction mix comprises 20 ng first strand cDNA; 25 nM GAPDH-F3, Forward Primer; 250 nM GAPDH-R1 Reverse Primer; 200 nM GAPDH-PVIC TAQMAN[®] Probe (fluorescent dye labeled oligonucleotide primer); 1X Buffer A (Applied Biosystems); 5.5 mM MgCl₂; 300 μM dATP, dGTP, dTTP, dCTP; and 1 U
- 10 AMPLITAQ GOLD[®] (Applied Biosystems). GAPDH (D-glyceraldehyde-3-phosphate dehydrogenase) is used as a control to normalize mRNA levels. Real-time TAQMAN[®] PCR is performed using an Applied Biosystems 7700 Sequence Detection System. Conditions are 95°C for 10 minutes (denaturation and activation of AMPLITAQ GOLD[®]), 40 cycles of PCR (95°C for 15 seconds, 60°C for 1 minute).
- 15 **[00322]** The sequences for the GAPDH oligonucleotides used in the TAQMAN[®] reactions are as follows:

GAPDH-F3: 5'-AGCCGAGCCACATCGCT-3' (SEQ ID NO:888);

GAPDH-R1: 5'-GTGACCAGGCGCCCAATAC-3' (SEQ ID NO:889); and

- 20 GAPDH-PVIC TAQMAN[®] Probe -VIC-5'-
CAAATCCGTTGACTCCGACCTTCACCTT-3' TAMRA (SEQ ID NO:890).

- [00323]** The Sequence Detection System generates a Ct (threshold cycle) value that is used to calculate a concentration for each input cDNA template. cDNA levels
- 25 for each polynucleotide of interest are normalized to GAPDH cDNA levels to compensate for variations in total cDNA quantity in the input sample. This is done by generating GAPDH Ct values for each cell line. Ct values for the polynucleotide of interest and GAPDH are inserted into a modified version of the $\delta\delta Ct$ equation (Applied Biosystems PRISM[®] 7700 Sequence Detection System User Bulletin #2),
- 30 which is used to calculate a GAPDH normalized relative cDNA level for each specific cDNA. The $\delta\delta Ct$ equation is as follows: relative quantity of nucleic acid

template $= 2^{\delta\delta Ct} = 2^{(\delta Ct_a - \delta Ct_b)}$, where $\delta Ct_a = Ct \text{ target} - Ct \text{ GAPDH}$, and $\delta Ct_b = Ct \text{ reference} - Ct \text{ GAPDH}$. (No reference cell line is used for the calculation of relative quantity; δCt_b is defined as 21).

5 **EXAMPLE 4 – PRODUCTION OF AN ANTIBODY DIRECTED AGAINST
PROTEIN TYROSINE KINASE BIOMARKER POLYPEPTIDES**

[00324] Anti-protein tyrosine kinase biomarker polypeptide antibodies of the present invention can be prepared by a variety of methods as detailed hereinabove. As one example of an antibody-production method, cells expressing a polypeptide of the present invention are administered to an animal as immunogen to induce the production of sera containing polyclonal antibodies directed against the expressed polypeptide. In a preferred method, the expressed polypeptide is prepared, preferably isolated and/or purified, to render it substantially free of natural contaminants using techniques commonly practiced in the art. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity for the expressed and isolated polypeptide.

[00325] In a most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof) and can be prepared using hybridoma technology as detailed hereinabove. Cells expressing the polypeptide can be cultured in any suitable tissue culture medium; however, it is frequently preferable to culture cells in Earle's modified Eagle's medium supplemented to contain 10% fetal bovine serum (inactivated at about 56°C), and supplemented to contain about 10 g/l nonessential amino acids, about 1.0 U/ml penicillin, and about 100 µg/ml streptomycin.

[00326] The splenocytes of immunized (and boosted) mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line can be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line SP2/0, available from the ATCC[®]. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described, for example, by Wands et al. (*Gastroenterology*, 80:225-232 (1981)). The hybridoma cells obtained through such a selection process

are then assayed to identify those cell clones that secrete antibodies capable of binding to the polypeptide immunogen, or a portion thereof.

[00327] Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain a second antibody that binds to a first antibody. In accordance with this method, protein-specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an immunized animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones that produce an antibody whose ability to bind to the protein-specific antibodies can be blocked by the protein. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce the formation of further protein-specific antibodies.

[00328] For *in vivo* use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known and practiced in the art. (See, e.g., for review, Morrison, *Science*, 229:1202 (1985)); Oi et al., *BioTechniques*, 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 87/02671; Boulianne et al., *Nature*, 312:643 (1984); and Neuberger et al., *Nature*, 314:268 (1985)).

EXAMPLE 5 – IMMUNOFLUORESCENCE ASSAYS

[00329] The following immunofluorescence protocol can be used, for example, to verify protein tyrosine kinase biomarker expression in cells, or, for example, to check for the presence of one or more antibodies that bind protein tyrosine kinase biomarkers (polypeptides or peptides) expressed on the surfaces of cells. Briefly, LAB-TEK[®] II chamber slides are coated overnight at 4°C with 10 µg/ml of bovine collagen Type II in DPBS containing calcium and magnesium (DPBS++). The slides are then washed twice with cold DPBS++ and seeded with approximately 8000 CHO cells transfected with a vector comprising the coding sequence for a protein tyrosine

kinase biomarker of the present invention or with CHO cells transfected with vector alone (control) in a total volume of 125 μ l and incubated at 37°C in the presence of 95% oxygen / 5% carbon dioxide.

[00330] Thereafter, the culture medium is gently removed by aspiration and the
5 adherent cells are washed twice with DPBS++ at ambient temperature. The slides are blocked with DPBS++ containing 0.2% BSA (blocker) at 0-4°C for one hour. The blocking solution is gently removed by aspiration, and 125 μ l of antibody containing solution (an antibody containing solution may be, for example, a hybridoma culture supernatant which is usually used undiluted, or serum/plasma which is usually
10 diluted, e.g., a dilution of about 1:50, 1:100, 1:1000, and the like). The slides are incubated for 1 hour at 0-4°C. Antibody solutions are then gently removed by aspiration and the cells are washed 5 times with 400 μ l of ice cold blocking solution. Next, 125 μ l of 1 μ g/ml rhodamine labeled secondary antibody (e.g., anti-human IgG) in blocker solution is added to the cells. Again, cells are incubated for 1 hour at 0-
15 4°C.

