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(54) Title: ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) BIOMARKERS

(57) Abstract: The present invention concerns the use of biomarkers for acute lymphoblastic leukemia (ALL) to prognose or evaluate a patient with ALL who is Ph+. Methods and compositions are provided that concern these ALL biomarkers. In specific embodiments, methods for determining whether an ALL patient should be treated with standard chemotherapy are provided.

DESCRIPTION

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) BIOMARKERS

BACKGROUND OF THE INVENTION

[0001] The present application claims the benefit of priority to U.S. Provisional Application Nos. 61/182,228, filed May 29, 2010, the entire contents of each of which are incorporated by reference herein.

1. Field of the Invention

[0002] The present invention relates generally to the fields of oncology and medicine. More particularly, methods and compositions involving one or more biomarkers for prognosing a patient with acute lymphoblastic leukemia (ALL), particularly ALL characterized by the presence of the Philadelphia chromosome (Ph+). ALL patients who are Ph+ may be evaluated using biomarkers to determine an appropriate course of treatment based on the likelihood that they will respond to chemotherapy.

2. Description of Related Art

[0003] Acute lymphoblastic leukemia (ALL) is characterized by different genetic characteristics. About 30-40% of adult ALL have the Philadelphia chromosome (Ph), resulting from BCR/ABL gene fusion. Ph positivity has a very negative prognostic impact in ALL. In about half of the Ph+ ALL patients, conventional chemotherapies fail to induce complete remission (CR), whereas in Ph- ALL, the CR rate is 80-90%. The 5-year overall survival (OS) rate for Ph+ ALL is only 0% to 30%. Combination of chemotherapy with imatinib, a tyrosine kinase inhibitor, has improved the CR rate in Ph+ ALL to the similar level as seen in Ph- ALL. However, up to 30% of patients are primary refractory. Among the initially responsive patients, 20-30% of them still relapse in a short time (a few months) and eventually die of the disease. Allogeneic stem cell transplantation (alloSCT) in first CR is potentially curative, but its value is profoundly limited by high primary resistance rate and rapid development of acquired resistance. Therefore, it is crucial to identify those patients who would most likely to respond poorly to chemotherapies and prone to relapse, and offer them alternative therapeutic options.

[0004] The description herein addresses this issue by providing methods and compositions that help identify those patients through the use of biomarkers.

SUMMARY OF THE INVENTION

[0005] A number of biomarkers that concern the prognosis of ALL patients have been identified. These ALL biomarkers are biological indicators that reflect a patient's state, including the likelihood of effective therapy or the risk of relapse or non-response to therapy. At least nine biomarkers have been identified, any one of which can be used singly or in combination to evaluate the likelihood that an ALL Ph+ patient will respond to standard chemotherapy. The term "standard chemotherapy" refers to a standard course of treatment with chemotherapeutic agents. Typically, standard chemotherapy involves multiple courses of therapy that may last days or weeks. This is in contrast to chemotherapy that is administered to prepare a patient for a bone marrow or cord blood transplant. An "ALL biomarker" refers to SLC2A3, ITPR1, TCF4, FLT3, CD69, NPM1, SPRY2, TP53, or PTGS1 in the context of embodiments discussed herein.

[0006] In some embodiments, there are methods for evaluating a patient with acute lymphoblastic leukemia (ALL) that is characterized by the presence of Philadelphia chromosome (Ph+) or suspected of being Ph+ comprising: a) generating an expression profile from a biological sample containing leukemic cells of the patient, wherein the expression profile comprises information about expression levels of SLC2A3, ITPR1, TCF4, and FLT3; and b)comparing the expression levels in the expression profile to a standard expression level, wherein the expression levels indicate if the patient is likely to respond to standard chemotherapy, likely not to respond to standard chemotherapy, or likely to relapse within four months. Methods involve determining the level of expression of one or more biomarkers. Determinations involve employing one or more physical assays on the biological sample, and will involve chemical reactions, chemical transformations, and/or machines or apparatuses.

[0007] Other embodiments include methods of generating an expression profile from a sample from an ALL patient, methods for evaluating a sample from an ALL patient, methods for assaying a sample from an ALL patient, methods for evaluating expression levels of one or more ALL biomarkers in an ALL patient, methods for screening an ALL patient, and methods for providing information relating to determining treatment for an ALL patient. Methods involve steps and embodiments discussed herein, such as determining levels of expression of one or more ALL biomarkers.

[0008] It is specifically contemplated that patients may be human patients. Moreover, it is contemplated that an "ALL patient" is a patient diagnosed with ALL. Embodiments discussed with respect to ALL patients may be applied to patients suspected of having ALL or patients who have symptoms of ALL.

[0009] It is contemplated that "expression level" refers to mRNA expression or to protein expression. In certain embodiments, the level of mRNA is evaluated, measured, and/or determined. This may be done using any method by which mRNA expression levels are evaluated, measured, or determined. A variety of such methods are well known to those of skill in the art, and these include, but are not limited to, those involving complementary probes or primers, amplification primers, cDNAs, *etc.* Such methods may involve RT-PCR, in situ hybridization (ISH), and/or arrays or biochips for evaluating RNA expression. In other embodiments, the level of protein is evaluated, measured, and/or determined. This may be done using any method by which protein expression levels are evaluated, measured, or determined. A variety of such methods are well known to those of skill in the art, and these include, but are not limited to, those involving an antibody or antibodies specific for the protein.

[0010] In certain embodiments, the expression profile further comprises information about the expression levels of one or more of: CD69, NPM1, SPRY2, TP53, or PTGS1, or any combination thereof. In some aspects, an expression profile comprises information about the expression level of at least CD69. In other aspects, the expression profile comprises information about the expression level of at least NPM1. In further aspects, the expression profile comprises information about the expression level of at least SPRY2. In additional aspects, the expression profile comprises information about the expression level of at least TP53. In other aspects, the expression profile comprises information about the expression level of at least PTGS1. In some embodiments, the expression profile comprises information about the expression level of any of SLC2A3, ITPR1, TCF4, FLT3, CD69, NPM1, SPRY2, TP53, or PTGS1, or any combination thereof. In certain embodiments, information about the expression levels of SLC2A3, ITPR1, TCF4, FLT3, CD69, NPM1, SPRY2, TP53, and PTGS1 is obtained or determined. In other embodiments, an expression profile of SLC2A3, ITPR1, TCF4, FLT3, and CD69 is obtained or determined. It is contemplated that NPM1, SPRY2, TP53, and/or PTGS1 may also be evaluated for expression levels. In some embodiments, the expression level of at least CD69 is obtained. In further embodiments,

information about the expression level of at least NPM1 is obtained or determined. In other embodiments, information about the expression level of at least SPRY2 is obtained or determined. In additional embodiments, information about the expression level of at least TP53 is obtained or determined. Moreover, other embodiments involve obtaining or generating information about the expression level of at least PTGS1. In some aspects, information about the expression levels of CD69, NPM1, SPRY2, TP53, and PTGS1 is obtained or determined. Aspects of the invention involve processing a biological sample to generate the information discussed herein.

[0011] In some embodiments, the expression profile comprises information of expression levels of gene transcripts, that is, RNA transcripts. Some aspects involve a process involving amplification of gene products. In some cases, an array or microarray is employed to determine expression levels and/or generate an expression profile.

[0012] In other embodiments, expression levels are determined by measuring, evaluating, and/or analyzing protein levels. This may be accomplished using antibodies specific for the protein. There is no limitation as to the source or type of antibody.

[0013] Embodiments involve a patient who is an adult suspected of being Ph+. In some cases, methods involve evaluating a biological sample to determine whether the patient is Ph+. Embodiments also concern diagnosing a patient with ALL based on one or more biomarkers discussed herein.

[0014] It is contemplated that methods may be performed by individuals in the medical field. This includes doctors, nurses, physician's assistants, laboratory personnel or laboratory technicians who may also perform activities associated with these roles in the practice of methods described herein. These include ordering a test to determine expression levels of an ALL biomarker, ordering other tests be conducted on the patient, diagnosing an ALL patient, treating an ALL patient, checking a patient for toxicity of a treatment, checking a patient for therapeutic efficacy, evaluating a patient's cancer or the occurrence or state of any remission, investigating transplant donors for a patient, HLA typing of a patient, perform other cytogenetic studies on a patient, evaluating the overall health of an ALL patient, taking a patient history, and obtaining any information or results from one or more of these activities. In some cases, a report of such information is prepared and/or provided. The report may be

reviewed by a clinician or a group of clinicians who then decide a course of treatment for the patient.

[0015] In some embodiments, methods involve obtaining a biological sample from the patient prior to generating the expression profile. It is contemplated that biological samples may contain leukemic cells, which can be evaluated for ALL biomarker expression levels. In some aspects, a biological sample is enriched or screened for leukemic cells. Methods may include assessing the level of white blood cells in the patient, and/or determining whether leukemic cells of the patient have abnormal ploidy, determining whether leukemic cells of the patient exhibit an 11q23 rearrangement.

[0016] In further embodiments of the invention, a biological sample is obtained from a patient. In other embodiments of the method, the entity evaluating the sample for ALL biomarkers does not directly obtain the sample from the patient. Therefore, methods of the invention involve obtaining the sample indirectly or directly from the patient. To achieve these methods, a doctor, medical practitioner, or their staff may obtain a biological sample for evaluation. The sample may be analyzed by the practitioner or their staff, or it may be sent to an outside or independent laboratory. The medical practitioner may be cognizant of whether the test is providing information regarding a quantitative level of ALL biomarker expression, or the medical practitioner may be aware that the test indicates directly or indirectly that the test was positive or negative for expression of a particular ALL biomarker.

[0017] In some embodiments, methods also involve reporting the expression profile or preparing a report regarding an expression profile or the levels of expression for ALL biomarkers. In any of these circumstances, the medical practitioner may know the relevant information that will allow him or her to determine whether the patient should be treated with standard chemotherapy or forego standard chemotherapy. In the latter case, the patient is treated with only conditional chemotherapy prior to more aggressive therapy involving a bone marrow or cord blood transplant. Prognosis and treatment regimen are based on quantitative or qualitative information about ALL biomarker expression. It is contemplated that, for example, a laboratory conducts the test to determine whether and/or to what extent one or more ALL biomarkers is expressed as an mRNA and/or protein. Laboratory personnel may report back to the practitioner with the specific result of the test performed or the laboratory may simply report that the patient is has upregulated or downregulated expression of one or more ALL biomarkers.

[0018] In some embodiments, the level of ALL biomarker expression may be evaluated quantitatively. In these cases, methods may involve comparing the level of an ALL biomarker expression in the biological sample of a patient to the level of expression in a normal sample or to the level of expression from a certain patient population, such as optimal responders, non-responders, or all ALL patients regardless of response. In some cases, normal or leukemic cells may be obtained from the patient, though they may also be from someone other than the patient. It is contemplated that the level of expression in a control sample may be evaluated, determined, or measured at the same time as the patient's sample, or it may be a level previously determined based on one or more such samples. In cases where more than one sample is evaluated, the level of an ALL biomarker expression in a normal sample may be a normalized value against which to compare the value from the patient. It is specifically contemplated that when levels of ALL biomarker expression are compared to a normal sample that the normal sample may be from the same kind of tissue or be the same kind of sample as the patient's sample. In other words, the levels of expression in homologous samples are compared. For example, the level of ALL biomarker protein in a biological sample obtained from a patient's bone marrow could be compared to the level of ALL biomarker protein in normal bone marrow. Moreover, it is assumed that amounts of biological material may be normalized when quantitative values are compared.

[0019] Alternatively, levels may be expressed relative to an internal standard. It could be assigned a number or value according to some normalized convention. For example, the level of FLT3 transcript levels may be determined to be approximately 5000 transcripts/cell or 1 transcript per 5 transcripts of an internal standard, like GADPH. It would be compared to FLT3 transcript levels in either nonleukemic cells or leukemic cells from either optimal responders or non-responders. A non-responder may express approximately 4000-6000 transcripts of FLT3/cell or 1 transcript per 5 transcripts of the same internal standard. In this example, the sample indicates that the level of FLT3 is similar to the level seen in a non-responder. A person of ordinary skill in the art would be able to evaluate the levels of expression based on the Examples below to classify the relative expression levels of ALL biomarkers. This includes being able to classify an expression level of an ALL biomarker as underexpressed or overexpressed relative to the same biomarker in a class of patients or to a standard that is not an ALL biomarker. It is further contemplated that expression levels may first be normalized. For example, expression levels of all the ALL biomarkers may be measured. The levels could then be normalized such that the sample is said to have an

expression level, for example, of 0.1 of FLT3, which could be compared to the expression level observed across a random ALL population. That level might be, for instance, 0.02, in which FLT3 would be considered to be overexpressed in the patient's sample.

[0020] Other embodiments include methods of treating a patient with acute lymphoblastic leukemia (ALL) that is characterized by the presence of Philadelphia chromosome (Ph+) or suspected of being Ph+ comprising: a) obtaining information about the patient's expression levels of SLC2A3, ITPR1, TCF4, and FLT3 in leukemic cells of the patient; b) treating the patient for ALL based on whether the expression levels of SLC2A3, ITPR1, TCF4, and FLT3 indicate the patient is an optimal responder or non-responder to ALL chemotherapy or is likely to relapse after ALL chemotherapy. As with methods discussed above, information about other ALL biomarkers may be relevant. In some embodiments, methods involve obtaining information about the expression levels of one or more of: CD69, NPM1, SPRY2, TP53, or PTGS1. It is contemplated that one, two, three, four, or all five biomarkers are evaluated in particular embodiments. In some embodiments, the expression level of at least CD69 is obtained. In further embodiments, information about the expression level of at least NPM1 is obtained. In other embodiments, information about the expression level of at least SPRY2 is obtained. In additional embodiments, information about the expression level of at least TP53 is obtained. Moreover, other embodiments involve obtaining information about the expression level of at least PTGS1. In some aspects, information about the expression levels of CD69, NPM1, SPRY2, TP53, and PTGS1 is obtained.

[0021] Treatment of ALL may be implemented in embodiments after an evaluation of biomarkers. The treatment may follow an evaluation within days, weeks or months of obtaining the results of the evaluation. In certain embodiments, treatment is based on the evaluation and begins within 1, 2, 3, 4, 5 weeks, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, and/or 1, or 2 years of receiving those results. Moreover, it is contemplated that treatment may have already begun at the time or been completed at the time evaluation of biomarkers occurs. Treatment may be resumed or commenced again after the results of the evaluation are obtained, and they may depend on the results. It is contemplated that treatment commenced after the results are obtained may be the same, similar, or different than any previous cancer treatment. It is contemplated that treatment after the biomarker evaluation may be a modification of a previous treatment. In some embodiments, the treatment is more

aggressive than the previous treatment. In others, treatment may be less aggressive than the previous treatment. Transplantation is considered a more aggressive treatment.

[0022] In some embodiments, the expression levels indicate the patient is likely to be an optimal responder and the patient is treated with standard chemotherapy. In other embodiments, the expression levels indicate the patient is likely to be to be a non-responder or likely to relapse and the patient is not treated with standard therapeutic chemotherapy. It is contemplated that methods may involve determining that a patient is likely to be an optimal responder or a non-responder or classifying the patient as likely to be an optimal responder or a non-responder. In some embodiments, a patient is treated with a bone marrow or cord blood transplant. In certain embodiments, a patient is processed for a transplant after a medical practitioner determines the patient is a likely non-responder to standard chemotherapy. In these cases, the patient does not undergo standard chemotherapy but may undergo conditional chemotherapy if a transplant is to be performed.

[0023] It is further contemplated that treatment may be determined based on the ALL biomarker information, but it may also include evaluating and considering the following in some embodiments: the level of white blood cells in the patient, whether leukemic cells of the patient have abnormal ploidy, whether leukemic cells of the patient exhibit an 11q23 rearrangement.

[0024] In some embodiments, information is obtained by taking a patient history or reviewing a report from a laboratory containing the information.

[0025] In some embodiments, methods involve obtaining and/or providing a sample containing leukemic cells from the patient to generate information about expression levels. The sample is provided to a laboratory for processing to generate information about expression levels in some embodiments. In other embodiments, methods may involve ordering a test from a laboratory to obtain information about the patient's expression levels or to obtain an expression profile. In some embodiments, methods involve ordering a test from a laboratory that determines whether the patient's ALL is Ph+.