[00331] The secondary antibody solution is then gently removed by aspiration and the cells are washed 3 times with 400 μ l of ice cold blocking solution, and 5 times with cold DPBS++. The cells are then fixed with 125 μ l of 3.7% formaldehyde in DPBS++ for 15 minutes at ambient temperature. Thereafter, the cells are washed 5
20 times with 400 μ l of DPBS++ at ambient temperature. Finally, the cells are mounted in 50% aqueous glycerol and viewed using a fluorescence microscope using rhodamine filters.

EXAMPLE 6 – COMPLIMENTARY SEQUENCES.

25 [00332] Antisense molecules or nucleic acid sequences complementary to the protein tyrosine kinase biomarker polypeptides-encoding sequence, or any part thereof, is used to decrease or to inhibit the expression of naturally occurring protein tyrosine kinase biomarker polypeptides. Although the use of antisense or complementary oligonucleotides comprising about 15 to 35 base-pairs is described,
30 essentially the same procedure is used with smaller or larger nucleic acid sequence fragments. An oligonucleotide based on the coding sequence of protein tyrosine

kinase biomarker polypeptides, as depicted in SEQ ID NO:1 to 356, for example, is used to inhibit expression of naturally occurring protein tyrosine kinase biomarker polypeptides. The complementary oligonucleotide is typically designed from the most unique 5' sequence and is used either to inhibit transcription by preventing promoter binding to the coding sequence, or to inhibit translation by preventing the ribosome from binding to the protein tyrosine kinase biomarker polypeptides-encoding transcript, among others. However, other regions may also be targeted.

[00333] Using an appropriate portion of the signal and 5' sequence of SEQ ID NO:1 to 356, an effective antisense oligonucleotide includes any of about 15-35 nucleotides spanning the region which translates into the signal or 5' coding sequence, among other regions, of the polypeptide as depicted in SEQ ID NO:1 to 356. Appropriate oligonucleotides may be designed using OLIGO 4.06 software and the protein tyrosine kinase biomarker polypeptides coding sequence (SEQ ID NO:1 to 356). Preferred oligonucleotides are deoxynucleotide, or chimeric deoxynucleotide/ribonucleotide based and are provided below. The oligonucleotides may be synthesized using chemistry essentially as described in U.S. Patent No. 5,849,902; which is hereby incorporated herein by reference in its entirety.

Representative RNAi reagent sequences are as follows:

Target Name	Sense Strand RNAi Reagent	SEQ ID NO:	Anti-Sense Strand RNAi Reagent	SEQ ID NO:
caveolin 1-1	CAGGGCAACAUCUACAAGCTT	891	GCUUGUAGAUGUUGCCUGTT	903
caveolin 1-2	GCAAGUGUACGACGCGCACTT	892	GUGCGCGUCGUACACUUGCTT	904
caveolin 1-3	CCGCUUGCUGUCUGCCUCTT	893	GAGGGCAGACAGCAAGCGGTT	905
caveolin 1-4	CAUCUGGGCAGUUGUACCATT	894	UGGUACAACUGCCCAGAUGTT	906
caveolin 2-1	CUACGCACUCCUUUGACAATT	895	UUGUCAAAGGAGUGCGUAGTT	907
caveolin 2-2	AGUGUGGAUCUGCAGCCAUTT	896	AUGGCUGCAGAUCCACACUTT	908
caveolin 2-3	GUUCCUGACGGUGUCCUGTT	897	CAGGAACACCGUCAGGAATT	909
caveolin 2-4	UUGCGGGAAUUCUCUUUGCTT	898	GCAAAGAGAAUCCCCGCAATT	910
ephA2-1	GGAAGUGGUACUGCUGGACTT	899	GUCCAGCAGUACCACUUCCTT	911
ephA2-2	CUUCCAGAAGCGCCUGUUCTT	900	GAACAGGCGCUUCUGGAAGTT	912
ephA2-3	GAGCCCCGUAUGCACUGUGTT	901	CACAGUGCAUACGGGGCUCTT	303
ephA2-4	CUACACCUUCACCGUGGAGTT	902	CUCCACGGUGAAGGUGUAGTT	304

20

Transfection of post-quiescent A549 cells with AntiSense Oligonucleotides

[00334] Materials needed:

- A549 cells can be maintained in DMEM with high glucose (Gibco-BRL) supplemented with 10% Fetal Bovine Serum, 2mM L-Glutamine, and 1X penicillin/streptomycin.
- Opti-MEM (Gibco-BRL)
- 5 • Lipofectamine 2000 (Invitrogen)
- Antisense oligomers (Qiagen)
- Polystyrene tubes.
- Tissue culture treated plates.

10 [00335] Quiescent cells are prepared as follows:

Day 0: 300, 000 A549 cells are seeded in a T75 tissue culture flask in 10 ml of A549 media (as specified above), and incubated in at 37°C, 5% CO₂ in a humidified incubator for 48 hours.

15 Day 2: The T75 flasks are rocked to remove any loosely adherent cells, and the A549 growth media removed and replenished with 10 ml of fresh A549 media. The cells are cultured for six days without changing the media to create a quiescent cell population.

Day 8: Quiescent cells are plated in multi-well format and transfected with antisense oligonucleotides.

20

[00336] A549 cells are transfected according to the following:

1. Trypsinize T75 flask containing quiescent population of A549 cells.
2. Count the cells and seed 24-well plates with 60K quiescent A549 cells per well.
- 25 3. Allow the cells to adhere to the tissue culture plate (approximately 4 hours).
4. Transfect the cells with antisense and control oligonucleotides according to the following:

30 a. A 10X stock of lipofectamine 2000 (10 ug/ml is 10X) may be prepared, and diluted lipid is allowed to stand at RT for 15 minutes.

Stock solution of lipofectamine 2000 is 1 mg/ml.

10 X solution for transfection is 10 ug/ml.

To prepare 10X solution, dilute 10 ul of lipofectamine 2000 stock per 1 ml of Opti-MEM (serum free media).

b. A 10X stock of each oligomer may be prepared for use in the transfection.