[0026] Embodiments include apparatuses or compositions that can be used to evaluate the expression level of an ALL biomarker. In some embodiments, there are kits comprising primers or probes that can be used to detect expression of one or more ALL biomarkers. In some embodiments there are primers or probes specific for SLC2A3, ITPR1, TCF4, and

FLT3. In certain embodiments, there is at least one primer pair (for example, for PCR) for each of SLC2A3, ITPR1, TCF4, and FLT3. Probes or primers may also be attached to an array or microarray. In other embodiments, these probes are attached to a solid support, such as a bead. It is contemplated that kits may also include reagents needed to use the probe or primer, such as buffers or reagents used for detection purposes.

[0027] Compositions also include cancer therapeutic agents for the use in the treatment of cancer after the patient has been evaluated for the likelihood of responding to conventional cancer treatment, such as chemotherapy. In certain embodiments, compositions include one or more chemotherapeutic agents used for the treatment of ALL after the patient has been evaluated and determined to be an optimal responder to chemotherapy. In other embodiments, a composition does not include a chemotherapeutic agent because the ALL patient has been determined not to be an optimal responder.

[0028] Any aspect discussed with respect to one embodiment applies to aspects of other embodiments as well.

[0029] The embodiments in the Example section are understood to be embodiments of the technology disclosed herein that are applicable to all aspects of the technology.

[0030] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

[0031] Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0032] Following long-standing patent law, the words "a" and "an," when used in conjunction with the word "comprising" in the claims or specification, denotes one or more, unless specifically noted.

[0033] Other objects, features and advantages of the claims will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the claims, are given by

way of illustration only, since various changes and modifications within the spirit and scope of the claims will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the claims. The claims may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0035] **FIG. 1**. Kaplan–Meier analysis of disease-free survival of the optimal responders and early relapse group (p=0.002).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

I. Acute Lymphoblastic Leukemia and Treatments

[0036] Acute lymphoblastic leukemia is cancer involving early lymphoid precursors that rapidly grow and replace normal hematopoietic cells in the bone marrow. While it is the most common leukemia in children, about one-third of the cases annually are diagnosed in adults. It is typically diagnosed based on the number and size of leukemia cells, the type of lymphocytes affects, and/or cytogenetics.

[0037] The cytogenetics may include an evaluation of whether leukemic cells are hyperdiploid or hypodiploid. It may involve or also involve checking for translocations, such as the following: t(12;21) (known as tel-AML-1 fusion); trisomies 4 and 10, or simultaneous trisomy 4 and 10; t(1;19) or E2A-PBX1, t(1;19); t(4;11); t(9;22) or Philadelphia chromosome (Ph+) (also known as BCR/ABL fusion); MLL (11q23) gene translocations such as t(4;11) (q21;q23) or t(11;19); t(8;14) (q24;q32); t(8;22)(q24;q11); and, t(2;8)(p11-p12;q24). Ways to evaluate cytogenetics are well known to those of skill in the art.

[0038] Chemotherapy treatment for ALL patients may involve one for more stages. Patients will typically receive treatment immediately, which is usually the "induction chemotherapy." Most ALL patients receive induction chemotherapy, which is to effect remission of the disease. It typically lasts a month and may be followed with a bone marrow transplant of consolidation therapy. Induction chemotherapy can be followed by "intensification therapy" or "consolidation therapy," which lasts four to eight months. Patients who go into remission may then have "maintenance therapy."

[0039] Standard chemotherapy involves a combination of prednisolone or dexamethasone (for children), asparaginase, vincristine, and daunorubicin (for adults). Another standard chemotherapy is Hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, and the steroid dexamethasone), which is an abbreviation of some of the drugs in a combination treatment.

[0040] Typically Hyper-CVAD treatment involves two different courses A and B, which are given up to four times with up to eight cycles total. Course A usually includes cyclophosphamide, which is an alkylating agent; vincristine, a mitotic inhibitor; doxorubicin, an antibiotic that counteracts tumors; dexamethasone, an immunosuppressant steroid; cytarabine or Ara-C, which is an antimetabolite; mesna, a drug that inhibits the occurrence of hemorrhagic cystitis (from cyclophosphamide); methotrexate, an antimetabolite. Course B typically involves methotrexate; leucovorin; sodium biocarbonate; cytarabine.

[0041] Tyrosine kinase inhibitors may be given with standard chemotherapy. These TKIs include imatinib mesylate, dasatinib, and/or nilotinib.

[0042] Patients may undergo a bone marrow or cord blood transplant following standard chemotherapy or, according to some embodiments, may undergo a bone marrow or cord blood transplant without undergoing a standard therapeutic course of chemotherapy. In such cases, a patient may not undergo a standard therapeutic course of chemotherapy but undergo a transplant that follows conditioning therapy. "Conditioning therapy" may include chemotherapy and/or radiation and it is administered within 10 days of undergoing a transplant. It is distinguishable from what is referred to herein as "standard therapeutic chemotherapy" or in the oncology field as "standard chemotherapy" because conditioning therapy is given to a patient within 3-10 days of undergoing a transplant. While it can have some therapeutic effect, conditioning therapy helps to suppress the immune system and prevent graft versus host disease.

[0043] In specific cases, a patient is processed for a bone marrow or cord blood transplant without undergoing chemotherapy. The transplant process will likely involve HLA typing of the patient and any potential allogeneic donor. The transplant process may involve the patient undertaking or undergoing any of the following procedures (or any combination of these steps): blood tests to measure kidney, liver, heart, or lung function or to measure hormone levels; blood tests to screen for infections; bone marrow evaluation; X-rays or CT scans; spinal tap; physical examination; dental examination; psychological evaluation; and

placement of a central venous catheter. Alternatively, a clinician such as a doctor, nurse, or physician's assistant may perform these procedures and/or order that one or more of these procedures be done. In some cases, laboratory personnel perform one or more of these procedures, including HLA typing. Embodiments may involve any of the steps and/or procedures described.

II. Biomarkers and Evaluating Levels of Biomarkers

[0044] Nine biomarkers for prognosing ALL Ph+ human patients have been identified. They include SLC2A3, ITPR1, TCF4, FLT3 (also known as FLK2 or STK1), CD69, NPM1, SPRY2, TP53 (or p53), and PTGS1.

[0045] It is contemplated that these biomarkers may be evaluated based on their gene products. In some embodiments, the gene product is the RNA transcript. In other embodiments, the gene product is the protein expressed by the RNA transcript.

[0046] The expression patterns can also be compared by using one or more ratios between the expression levels of different ALL biomarkers. Other suitable measures or indicators can also be employed for assessing the relationship or difference between different expression patterns.

[0047] The FLT3 nucleic acid and protein sequences are provided in GenBank accession numbers (NM_004119.2, U02687.1, Z26652.1, BC036028.1, BC126350.1). The ITPR1 nucleic acid and protein sequences are provided in GenBank accession numbers (NM_001099952.1, NM_002222.4, D26070.1, L38019.2, U23850.1, AB208868.1). The SLC2A3 sequence nucleic acid and protein sequences are provided in GenBank accession numbers (NM_006931.1, M20681.1, CR621471.1, AB209607.1, BC039196.1). The TCF4 nucleic acid and protein sequences are provided in GenBank accession numbers (NM_001083962.1, NM_003199.2, M74718.1, M74719.1, X52079.1, CR614823.1, CR624281.1, AB209741.1, BC031056.1, AK122765.1, AK095041.1, AK096862.1, BC125084.1, BC125085.1). The CD69 nucleic acid and protein sequences are provided in GenBank accession numbers (NM_001781.1, Z22576.1, L07555.1,AY238518.1, AK291869.1, BC007037.1). The NPM1 nucleic acid and protein sequences are provided in GenBank accession numbers (NM_199185.2, NM_001037738.1, NM_002520.5, M28699.1, M23613.1, M26697.1, X16934.1, AB042278.1, BC008495.1, AK000472.1, BC003670.1, BC002398.2, CR590741.1, R594093.1, CR595866.1, CR596514.1, CR597478.1,

CR601970.1, CR60). The PTGS1 nucleic acid and protein sequences are provided in GenBank accession numbers (NM_080591.1, NM_000962.2, U63846.1, AJ420464.1). The SPRY2 nucleic acid and protein sequences are provided in GenBank accession numbers (NM_005842.2, AF039843.1, BC015745.1). The TP53 nucleic acid and protein sequences are provided in GenBank accession numbers (NM_000546.3, AY627884.1, DQ186648.1, DQ186649.1, DA308036.1, DQ191317.1, DQ286964.1, DQ648884.1, AK225838.1, K03199.1, M14694.1, M14695.1, X02469.1, AF307851.1, BC003596.1). The content of all of these GenBank Accession numbers is specifically incorporated herein by reference.

[0048] The following biomarkers and SEQ ID NOs are provided for implementation with embodiments discussed herein. All of them are nucleic acid sequences unless two sequences are identified for a specific Accession number, in which case the second sequence is a polypeptide sequence.

FLT3:
NM_004119.2 (SEQ ID 1 and 2)
U02687.1 (SEQ ID NP 3)
Z26652.1 (SEQ ID NO 4)
BC036028.1, ((SEQ ID NO 5)
BC126350.1 (SEQ ID NO 6)

ITPR1:
NM_001099952.1 (SEQ ID NO 7 and 8),
NM_002222.4 (SEQ ID NO 9 and 10),
D26070.1 (SEQ ID NO 11)
L38019.2 (SEQ ID NO 12 and 13)
U23850.1 (SEQ ID NO 14)
AB208868.1 (SEQ ID NO 15 and 16)

SLC2A3:
NM_006931.1 (SEQ ID NO 17 and 18
M20681.1 (SEQ ID NO19)
CR621471.1 (SEQ ID NO 20)
AB209607.1 (SEQ ID NO 21 and 22)
BC039196.1 (SEQ ID NO 23)

TCF4:
NM_001083962.1 (SEQ ID NO 24 and 25)
NM_003199.2 (SEQ ID NO 26 and 27)
M74718.1 (SEQ ID NO 28)
M74719.1 (DUPLICATE)
X52079.1 (SEQ ID NO 29 and 30)
CR614823.1 (SEQ ID NO 31)
CR624281.1 (SEQ ID NO 32)

AB209741.1, (SEQ ID NO 33 and 34)
BC031056.1 (SEQ ID NO 35)
AK122765.1 (SEQ ID NO 36)
AK095041.1 (SEQ ID NO 69 and 70)
AK096862.1 (SEQ ID NO 71 and 72)
BC125084.1 (SEQ ID NO 73)
BC125085.1 (SEQ ID NO 74)

CD69:	NM_001781.1 (SEQ ID NO 37 and 38) Z22576.1 (SEQ ID NO 39) L07555.1 (SEQ ID NO 40) AY238518.1 (SEQ ID NO 41) AK291869.1 (SEQ ID NO 42) BC007037.1 (SEQ ID NO 43)
NPM1	NM_199185.2 (SEQ ID NO 44 and 45) NM_001037738.1 (SEQ ID NO 46 and 47) NM_002520.5 (SEQ ID NO 48 and 49) M28699.1 (SEQ ID NO 50) M23613.1 (SEQ ID NO 51) M26697.1 (SEQ ID NO 52) X16934.1 (SEQ ID NO 53 and 54) AB042278.1 (SEQ ID NO 55 and 56) BC008495.1 (SEQ ID NO 57) AK000472.1 (SEQ ID NO 58 and 59 BC003670.1 (SEQ ID NO 60 and 61) BC002398.2 (SEQ ID NO 62) CR590741.1 (SEQ ID NO 63) CR594093.1, (SEQ ID NO 64) CR595866.1 (SEQ ID NO 65) CR596514.1 (SEQ ID NO 66) CR597478.1 (SEQ ID NO 67) CR601970.1 (SEQ ID NO 68)
PTGS1	NM_080591.1 (SEQ ID NO 75 and 76) NM_000962.2 (SEQ ID NO 77 and 78) U63846.1 (SEQ ID NO 79) AJ420464.1 (SEQ ID NO 80)
SPRY2	NM_005842.2 (SEQ ID NO 81 and 82) AF039843.1 (SEQ ID NO 83) BC015745.1 (SEQ ID NO 84)
TP53	NM_000546.3 (SEQ ID NO 85 and 86) AY627884.1 (SEQ ID NO 87 and 88) DQ186648.1 (SEQ ID NO 89 and 90) DQ186649.1 (SEQ ID NO 91 and 92) DA308036.1 (SEQ ID NO 93) DQ191317.1 (SEQ ID NO 94) DQ286964.1 (SEQ ID NO 95) DQ648884.1 (SEQ ID NO 96 and 97) AK225838.1 (SEQ ID NO 98) K03199.1 (SEQ ID NO 99) M14694.1 (SEQ ID NO 100) M14695.1 (SEQ ID NO 101) X02469.1 (SEQ ID NO 103) AF307851.1 (SEQ ID NO 104)

BC003596.1 (SEQ ID NO 105)

[0049] One or more of the biomarkers can be used to prognose a human patient with ALL. The expression pattern of these biomarkers in leukemic cells may be used to evaluate a patient to determine whether they are likely to respond to standard chemotherapy, likely not to respond to standard chemotherapy, or likely to relapse after standard chemotherapy.

[0050] The expression levels of ALL biomarkers can be compared to reference expression levels using various methods. These reference levels can be determined using expression levels of a reference based on all ALL patients or all ALL Ph+ patients, regardless of their prognosis. Alternatively, it can be based on an internal reference such as a gene that is expressed in all cells. In some embodiments, the reference is a gene expressed in leukemic cells at a higher level than any biomarker. Any comparison can be performed using the fold change or the absolute difference between the expression levels to be compared. One or more ALL biomarkers can be used in the comparison. It is contemplated that 1, 2, 3, 4, 5, 6, 7, 8, and/or 9 biomarkers may be compared to each other and/or to a reference that is internal or external. A person of ordinary skill in the art would know how to do such comparisons.

[0051] Comparisons or results from comparisons may reveal or be expressed as x-fold increase or decrease in expression relative to a standard or relative to another biomarker or relative to the same biomarker but in a different class of prognosis. In some embodiments, optimal responders have a relatively high level of expression (overexpression) or relatively low level of expression (underexpression) when compared to non-responders, or vice versa.

[0052] Fold increases or decreases may be, be at least, or be at most 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-, 20-, 25-, 30-, 35-, 40-, 45-, 50-, 55-, 60-, 65-, 70-, 75-, 80-, 85-, 90-, 95-, 100- or more, or any range derivable therein. Alternatively, differences in expression may be expressed as a percent decrease or increase, such as at least or at most 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900, 1000% difference, or any range derivable therein.

[0053] Other ways to express relative expression levels are by normalized or relative numbers such as 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03. 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2,

1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7. 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, or any range derivable therein.

[0054] For example, if expression levels of the biomarkers are normalized based on GAPDH levels, the following levels of relative expression are seen:

Biomarker	Optimal Responders	Non-Responders
SLC2A3	0.01-0.1	0.2-2
ITPR1	0.05-0.25	0.004-0.03
TCF4	0.05-0.85	<0.01
FLT3	0.01-0.25	<0.008
CD69	>0.06	<0.18
NPM1	>0.7	<0.8
SPRY2	>0.008	<0.022
TP53	>0.04	<0.04
PTGS1	<0.008	>0.002

[0055] The Example shows the following: SLC2A3 is downregulated in optimal responders, which means that expression of SLC2A3 is about 10-20-fold lower than in non-responders; ITPR1 is upregulated in optimal responders, which means that expression of ITPR1 is about 10-20-fold higher than in non-responders; TCF4 is upregulated in optimal responders, which means that expression of TCF4 is about 5-100-fold higher than in non-responders; FLT3 is upregulated in optimal responders, which means that expression of FLT3 is about 2-30-fold higher than in non-responders; CD69 is downregulated in optimal responders, which means that expression of CD69 is about 3-5-fold lower than in non-responders; NPM1 is upregulated in optimal responders, which means that expression of NPM1 is higher than in non-responders; SPRY2 is upregulated in optimal responders, which

means that expression of SPRY2 is about 2-5-fold higher than in non-responders; TP53 is upregulated in optimal responders, which means that expression of TP53 is higher than in non-responders; PTGS1 is upregulated in optimal responders, which means that expression of PTGS1 is higher than in non-responders.