5 Stock solutions of oligomers are at 100 uM in 20 mM HEPES, pH 7.5.
10X concentration of oligomer may be 0.25 uM.

To prepare the 10X solutions, dilute 2.5 ul of oligomer per 1 ml of Opti-MEM.

c. Equal volumes of the 10X lipofectamine 2000 stock and the
10 10X oligomer solutions are mixed well, and incubated for 15 minutes at RT to allow complexation of the oligomer and lipid. The resulting mixture is 5X.

d. After the 15 minute complexation, 4 volumes of full growth media is added to the oligomer/lipid complexes (solution may be 1X).

e. The media may be aspirated from the cells, and 0.5 ml of the
15 1X oligomer/lipid complexes added to each well.

f. The cells are incubated for 16-24 hours at 37°C in a humidified CO₂ incubator.

g. Cell pellets are harvested for RNA isolation and TAQMAN[®]
analysis of the expression of the protein tyrosine kinase biomarker
20 polypeptides to assess level of knock-down.

TAQMAN[®] Reactions

[00337] Quantitative RT-PCR analysis may be performed on total RNA preps that are treated with DNaseI or poly A selected RNA. The Dnase treatment may be
25 performed using methods known in the art, though preferably using a Qiagen RNeasy kit to purify the RNA samples, wherein DNase I treatment is performed on the column.

[00338] Briefly, a master mix of reagents may be prepared according to the following table:

30

Dnase I Treatment

Reagent	Per r'xn (in uL)
---------	------------------

10x Buffer	2.5
Dnase I (1 unit/ul @1 unit per ug sample)	2
DEPC H ₂ O	0.5
RNA sample @ 0.1 ug/ul (2-3 ug total)	20
Total	25

- [00339] Next, 5 ul of master mix may be aliquoted per well of a 96-well PCR reaction plate (PE part # N801-0560). RNA samples are adjusted to 0.1 ug/ul with DEPC treated H₂O (if necessary), and 20 ul may be added to the aliquoted master mix for a final reaction volume of 25 ul.

[00340] The wells are capped using strip well caps (PE part # N801-0935), placed in a plate, and briefly spun in a plate centrifuge (Beckman) to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a SORVALL[®] RT is sufficient

- 10 [00341] The plates are incubated at 37°C for 30 mins. Then, an equal volume of 0.1mM EDTA in 10mM Tris may be added to each well, and heat inactivated at 70°C for 5 min. The plates are stored at -80°C upon completion.

RT reaction

- 15 [00342] A master mix of reagents may be prepared according to the following table:

RT Reaction

Reagent	RT Per Rx'n (in ul)	No RT Per Rx'n (in ul)
10x RT buffer	5	2.5
MgCl ₂	11	5.5
DNTP mixture	10	5
Random Hexamers	2.5	1.25
Rnase inhibitors	1.25	0.625
RT enzyme	1.25	-
Total RNA 500ng (100ng no RT)	19.0 max	10.125 max
DEPC H ₂ O	-	-
Total	50uL	25uL

[00343] Samples are adjusted to a concentration so that 500ng of RNA is added to each RT rx'n (100ng for the no RT). A maximum of 19 ul can be added to the RT rx'n mixture (10.125 ul for the no RT.) Any remaining volume up to the maximum values may be filled with DEPC treated H₂O, so that the total reaction volume is 50 ul
5 (RT) or 25 ul (no RT).

[00344] On a 96-well PCR reaction plate (PE part # N801-0560), 37.5 ul of master mix may be aliquoted (22.5 ul of no RT master mix), and the RNA sample added for a total reaction volume of 50ul (25 ul, no RT). Control samples are loaded into two or even three different wells in order to have enough template for generation of a
10 standard curve.

[00345] The wells are capped using strip well caps (PE part # N801-0935), placed in a plate, and spin briefly in a plate centrifuge (Beckman) to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a SORVALL[®] RT is sufficient.

15 [00346] For the RT-PCR reaction, the following thermal profile may be used:

- a. 25°C for 10 min
- b. 48°C for 30 min
- c. 95°C for 5 min
- d. 4°C hold (for 1 hour)
- 20 e. Store plate @-20°C or lower upon completion.

TAQMAN[®] reaction (Template comes from RT plate)

[00347] A master mix may be prepared according to the following table:

25

TAQMAN[®] reaction (per well)

Reagent	Per Rx'n (in ul)
TAQMAN [®] Master Mix	4.17
100 uM Probe	.025
100 uM Forward primer	.05
100 uM Reverse primer	.05
Template	-
DEPC H ₂ O	18.21
Total	22.5

[00348] Appropriate forward, reverse, and probe primers may be designed for each protein tyrosine kinase biomarker polypeptides coding region for use in the RT-PCR reaction

5 [00349] Using a GILSON[®] P-10 repeat pipetter, 22.5 ul of master mix is aliquouted per well of a 96-well optical plate. Then, using P-10 pipetter, 2.5 ul of sample is added to individual wells. Generally, RT samples are run in triplicate with each primer/probe set used, and no RT samples are run once and only with one primer/probe set, often gapdh (or other internal control).

10 [00350] A standard curve is then constructed and loaded onto the plate. The curve has five points plus one no template control (NTC, =DEPC treated H₂O). The curve may be made with a high point of 50 ng of sample (twice the amount of RNA in unknowns), and successive samples of 25, 10, 5, and 1 ng. The curve may be made from a control sample(s) (see above).

15 [00351] The wells are capped using optical strip well caps (PE part # N801-0935), placed in a plate, and spun in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a SORVALL[®] RT is sufficient.

[00352] Plates are loaded onto a PE 5700 sequence detector making sure the plate is aligned properly with the notch in the upper right hand corner. The lid may be tightened down and run using the 5700 and 5700 quantitation program and the
20 SYBR[®] probe using the following thermal profile:

50°C for 2 min

95°C for 10 min

and the following for 40 cycles:

95°C for 15 sec

25 60°C for 1 min

Change the reaction volume to 25ul.

[00353] Once the reaction may be complete, a manual threshold of around 0.1 may be set to minimize the background signal. Additional information relative to operation of the GENEAMP[®] 5700 machine may be found in reference to the following
30 manuals: "GeneAmp 5700 Sequence Detection System Operator Training CD"; and the "User's Manual for 5700 Sequence Detection System"; available from Perkin-Elmer and hereby incorporated by reference herein in their entirety.

[00354] The skilled artisan would acknowledge that modifications to the above protocol may be required for each protein tyrosine kinase biomarker polypeptide of the present invention. The skilled artisan would also acknowledge that cell lines other than A549 could be used and that A549 are only provided as an example. The skilled
5 artisan would also acknowledge that other means may be used to assess the ability of a complimentary oligonucleotide, such as the RNAi reagents provided in SEQ ID NO:534 to 557, which include, but are not limited to western blots and ELISA assays, among others.

10 **EXAMPLE 7 – ALTERNATIVE METHOD OF ASSESSING ABILITY OF
COMPLIMENTARY SEQUENCES TO MODULATE EXPRESSION LEVELS
OF THE PROTEIN TYROSINE KINASE BIOMARKER POLYPEPTIDES OF
THE PRESENT INVENTION**

[00355] Preferred complimentary sequences that may be assessed for their ability
15 to modulate the expression levels the protein tyrosine kinase biomarker polypeptides of the present invention are provided as SEQ ID NO:14 to 37. Other complimentary sequences may be designed based upon the coding region of the protein tyrosine kinase biomarker polypeptides of the present invention as provided as SEQ ID NO:1 to 356, and are specifically contemplated by the present invention.

20

Co-Transfection RNAi

Transfection:

[00356] Day prior to transfection, seed 2×10^5 HeLa cells per well of a 24 well dish. The following day, cells should be 90-95% confluent. Dilute 4.5uL of 20uM
25 stock RNAi (one or more of SEQ ID NO:14 to 37) in 50uL Optimem in a polystyrene tube for each RNAi to be transfected (tube A). Mix by gentle tapping. In another polystyrene tube combine 2uL Lipofectamine 2000 with 50 uL Optimem (tube B). Mix by gentle tapping. Allow to sit at RT for 5'. Combine 50uL tube B with the 50uL for each tube A. Mix by gentle tapping. Allow to sit at RT for 15'. Add 500uL
30 serum/antibiotic-free MEM to each tube to give a final RNAi concentration of 150nM. (For cotransfections of RNAi with plasmid, use 1uL of 20uM stock RNAi (final concentration of 33nM) along with 1ug vector DNA in tube A, and then

proceed with transfection protocol above). Remove the media from HeLa plates and replace with the 600uL transfection mix. Put in 37°C 5% CO₂ incubator for 4-5 hours. Replace the media with MEM containing 10% FBS.

- [00357] Controls to include in the transfection include a fluorescent oligonucleotide control (1U/uL=20uM) to calculate transfection efficiency, GFP B as a non-specific negative control, CDC2 as a normalizing knockdown control, and an untransfected control receiving no DNA.

Lysis:

- 10 [00358] 48 hours post-transfection, aspirate media and wash cells 1X with approx. 500uL cold 1xPBS per well. Aspirate and replace with 100uL cold RIPA containing protease inhibitors (1 mini BM protease inhibitor tablet/10mL 1x RIPA). Rock and tap the plate a few times and place at 4°C for 10-15 minutes. Tap/rock the plate several more times. Using a 200uL pipetteman, aspirate 5-10 times and wash the well
- 15 to ensure complete lysis and transfer of all material. Transfer lysate to an eppendorf tube and pipette up and down 5-10 times. If sample is still viscous, pipette up and down several more times. Spin samples down for 10' at 14000 RPM 4°C. Samples can now be stored at -20°C or prepared for loading.

20 **Western blotting/Novex:**

- [00359] Prepare sample by combining 20uL lysate with 3uL reducing reagent and 7uL 4X gel loading dye. Heat at 70°C for 10' and then place samples on ice. While samples are heating, prepare desired gel (usually a 4-12% Bis-Tris gel) by removing comb and sealing tape. Place gels in gel box and fill inner and outer chambers with
- 25 desired buffer (either 1x MES or MOPS- Add 50mL 20x buffer to 950mL dH₂O for each gel box).. Add 600uL Oxidizing reagent to the inner chamber. Wash out each well by blasting with 500uL buffer. In well one, load 5uL marker- Invitrogen's SEEBLUE[®] Plus2. Load samples in subsequent lanes. Run gel at 200V for 45-50 minutes. Make up 1X transfer buffer- 50mL 20x transfer buffer, Methanol (100mL if
- 30 transferring one gel, 200mL if transferring 2 gels in the same apparatus) and dH₂O to 1000mL. Soak blotting pads in dH₂O and then transfer buffer- make sure to push down on pads to rid of air bubbles. Soak precut Hybond-ECL membrane (Amersham

nitrocellulose) in dH2O and then in transfer buffer. Cut the end off of Biorad filter paper to match size of transfer membrane. If transferring one gel, place 2 blotting pads into blotting chamber. For 2 gels, place down 1 pad. Briefly soak a filter paper in transfer buffer and carefully lay on blotting pad. Open gel cassette with cracking tool,
5 cut off top, bottom and sides of gel. Briefly rinse it in transfer buffer and then lay it on filter paper carefully making sure no air bubbles are present. Lay transfer membrane on top again being careful there are no bubbles. Put down filter paper. Put down 2 blotting pads if transferring one gel to complete the sandwich. If transferring 2, put down 1 blotting pad, filter paper, gel, membrane, filter paper, blotting pad. Gels
10 are now ready for transfer. Squeeze together the gel sandwich and place in transfer apparatus. Fill inner and outer chambers with transfer buffers. Transfer gels for 1 hour at 30V.

[00360] Remove membranes and place them in SUPERBLOCK[®] (Pierce) and rock at RT for a minimum of 1 hour-overnight. (I have stored membranes in
15 SUPERBLOCK[®] at 4°C over the weekend). Primary antibody and normalizing antibody are diluted in a 1:10 mix of SUPERBLOCK[®]:1xPBS/0.3% Tween-20. Membranes are incubated and rocked at RT in primary antibody for a minimum of 1 hour-overnight. Membranes are then washed thoroughly in 1xPBS/0.3% Tween-20. I usually give several quick rinses and then rinse 3X 5' in 1xPBS/0.3% Tween-20.
20 During the final wash, HRP-conjugated secondary antibody is diluted in 1xPBS/0.3% Tween-20. Add this to membrane and rock at RT for a minimum of 30'. Wash membranes thoroughly. I usually give several quick rinses and then rinse 3X 5' in 1xPBS/0.3% Tween-20. Membranes are removed from wash buffer and the excess buffer drained by holding the edge of the membranes on a paper towel. Membranes
25 are placed on Saran Wrap that has been smoothed on benchtop to remove air bubbles. Enough ECL reagent is added to cover the membrane for 1 minute. Remove membranes and drain off excess ECL on paper towel. Place membranes in between two transparency sheets, being careful to smooth out air bubbles.