[0056] Algorithms, such as the weighted voting programs, can be used to facilitate the evaluation of biomarker levels. In addition, other clinical evidence can be combined with the biomarker-based test to reduce the risk of false evaluations. Other cytogenetic evaluations may be considered in some embodiments of the invention.

[0057] Any biological sample from the patient that contains leukemic cells may be used to evaluate the expression pattern of any biomarker discussed herein. In some embodiments, a biological sample from bone marrow is used. In other embodiments, peripheral blood can be used as the biological sample. Evaluation of the sample may involve, though it need not involve, panning (enriching) for leukemic cells or isolating the leukemic cells. The peripheral blood samples can be either whole blood, or blood samples enriched for blast cells.

A. Nucleic Acids

[0058] Screening methods based on differentially expressed gene products are well known in the art. In accordance with one aspect of the present invention, the differential expression patterns of ALL biomarkers can be determined by measuring the levels of RNA transcripts of these genes in the patient's leukemic cells. Suitable methods for this purpose include, but are not limited to, RT-PCTR, Northern Blot, in situ hybridization, Southern Blot, slot-blotting, nuclease protection assay and oligonucleotide arrays.

[0059] In general, RNA isolated from leukemic or blast cells can be amplified to cDNA or cRNA before detection and/or quantitation. The isolated RNA can be either total RNA or mRNA. The RNA amplification can be specific or non-specific. Suitable amplification methods include, but are not limited to, reverse transcriptase PCR, isothermal amplification, ligase chain reaction, and Qbeta replicase. The amplified nucleic acid products can be detected and/or quantitated through hybridization to labeled probes. In some embodiments, detection may involve fluorescence resonance energy transfer (FRET) or some other kind of quantum dots.

[0060] Amplification primers or hybridization probes for an ALL biomarker can be prepared from the gene sequence. In certain embodiments the gene sequence is identical or complementary to at least 8 contiguous nucleotides of the coding sequence.

[0061] Sequences suitable for making probes/primers for the detection of their corresponding ALL biomarkers include those that are identical or complementary to all or part of SEQ ID NOs:1, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 17, 19, 20, 21, 23, 24, 26, 28, 29, 31, 32, 33, 35, 36, 37, 39, 40, 41, 42, 43, 44, 46, 48, 50, 51, 52, 53, 55, 57, 58, 60, 62, 63, 64, 65, 66, 67, 68, 69, 71, 73, 74, 75, 77, 79, 80, 81, 83, 84, 85, 87, 89, 91, 93, 94, 95, 96, 98, 99, 100, 101, 103, 104, and 105. These sequences are all nucleic acid sequences of ALL biomarkers. A number of them represent slight differences in sequence that have been observed in humans. It is contemplated that in some embodiments, primers or probes that are used in embodiments of the invention have a sequence that is common to the different sequences of that same biomarker. For instance, a probe or primer may have a sequence for FLT3 that is common to SEQ ID NOs: 1, 3, 4, 5, and 6.

[0062] The use of a probe or primer of between 13 and 100 nucleotides, preferably between 17 and 100 nucleotides in length, or in some aspects of the invention up to 1-2 kilobases or more in length, allows the formation of a duplex molecule that is both stable and selective. Molecules having complementary sequences over contiguous stretches greater than 20 bases in length are generally preferred, to increase stability and/or selectivity of the hybrid molecules obtained. One will generally prefer to design nucleic acid molecules for hybridization having one or more complementary sequences of 20 to 30 nucleotides, or even longer where desired. Such fragments may be readily prepared, for example, by directly synthesizing the fragment by chemical means or by introducing selected sequences into recombinant vectors for recombinant production.

[0063] In one embodiment, each probe/primer comprises at least 15 nucleotides. For instance, each probe can comprise at least or at most 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 400 or more nucleotides (or any range derivable therein). They may have these lengths and have a sequence that is identical or complementary to SEQ ID NOs: SEQ ID NOs:1, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 17, 19, 20, 21, 23, 24, 26, 28, 29, 31, 32, 33, 35, 36, 37, 39, 40, 41, 42, 43, 44, 46, 48, 50, 51, 52, 53, 55, 57, 58, 60, 62, 63, 64, 65, 66, 67, 68, 69, 71, 73, 74, 75, 77, 79, 80, 81, 83, 84, 85, 87, 89, 91, 93, 94, 95, 96, 98, 99, 100, 101, 103, 104, or 105. Preferably, each probe/primer has relatively high sequence

complexity and does not have any ambiguous residue (undetermined "n" residues). The probes/primers preferably can hybridize to the target gene, including its RNA transcripts, under stringent or highly stringent conditions. In some embodiments, because each of the biomarkers has more than one human sequence, it is contemplated that probes and primers may be designed for use with each on of these sequences. For example, inosine is a nucleotide frequently used in probes or primers to hybridize to more than one sequence. It is contemplated that probes or primers may have inosine or other design implementations that accommodate recognition of more than one human sequence for a particular biomarker.

[0064] For applications requiring high selectivity, one will typically desire to employ relatively high stringency conditions to form the hybrids. For example, relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.10 M NaCl at temperatures of about 50°C to about 70°C. Such high stringency conditions tolerate little, if any, mismatch between the probe or primers and the template or target strand and would be particularly suitable for isolating specific genes or for detecting specific mRNA transcripts. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

[0065] In another embodiment, the probes/primers for a gene are selected from regions which significantly diverge from the sequences of other genes. Such regions can be determined by checking the probe/primer sequences against a human genome sequence database, such as the Entrez database at the NCBI. One algorithm suitable for this purpose is the BLAST algorithm. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold. These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence to increase the cumulative alignment score. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. These parameters can be adjusted for different purposes, as appreciated by one of ordinary skill in the art.

[0066] In one embodiment, quantitative RT-PCR (such as TaqMan, ABI) is used for detecting and comparing the levels of RNA transcripts of the RCC disease genes in peripheral blood samples. Quantitative RT-PCR involves reverse transcription (RT) of RNA to cDNA followed by relative quantitative PCR (RT-PCR).

[0067] The concentration of the target DNA in the linear portion of the PCR process is proportional to the starting concentration of the target before the PCR was begun. By determining the concentration of the PCR products of the target DNA in PCR reactions that have completed the same number of cycles and are in their linear ranges, it is possible to determine the relative concentrations of the specific target sequence in the original DNA mixture. If the DNA mixtures are cDNAs synthesized from RNAs isolated from different tissues or cells, the relative abundances of the specific mRNA from which the target sequence was derived may be determined for the respective tissues or cells. This direct proportionality between the concentration of the PCR products and the relative mRNA abundances is true in the linear range portion of the PCR reaction.

[0068] The final concentration of the target DNA in the plateau portion of the curve is determined by the availability of reagents in the reaction mix and is independent of the original concentration of target DNA. Therefore, the sampling and quantifying of the amplified PCR products preferably are carried out when the PCR reactions are in the linear portion of their curves. In addition, relative concentrations of the amplifiable cDNAs preferably are normalized to some independent standard, which may be based on either internally existing RNA species or externally introduced RNA species. The abundance of a particular mRNA species may also be determined relative to the average abundance of all mRNA species in the sample.

[0069] In one embodiment, the PCR amplification utilizes one or more internal PCR standards. The internal standard may be an abundant housekeeping gene in the cell or it can specifically be GAPDH, GUSB and β-2 microglobulin. These standards may be used to normalize expression levels so that the expression levels of different gene products can be compared directly. A person of ordinary skill in the art would know how to use an internal standard to normalize expression levels.

[0070] This strategy is especially effective if the products of the PCR amplifications are sampled during their linear phases. If the products are sampled when the reactions are

approaching the plateau phase, then the less abundant product may become relatively over-represented. Comparisons of relative abundances made for many different RNA samples, such as is the case when examining RNA samples for differential expression, may become distorted in such a way as to make differences in relative abundances of RNAs appear less than they actually are. This can be improved if the internal standard is much more abundant than the target. If the internal standard is more abundant than the target, then direct linear comparisons may be made between RNA samples.

[0071] A problem inherent in clinical samples is that they are of variable quantity and/or quality. This problem can be overcome if the RT-PCR is performed as a relative quantitative RT-PCR with an internal standard in which the internal standard is an amplifiable cDNA fragment that is similar or larger than the target cDNA fragment and in which the abundance of the mRNA encoding the internal standard is roughly 5-100 fold higher than the mRNA encoding the target. This assay measures relative abundance, not absolute abundance of the respective mRNA species.