30 **Quantitation:**

[00361] Expose membranes using FluorS-Max. Relative percent inhibition may be determined by comparing the intensity of each band with RNAi treatment to the

intensity of each band without RNAi treatment (control). Normalize lanes by dividing band of interest by normalizing band for each lane. Divide the normalized value for each treated sample by the normalized value of the control. Percent inhibition can then be calculated by using the formula $(1 - \text{above value}) \times 100$.

5

[00362] The skilled artisan would acknowledge that modifications to the above protocol may be required for each protein tyrosine kinase biomarker polypeptide of the present invention.

[00363] The contents of all patents, patent applications, published PCT applications and articles, books, references, reference manuals, abstracts, the Sequence Listing, and internet websites cited herein are hereby incorporated by reference in their entirety to more fully describe the state of the art to which the invention pertains.

[00364] As various changes can be made in the above-described subject matter without departing from the scope and spirit of the present invention, it is intended that all subject matter contained in the above description, or defined in the appended claims, be interpreted as descriptive and illustrative of the present invention. Many modifications and variations of the present invention are possible in light of the above teachings.

20

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

[00365] Incorporated herein by reference in its entirety is a Sequence Listing, comprising SEQ ID NO:1 through SEQ ID NO:912, which include nucleic acid and amino acid sequences of the protein tyrosine kinase biomarkers as presented in Table 2 herein and the nucleotide sequences of forward and reverse primer pairs for the polynucleotide markers, probes, and RNAi reagents as described herein. The Sequence Listing is contained on a compact disc, i.e., CD-ROM, three identical copies of which are filed herewith. The Sequence Listing, in IBM/PC MS-WINDOWS text format, was first created on February 1, 2007, and is 153 MB in size.

25

WHAT IS CLAIMED IS:

1. A predictor set comprising a plurality of polynucleotides whose expression pattern is predictive of the response of cells to treatment with N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide.
2. The predictor set according to Claim 1 wherein the polynucleotides are selected from the group consisting of:
 - a) the polynucleotides provided in Table 2;
 - b) the sensitive predictor polynucleotides provided in Table 2;
 - 10 c) the resistant predictor polynucleotides provided in Table 2
 - d) the polynucleotides provided in Table 3;
 - e) the sensitive predictor polynucleotides provided in Table 3;
 - f) the resistant predictor polynucleotides provided in Table 3;
 - g) the polynucleotides provided in Table 4;
 - 15 h) the sensitive predictor polynucleotides provided in Table 4;
 - i) the resistant predictor polynucleotides provided in Table 4;
 - j) the polynucleotides provided in Table 5;
 - k) the sensitive predictor polynucleotides provided in Table 5;
 - l) the resistant predictor polynucleotides provided in Table 5;
 - 20 m) CK5, PSA, AR polynucleotides;
 - n) PSA and AR polynucleotides;
 - o) CK5 polynucleotides;
 - p) uPA polynucleotides;
 - q) EPHA2 polynucleotides;
 - 25 r) PSA polynucleotides;
 - s) AR polynucleotides; and
 - t) any combination of the above polynucleotides.
3. A predictor set comprising a plurality of polypeptides whose expression pattern is predictive of the response of cells to treatment with N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide.
- 30

4. The predictor set according to Claim 3 wherein the polypeptides are selected from the group consisting of:

- a) the polypeptides provided in Table 2;
- b) the sensitive predictor polypeptides provided in Table 2;
- 5 c) the resistant predictor polypeptides provided in Table 2
- d) the polypeptides provided in Table 3;
- e) the sensitive predictor polypeptides provided in Table 3;
- f) the resistant predictor polypeptides provided in Table 3;
- 10 g) the polypeptides provided in Table 4;
- h) the sensitive predictor polypeptides provided in Table 4;
- i) the resistant predictor polypeptides provided in Table 4;
- j) the polypeptides provided in Table 5;
- k) the sensitive predictor polypeptides provided in Table 5;
- 15 l) the resistant predictor polypeptides provided in Table 5;
- m) CK5, PSA, AR polypeptides;
- n) PSA and AR polypeptides;
- o) CK5 polypeptides;
- p) uPA polypeptides;
- q) EPHA2 polypeptides;
- 20 r) PSA polypeptides;
- s) AR polypeptides; and
- t) any combination of the above polypeptides.

5. A method for predicting whether a compound is capable of modulating the activity of cells, comprising the steps of:

- 25 a) obtaining a sample of cells;
- b) determining whether said cells express a plurality of markers; and
- c) correlating the expression of said markers to the compounds ability to modulate the activity of said cells.

6. The method according to Claim 5 wherein the plurality of markers are one or more of the polynucleotides according to Claim 2.

7. The method according to Claim 9 wherein the plurality of markers are one or more of the polypeptides according to Claim 5.

8. A method of identifying polynucleotides and polypeptides that predict compound sensitivity or resistance of cells associated with a disease state, comprising the steps of:

- a) subjecting the plurality of cell lines to one or more compounds;
- 5 b) analyzing the expression pattern of a microarray of polynucleotides or polypeptides; and
- c) selecting polynucleotides or polypeptides that predict the sensitivity or resistance of cells associated with a disease state by using said expression pattern of said microarray.

10 9. A method for predicting whether an individual requiring treatment for a disease state, will successfully respond or will not respond to said treatment comprising the steps of:

- a) obtaining a sample of cells from said individual;
- b) determining whether said cells express a plurality of markers; and
- 15 c) correlating the expression of said markers to the individuals ability to respond to said treatment.

10. The method according to Claim 9 wherein the disease state is prostate cancer, breast cancer, or lung cancer.

11. A method of screening for candidate compounds capable of binding to
20 and/or modulating the activity of a protein tyrosine kinase biomarker polypeptide, comprising:

- a) contacting a test compound with a polypeptide according to Claim 4;
and
- b) selecting as candidate compounds those test compounds that bind to
25 and/or modulate activity of the polypeptide.

12. A method of treating prostate cancer in a subject, comprising administering a modulator of one or more protein tyrosine kinase biomarker polypeptides as provided in Claim 4.