[0072] In another embodiment, the relative quantitative RT-PCR uses an external standard protocol. Under this protocol, the PCR products are sampled in the linear portion of their amplification curves. The number of PCR cycles that are optimal for sampling can be empirically determined for each target cDNA fragment. In addition, the reverse transcriptase products of each RNA population isolated from the various samples can be normalized for equal concentrations of amplifiable cDNAs.

[0073] Nucleic acid arrays can also be used to detect and compare the differential expression patterns of ALL biomarkers in leukemic cells. The probes suitable for detecting the corresponding ALL biomarkers can be stably attached to known discrete regions on a solid substrate. As used herein, a probe is "stably attached" to a discrete region if the probe maintains its position relative to the discrete region during the hybridization and the subsequent washes. Construction of nucleic acid arrays is well known in the art. Suitable substrates for making polynucleotide arrays include, but are not limited to, membranes, films, plastics and quartz wafers.

[0074] A nucleic acid array of the present invention can comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more different polynucleotide probes, which may hybridize to different and/or the same biomarkers.

Multiple probes for the same gene can be used on a single nucleic acid array. Probes for other disease genes can also be included in the nucleic acid array. The probe density on the array can be in any range. In some embodiments, the density may be 50, 100, 200, 300, 400, 500 or more probes/cm^2.

[0075] Specifically contemplated by the present inventors are chip-based nucleic acid technologies such as those described by Hacia *et al.* (1996) and Shoemaker *et al.* (1996). Briefly, these techniques involve quantitative methods for analyzing large numbers of genes rapidly and accurately. By tagging genes with oligonucleotides or using fixed probe arrays, one can employ chip technology to segregate target molecules as high density arrays and screen these molecules on the basis of hybridization (see also, Pease *et al.*, 1994; and Fodor *et al*, 1991). It is contemplated that this technology may be used in conjunction with evaluating the expression level of one or more ALL biomarkers with respect to diagnostic, prognostic, and treatment methods of the invention.

[0076] The present invention may involve the use of arrays or data generated from an array. Data may be readily available. Moreover, an array may be prepared in order to generate data that may then be used in correlation studies.

[0077] An array generally refers to ordered macroarrays or microarrays of nucleic acid molecules (probes) that are fully or nearly complementary or identical to a plurality of mRNA molecules or cDNA molecules and that are positioned on a support material in a spatially separated organization. Macroarrays are typically sheets of nitrocellulose or nylon upon which probes have been spotted. Microarrays position the nucleic acid probes more densely such that up to 10,000 nucleic acid molecules can be fit into a region typically 1 to 4 square centimeters. Microarrays can be fabricated by spotting nucleic acid molecules, *e.g.*, genes, oligonucleotides, *etc.*, onto substrates or fabricating oligonucleotide sequences *in situ* on a substrate. Spotted or fabricated nucleic acid molecules can be applied in a high density matrix pattern of up to about 30 non-identical nucleic acid molecules per square centimeter or higher, *e.g.* up to about 100 or even 1000 per square centimeter. Microarrays typically use coated glass as the solid support, in contrast to the nitrocellulose-based material of filter arrays. By having an ordered array of complementing nucleic acid samples, the position of each sample can be tracked and linked to the original sample. A variety of different array devices in which a plurality of distinct nucleic acid probes are stably associated with the surface of a solid support are known to those of skill in the art. Useful substrates for arrays

include nylon, glass and silicon Such arrays may vary in a number of different ways, including average probe length, sequence or types of probes, nature of bond between the probe and the array surface, *e.g.* covalent or non-covalent, and the like. The labeling and screening methods of the present invention and the arrays are not limited in its utility with respect to any parameter except that the probes detect expression levels; consequently, methods and compositions may be used with a variety of different types of genes.

[0078] Representative methods and apparatus for preparing a microarray have been described, for example, in U.S. Patent Nos. 5,143,854; 5,202,231; 5,242,974; 5,288,644; 5,324,633; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,432,049; 5,436,327; 5,445,934; 5,468,613; 5,470,710; 5,472,672; 5,492,806; 5,525,464; 5,503,980; 5,510,270; 5,525,464; 5,527,681; 5,529,756; 5,532,128; 5,545,531; 5,547,839; 5,554,501; 5,556,752; 5,561,071; 5,571,639; 5,580,726; 5,580,732; 5,593,839; 5,599,695; 5,599,672; 5,610;287; 5,624,711; 5,631,134; 5,639,603; 5,654,413; 5,658,734; 5,661,028; 5,665,547; 5,667,972; 5,695,940; 5,700,637; 5,744,305; 5,800,992; 5,807,522; 5,830,645; 5,837,196; 5,871,928; 5,847,219; 5,876,932; 5,919,626; 6,004,755; 6,087,102; 6,368,799; 6,383,749; 6,617,112; 6,638,717; 6,720,138, as well as WO 93/17126; WO 95/11995; WO 95/21265; WO 95/21944; WO 95/35505; WO 96/31622; WO 97/10365; WO 97/27317; WO 99/35505; WO 09923256; WO 09936760; WO0138580; WO 0168255; WO 03020898; WO 03040410; WO 03053586; WO 03087297; WO 03091426; WO03100012; WO 04020085; WO 04027093; EP 373 203; EP 785 280; EP 799 897 and UK 8 803 000; the disclosures of which are all herein incorporated by reference.

[0079] It is contemplated that the arrays can be high density arrays, such that they contain 100 or more different probes. It is contemplated that they may contain 1000, 16,000, 65,000, 250,000 or 1,000,000 or more different probes. The probes can be directed to targets in one or more different organisms. The oligonucleotide probes range from 5 to 50, 5 to 45, 10 to 40, or 15 to 40 nucleotides in length in some embodiments. In certain embodiments, the oligonucleotide probes are 20 to 25 nucleotides in length.

[0080] The location and sequence of each different probe sequence in the array are generally known. Moreover, the large number of different probes can occupy a relatively small area providing a high density array having a probe density of generally greater than about 60, 100, 600, 1000, 5,000, 10,000, 40,000, 100,000, or 400,000 different

oligonucleotide probes per cm^2. The surface area of the array can be about or less than about 1, 1.6, 2, 3, 4, 5, 6, 7, 8, 9, or 10 cm^2.

[0081] Moreover, a person of ordinary skill in the art could readily analyze data generated using an array. Such protocols are disclosed above, and include information found in WO 9743450; WO 03023058; WO 03022421; WO 03029485; WO 03067217; WO 03066906; WO 03076928; WO 03093810; WO 03100448A1, all of which are specifically incorporated by reference.

[0082] In one embodiment, nuclease protection assays are used to quantify RNAs derived from the peripheral blood samples. There are many different versions of nuclease protection assays known to those practiced in the art. The common characteristic that these nuclease protection assays have is that they involve hybridization of an antisense nucleic acid with the RNA to be quantified. The resulting hybrid double-stranded molecule is then digested with a nuclease that digests single-stranded nucleic acids more efficiently than double-stranded molecules. The amount of antisense nucleic acid that survives digestion is a measure of the amount of the target RNA species to be quantified. An example of a nuclease protection assay that is commercially available is the RNase protection assay manufactured by Ambion, Inc. (Austin, Tex.).

B. Proteins and Polypeptides

[0083] In other embodiments, the differential expression patterns of ALL biomarkers can be determined by measuring the levels of polypeptides encoded by these genes in leukemic cells. Methods suitable for this purpose include, but are not limited to, immunoassays such as ELISA, RIA, FACS, dot blot, Western Blot, immunohistochemistry, and antibody-based radioimaging. Protocols for carrying out these immunoassays are well known in the art. Other methods such as 2-dimensional SDS-polyacrylamide gel electrophoresis can also be used. These procedures may be used to recognize any of the polypeptides encoded by the ALL biomarker genes described herein. In specific embodiments, all or part of the following protein sequences are used to evaluate gene product expression of an ALL biomarker: SEQ ID NOs:2, 8, 10, 13, 16, 18, 22, 25, 27, 34, 38, 45, 47, 49, 54, 56, 59, 61, 70, 72, 76, 78, 82, 86, 88, 90, 92, and 97.