13. A method of predicting whether patients may be responsive to a
30 protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, using a method selected from the group consisting of:

a) measuring the expression level of kallikrein 3 and androgen receptor in a patient sample, wherein an elevated expression level of kallikrein 3 and/or androgen receptor relative to the level observed in a standard sample, is indicative of at least partial resistance to said protein tyrosine kinase inhibitor, and wherein decreased
5 expression of kallikrein 3 and/or androgen receptor relative to the level observed in a standard sample, is indicative of sensitivity to said protein tyrosine kinase inhibitor;

b) measuring the expression level of cytokeratin 5 in a patient sample, wherein a decreased expression level of cytokeratin 5 relative to the level observed in a standard sample, is indicative of at least partial resistance to said protein tyrosine
10 kinase inhibitor, and wherein elevated expression of cytokeratin 5 relative to the level observed in a standard sample, is indicative of sensitivity to said protein tyrosine kinase inhibitor;

c) determining whether a patient sample shows signs of basal cell type phenotype for the prostatic cell lineage, wherein the presence of such signs is
15 indicative of sensitivity to said protein tyrosine kinase inhibitor;

d) measuring the expression level of kallikrein 3, androgen receptor, and cytokeratin 5 in a patient sample, wherein an elevated expression level of kallikrein 3 and/or androgen receptor relative to the level observed in a standard sample and wherein a decreased expression level relative to the level observed in a standard
20 sample, is indicative of at least partial resistance to said protein tyrosine kinase inhibitor, and wherein decreased expression of kallikrein 3 and/or androgen receptor relative to the level observed in a standard sample and wherein an elevated expression level of cytokeratin 5 relative to the level observed in a standard sample, is indicative of sensitivity to said protein tyrosine kinase inhibitor;

e) measuring the expression level of SCEL, ANXA3, CST6, LAMC2, ZBED2, EREG, AXL, FHL2, PLAU, and/or ARNTL2 in a patient sample, wherein a decreased expression level of SCEL, ANXA3, CST6, LAMC2, ZBED2, EREG, AXL, FHL2, PLAU, and/or ARNTL2 relative to the level observed in a standard sample, is
25 indicative of at least partial resistance to said protein tyrosine kinase inhibitor, and
30 wherein elevated expression of SCEL, ANXA3, CST6, LAMC2, ZBED2, EREG, AXL, FHL2, PLAU, and/or ARNTL2 relative to the level observed in a standard sample, is indicative of sensitivity to said protein tyrosine kinase inhibitor;

f) measuring the expression level of EPHA2 in a patient sample, wherein a decreased expression level of EPHA2 relative to the level observed in a standard sample, is indicative of at least partial resistance to said protein tyrosine kinase inhibitor, and wherein elevated expression of EPHA2 relative to the level observed in a standard sample, is indicative of sensitivity to said protein tyrosine kinase inhibitor; and

g) measuring the expression level of UPA in a patient sample, wherein a decreased expression level of UPA relative to the level observed in a standard sample, is indicative of at least partial resistance to said protein tyrosine kinase inhibitor, and wherein elevated expression of UPA relative to the level observed in a standard sample, is indicative of sensitivity to said protein tyrosine kinase inhibitor.

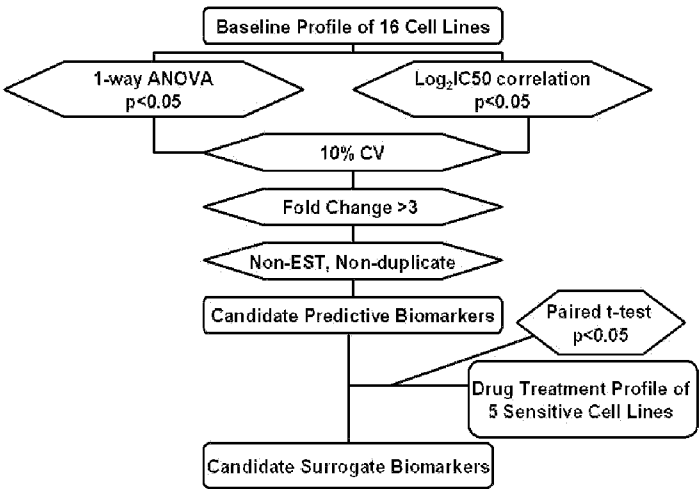
14. A method of establishing a treatment regimen for a patient suffering from cancer comprising the step of determining whether a patient is predicted to be responsive to a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide according to any one of Claims 5, 6, 7, 8, 9, 10, 11, 12, or 13, and administering a more aggressive treatment regimen if the patient sample is predicted to be resistant to said protein tyrosine kinase inhibitor, or administering a typical treatment regimen if the patient sample is predicted to be sensitive to said protein tyrosine kinase inhibitor.

15. A method of using a surrogate marker to predict the responsiveness of a patient suffering from cancer to a protein tyrosine kinase inhibitor, comprising the step of comparing the expression level of a predictive polynucleotide or polypeptide marker both prior to and subsequent to the administration of a protein tyrosine kinase inhibitor, such as N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazolecarboxamide, wherein a decrease in expression of said predictive marker subsequent to said administration relative to the absence of said administration is indicative of said patient being responsive to said protein tyrosine kinase inhibitor, wherein said predictive marker is represented by one or more sensitive markers provided in Tables 2, 3, 4, or 5.