[0084] One exemplary method suitable for detecting the levels of target proteins in peripheral blood samples is ELISA. In an exemplifying ELISA, antibodies capable of binding

to the target proteins encoded by one or more ALL biomarker genes are immobilized onto a selected surface exhibiting protein affinity, such as wells in a polystyrene or polyvinylchloride microtiter plate. Then, leukemic cell samples to be tested are added to the wells. After binding and washing to remove non-specifically bound immunocomplexes, the bound antigen(s) can be detected. Detection can be achieved by the addition of a second antibody which is specific for the target proteins and is linked to a detectable label. Detection may also be achieved by the addition of a second antibody, followed by the addition of a third antibody that has binding affinity for the second antibody, with the third antibody being linked to a detectable label. Before being added to the microtiter plate, cells in the peripheral blood samples can be lysed using various methods known in the art. Proper extraction procedures can be used to separate the target proteins from potentially interfering substances.

[0085] In another ELISA embodiment, the leukemic cell samples containing the target proteins are immobilized onto the well surface and then contacted with the antibodies of the invention. After binding and washing to remove non-specifically bound immunocomplexes, the bound antigen is detected. Where the initial antibodies are linked to a detectable label, the immunocomplexes can be detected directly. The immunocomplexes can also be detected using a second antibody that has binding affinity for the first antibody, with the second antibody being linked to a detectable label.

[0086] Another typical ELISA involves the use of antibody competition in the detection. In this ELISA, the target proteins are immobilized on the well surface. The labeled antibodies are added to the well, allowed to bind to the target proteins, and detected by means of their labels. The amount of the target proteins in an unknown sample is then determined by mixing the sample with the labeled antibodies before or during incubation with coated wells. The presence of the target proteins in the unknown sample acts to reduce the amount of antibody available for binding to the well and thus reduces the ultimate signal.

[0087] Different ELISA formats can have certain features in common, such as coating, incubating or binding, washing to remove non-specifically bound species, and detecting the bound immunocomplexes. For instance, in coating a plate with either antigen or antibody, the wells of the plate can be incubated with a solution of the antigen or antibody, either overnight or for a specified period of hours. The wells of the plate are then washed to remove incompletely adsorbed material. Any remaining available surfaces of the wells are then "coated" with a nonspecific protein that is antigenically neutral with regard to the test

samples. Examples of these nonspecific proteins include bovine serum albumin (BSA), casein and solutions of milk powder. The coating allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific binding of antisera onto the surface.

[0088] In ELISAs, a secondary or tertiary detection means can also be used. After binding of a protein or antibody to the well, coating with a non-reactive material to reduce background, and washing to remove unbound material, the immobilizing surface is contacted with the control and/or clinical or biological sample to be tested under conditions effective to allow immunocomplex (antigen/antibody) formation. These conditions may include, for example, diluting the antigens and antibodies with solutions such as BSA, bovine gamma globulin (BGG) and phosphate buffered saline (PBS)/Tween and incubating the antibodies and antigens at room temperature for about 1 to 4 hours or at 49°C overnight. Detection of the immunocomplex then requires a labeled secondary binding ligand or antibody, or a secondary binding ligand or antibody in conjunction with a labeled tertiary antibody or third binding ligand.

[0089] After all of the incubation steps in an ELISA, the contacted surface can be washed so as to remove non-complexed material. For instance, the surface may be washed with a solution such as PBS/Tween, or borate buffer. Following the formation of specific immunocomplexes between the test sample and the originally bound material, and subsequent washing, the occurrence of the amount of immunocomplexes can be determined.

[0090] To provide a detecting means, the second or third antibody can have an associated label to allow detection. In one embodiment, the label is an enzyme that generates color development upon incubating with an appropriate chromogenic substrate. Thus, for example, one may contact and incubate the first or second immunocomplex with a urease, glucose oxidase, alkaline phosphatase or hydrogen peroxidase-conjugated antibody for a period of time and under conditions that favor the development of further immunocomplex formation (e.g., incubation for 2 hours at room temperature in a PBS-containing solution such as PBS-Tween).

[0091] After incubation with the labeled antibody, and subsequent to washing to remove unbound material, the amount of label is quantified, e.g., by incubation with a chromogenic substrate such as urea and bromocresol purple or 2,2'-azido-di-(3-ethyl)-benzhiazoline-6-

sulfonic acid (ABTS) and hydrogen peroxide, in the case of peroxidase as the enzyme label. Quantitation can be achieved by measuring the degree of color generation, e.g., using a spectrophotometer.

[0092] Another suitable method is RIA (radioimmunoassay). An exemplary RIA is based on the competition between radiolabeled-polypeptides and unlabeled polypeptides for binding to a limited quantity of antibodies. Suitable radiolabels include, but are not limited to, I^{125}. In one embodiment, a fixed concentration of I^{125}-labeled polypeptide is incubated with a series of dilution of an antibody specific to the polypeptide. When the unlabeled polypeptide is added to the system, the amount of the I^{125}-polypeptide that binds to the antibody is decreased. A standard curve can therefore be constructed to represent the amount of antibody-bound I^{125}-polypeptide as a function of the concentration of the unlabeled polypeptide. From this standard curve, the concentration of the polypeptide in unknown samples can be determined. Various protocols for conducting RIA to measure the levels of polypeptides in leukemic cell samples are well known in the art.

[0093] Suitable antibodies for this invention include, but are not limited to, polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) can also be used.

[0094] The antibodies of this invention can be labeled with one or more detectable moieties to allow for detection of antibody-antigen complexes. The detectable moieties can include compositions detectable by spectroscopic, enzymatic, photochemical, biochemical, bioelectronic, immunochemical, electrical, optical or chemical means. The detectable moieties include, but are not limited to, radioisotopes, chemiluminescent compounds, labeled binding proteins, heavy metal atoms, spectroscopic markers such as fluorescent markers and dyes, magnetic labels, linked enzymes, mass spectrometry tags, spin labels, electron transfer donors and acceptors, and the like.

[0095] Protein array technology is discussed in detail in Pandey and Mann (2000) and MacBeath and Schreiber (2000), each of which is herein specifically incorporated by reference.

[0096] These arrays typically contain thousands of different proteins or antibodies spotted onto glass slides or immobilized in tiny wells and allow one to examine the biochemical

activities and binding profiles of a large number of proteins at once. To examine protein interactions with such an array, a labeled protein is incubated with each of the target proteins immobilized on the slide, and then one determines which of the many proteins the labeled molecule binds. In certain embodiments such technology can be used to quantitate a number of proteins in a sample, such as an ALL biomarker proteins.

[0097] The basic construction of protein chips has some similarities to DNA chips, such as the use of a glass or plastic surface dotted with an array of molecules. These molecules can be DNA or antibodies that are designed to capture proteins. Defined quantities of proteins are immobilized on each spot, while retaining some activity of the protein. With fluorescent markers or other methods of detection revealing the spots that have captured these proteins, protein microarrays are being used as powerful tools in high-throughput proteomics and drug discovery.

[0098] The earliest and best-known protein chip is the ProteinChip by Ciphergen Biosystems Inc. (Fremont, Calif.). The ProteinChip is based on the surface-enhanced laser desorption and ionization (SELDI) process. Known proteins are analyzed using functional assays that are on the chip. For example, chip surfaces can contain enzymes, receptor proteins, or antibodies that enable researchers to conduct protein-protein interaction studies, ligand binding studies, or immunoassays. With state-of-the-art ion optic and laser optic technologies, the ProteinChip system detects proteins ranging from small peptides of less than 1000 Da up to proteins of 300 kDa and calculates the mass based on time-of-flight (TOF).

[0099] The ProteinChip biomarker system is the first protein biochip-based system that enables biomarker pattern recognition analysis to be done. This system allows researchers to address important clinical questions by investigating the proteome from a range of crude clinical samples (*i.e.*, laser capture microdissected cells, biopsies, tissue, urine, and serum). The system also utilizes biomarker pattern software that automates pattern recognition-based statistical analysis methods to correlate protein expression patterns from clinical samples with disease phenotypes.

[00100] In other aspects of screening methods, the levels of polypeptides in peripheral blood samples can be determined by detecting the biological activities associated with the polypeptides. If a biological function/activity of a polypeptide is known, suitable in vitro

bioassays can be designed to evaluate the biological function/activity, thereby determining the amount of the polypeptide in the sample.