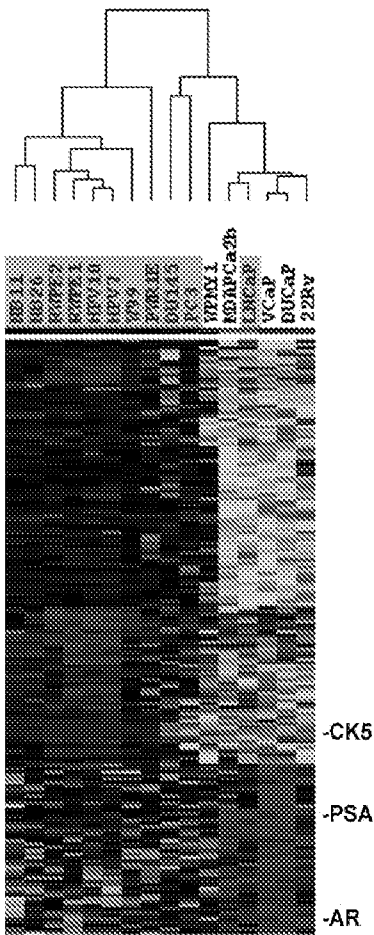
FIG. 1

Biomarker strategy and subtype identification

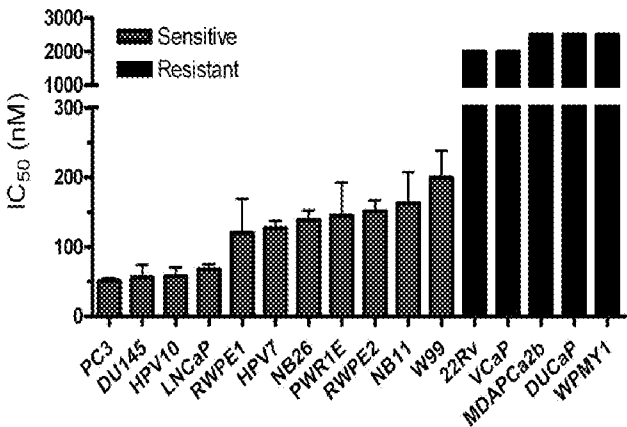
A. Biomarker discovery strategy



C. Cluster analysis with 174 genes



B. Sensitivity of prostate cell lines to Dasatinib



D. Relative expression of CK5, PSA and AR

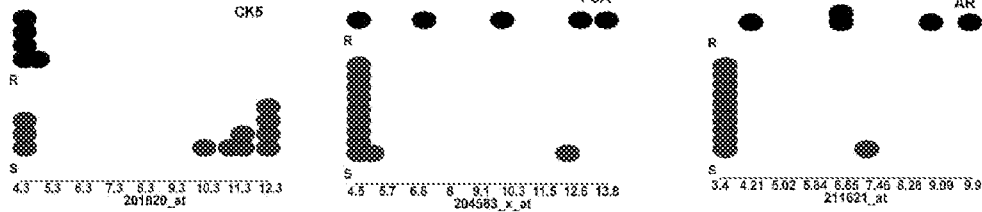


FIG. 2

Expression of EphA2 as a predictive marker

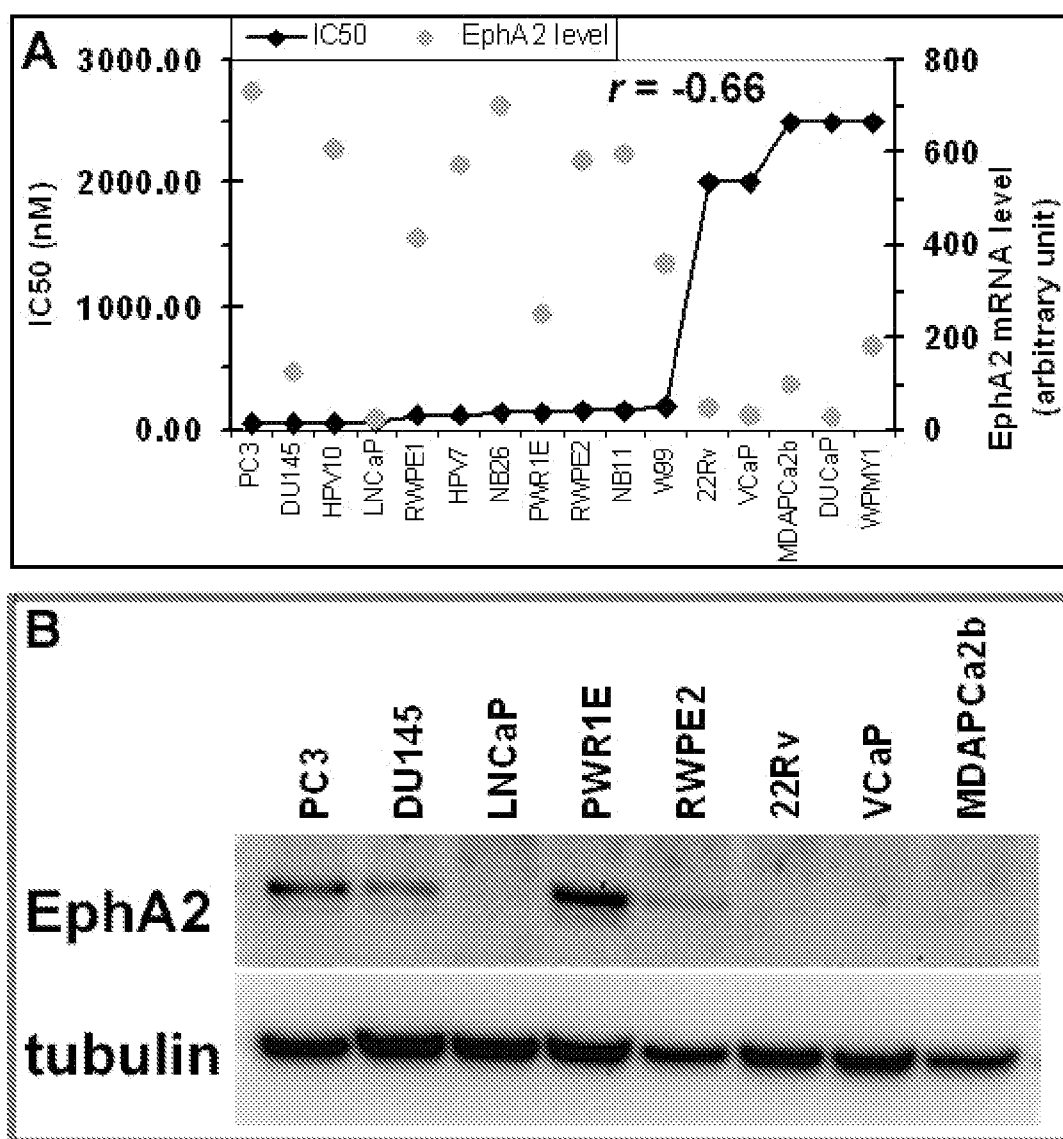


FIG. 3

Specific down-regulation of PLAU expression by Dasatinib

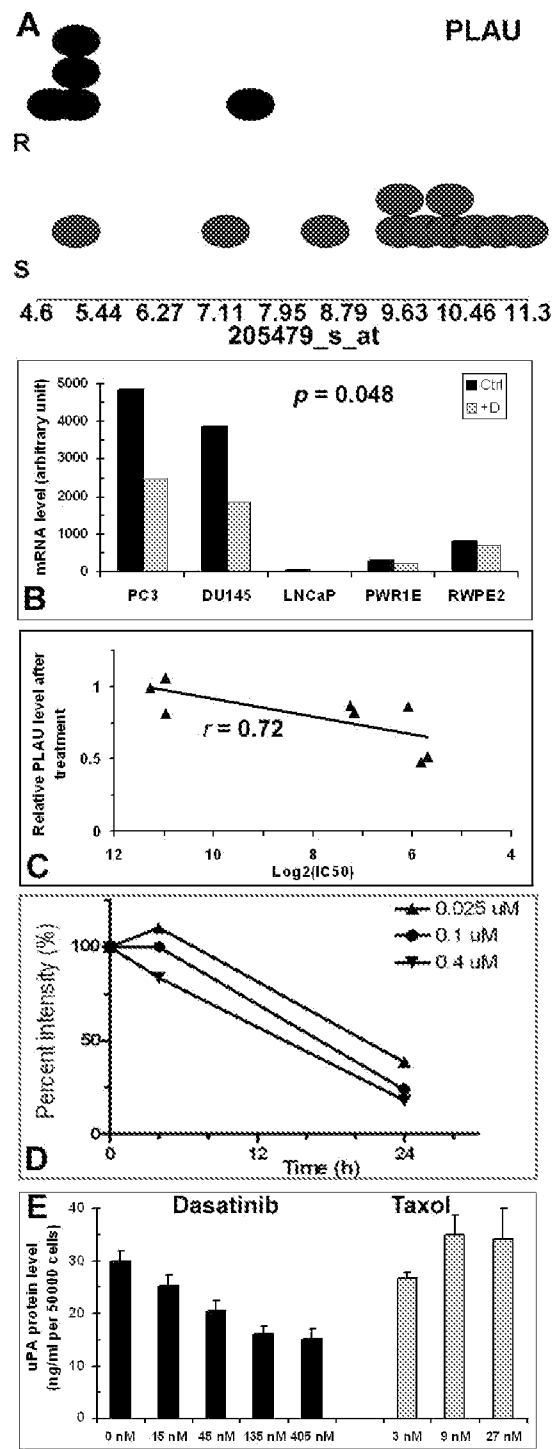


FIG. 4

Expression of 5 genes in cell lines and tumors

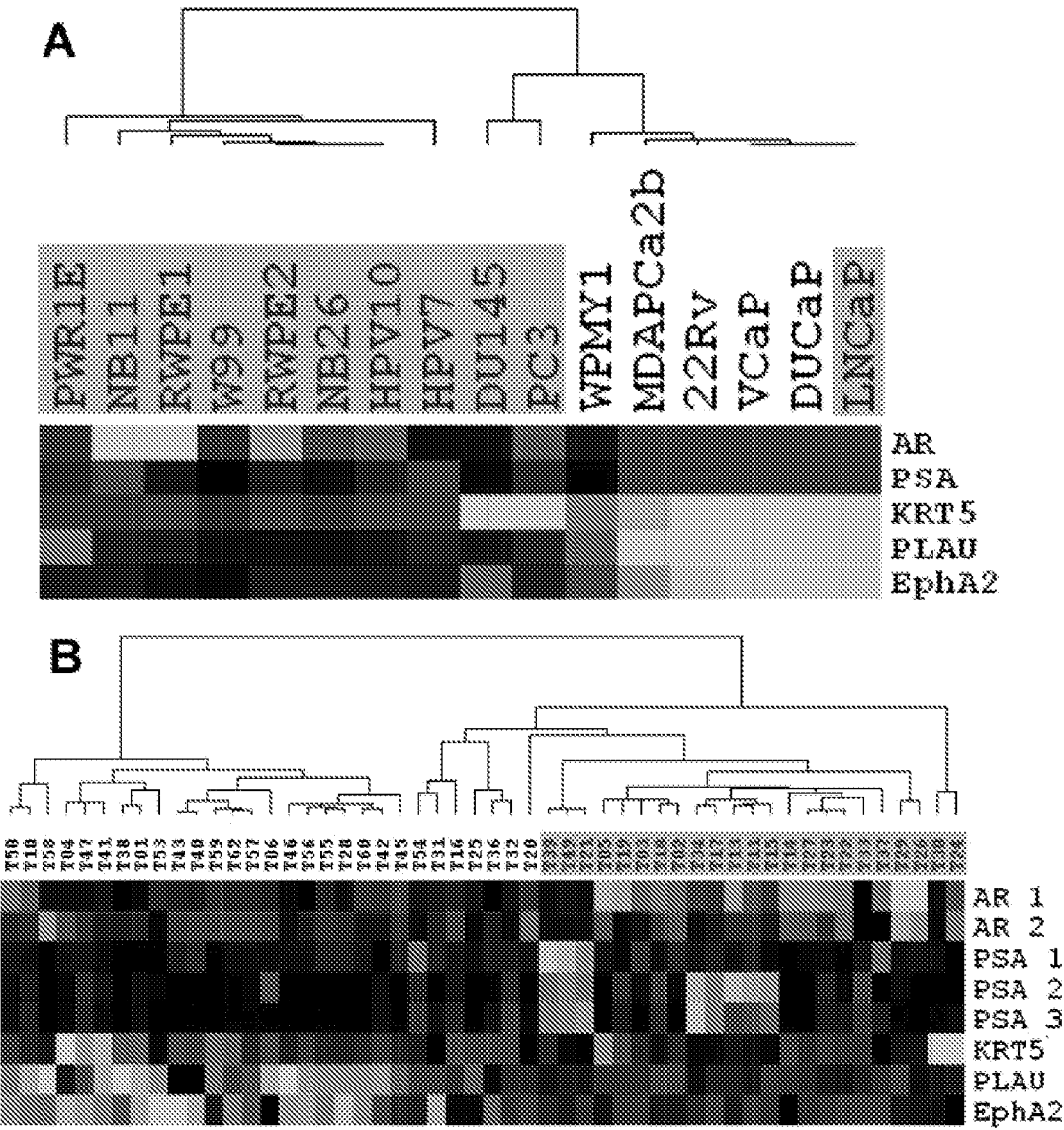


FIG. 5

Down-regulation of uPA serum level by dasatinib in PC3 xenograft