[00101] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

IDENTIFICATION OF BIOMARKERS

[00102] Normalized gene expression data from previously published studies of 672 ALL patients were analyzed to identify genes associated with therapy response. Expression of the selected genes was assessed using Applied Biosystems low density reverse transcription quantitative PCR (RT-qPCR) arrays in bone marrow (BM) samples from 43 adult Ph+ ALL patients treated with standard chemotherapy plus a tyrosine kinase inhibitor (TKI). Information about the patients is provided in Table 1. Treatment was hyper CVAD and one form of TKI, imatinib or dasatinib.

Table 1. Clinical characteristics of the patients at time of diagnosis.

	Optimal	**Persistent**	**Early relapse**
Total cases	19	17	7
Median age at diagnosis (range, years)	54 (19-85)	48 (21-67)	56 (30-68)
Median Bcr/Abl1 level	84.8	71.7	57.2
Median presenting BM blast count (%)	87	90	89
Median presenting WBC count ($x10^9$/L)	17.0	20.8	25.0

[00103] Therapy responses were defined at molecular level by monitoring BCR/ABL1 transcript levels, and categorized into 3 groups: optimal, persistent and relapse.

[00104] **Optimal responders**: undetectable within 3 months of therapy, and no increase in the next 6 months.

[00105] **Persistent or Non-responders**: BCR/ ABL1 level persisted at the detectable level within 3 months of therapy.

[00106] **Early relapse**: undetectable within 3 months of therapy but turned detectable in the next 6 months.

[00107] Median follow up was 6 months (range 4-15). Median disease-free survival among the optimal and relapse groups were 12 and 5 months respectively ($p=0.002$). There was no statistical difference in age, initial peripheral white blood cell and BM blast counts, and initial normalized BCR/ABL1 levels between groups. Differentially expressed genes were selected using the significance analysis of microarrays (SAM). Hierarchical clustering and principal component analysis were applied to assess the correlation between gene expression pattern and therapy response. A predictive model was built using support vector machines. Differences in survival among groups were compared by Kaplan-Meier analysis.

[00108] Data mining and pathway analysis of the published data identified 46 genes in 7 pathways potentially associated with therapy response ($p<0.001$). Gene expression profiling data from the literature (6 studies) were pooled and normalized. Relative expression levels were calculated, and associated to outcomes by hierarchical clustering. Associations were further scored by Cox proportionate hazard regression, and the top associated genes were selected as test genes for this study. Each of the test genes was then be assigned to Gene Ontology (GO) pathways. The GO classifications of interest in this study included: cell growth and proliferation, cell communication, metabolism and development, cell motility, response to stress, and cell death. Final selection was based on network analysis of the pathways using Ingenuity Pathway Analysis software, in combination with expert knowledge of the disease mechanism. 46 test genes plus 2 normalizing genes (GAPDH, and GUSB) were used to start the initial screen.

[00109] Total RNA was extracted from bone marrow specimen. A custom-designed TaqMan low density quantitative RT-qPCR array (LDA) (Applied Biosystems, Foster City, CA) was

used to evaluate the 46 identified genes. Expression profiling was done on diagnostic Ph+ ALL samples prior to the initiation of TKI-combined chemotherapy using a custom-designed TaqMan low density quantitative RQ-PCR array (LDA) containing a gene-specific forward and reverse primer pair and TaqMan MGB probe (6-FAM dye-labeled) in each well (Applied Biosystems, Foster City, CA). Total RNA was extracted from bone marrow specimen using the guanidium solubilization method (Trizol, Invitrogen, Carlsbad, CA) and complementary DNA (cDNA) synthesized using Superscript III reverse transcriptase (Invitrogen) using random hexamers for priming. RQ-PCR was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems) with 1 μg of cDNA from each sample. Thermal cycling conditions were as follows: 2 minutes at 50°C, 10 minutes at 95°C, 40 cycles of denaturation at 95°C for 15 seconds, and annealing and extension at 60°C for 1 minute.

[00110] The relative expression level of a particular gene in a given sample on the array was calculated by the delta (Δ)Ct method. Using the approach previously described for LDA arrays, the ΔCt value was obtained by normalizing against the Ct value of GAPDH for each sample.

[00111] One-way analysis of variance (ANOVA) or Student's t-test were used to test against null hypothesis of no significant difference for any given gene expression among three treatment response groups, optimal, suboptimal and resistant group, or between two groups when combining the optimal and the suboptimal into one group. Holm's method was applied to adjust p-values of ANOVA and t-tests to correct multiple comparisons.

[00112] Support vector machine was used to model multiple gene effects regarding response groups. To get the unbiased estimation of classification performance, we applied 5-fold cross validation. . In addition, we repeated the process for 7 iterations. Thus, we have totally 35 different learning and test sets.

[00113] .RT-qPCR results from 15 training cases, 5 in each outcome group identified 9 genes ($p<0.001$) that classified the cases with 100% accuracy. Table 2. Validation using an additional 28 cases showed 92.9% prediction accuracy (ROC error=0.035). Compared to initial diagnostic samples, gene expression pattern in relapsed specimens shifted to that resembling persistent group. Further analysis of the biological functions of our signature genes revealed that optimal responders tend to overexpress genes associated with

proliferation and apoptosis pathways, while poor responders have higher expression of cation drug transporter genes.

[00114] Relative expression level of a particular gene of a given sample on the array was calculated by the delta (D)Ct method. The data was analyzed by significance analysis of microarrays (SAM), unsupervised hierarchical clustering, principal component analysis, and support vector machine (SVM) using R, version 2.7.0 software. Optimal responders over-express CD69, FLT3, ITPR1, NPM1, SPRY2, TCF4, and TP53, with decreased expression of PTGS1 and SLC2A3. Persistent group (*i.e.*, resistant to therapy) shows the opposite pattern. The early relapse group has a mixed pattern that set them in between the above 2 groups.

Table 2. Characteristics of the signature genes.

Gene Symbol	Gene Name	Group	Major Pathways
CD69	CD69 molecule	Transmembrane receptor	Cytotoxicity, apoptosis
FLT3	fms-related tyrosine kinase 3	Protein kinase receptor	Growth, apoptosis
ITPR1	inositol 1,4,5-triphosphate receptor, type 1	Ion channel, cation transporter	Apoptosis, growth
NPM1	nucleophosmin	Chaperone, transcription regulator	Apoptosis, growth
PTGS1	prostaglandin-endoperoxide synthase 1	Synthase	Apoptosis, drug resistance
SLC2A3	solute carrier family 2, member 3	Carbohydrate transporter	Apoptosis, Hif1a signaling
SPRY2	sprouty homolog 2	signaling molecule	Growth, migration
TCF4	transcription factor 4	Helix-loop-helix transcription factor	Growth, acute phase response
TP53	tumor protein p53	Transcription factor	Apoptosis, growth

[00115] Using data-mining and meta-analysis of whole genome expression studies, a 9-gene signature was defined and validated that is an independent predictive marker for therapy response in adult Ph+ ALL patients.

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[00116] The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

1. A method for evaluating a patient with acute lymphoblastic leukemia (ALL) that is characterized by the presence of Philadelphia chromosome (Ph+) or suspected of being Ph+ comprising:

a) generating an expression profile from a biological sample containing leukemic cells of the patient, wherein the expression profile comprises information about expression levels of SLC2A3, ITPR1, TCF4, and FLT3;

b) comparing the expression levels in the expression profile to standard expression levels, wherein the expression levels indicate if the patient is likely to respond to conventional chemotherapy, likely not to respond to conventional chemotherapy, or likely to relapse within four months.

2. The method of claim 1, wherein the expression profile further comprises information about the expression levels of one or more of: CD69, NPM1, SPRY2, TP53, or PTGS1.

3. The method of claim 2, wherein the expression profile further comprises information about the expression level of at least CD69.

4. The method of claim 2, wherein the expression profile further comprises information about the expression level of at least NPM1.

5. The method of claim 2, wherein the expression profile further comprises information about the expression level of at least SPRY2.

6. The method of claim 2, wherein the expression profile further comprises information about the expression level of at least TP53.

7. The method of claim 2, wherein the expression profile further comprises information about the expression level of at least PTGS1.

8. The method of claim 2, wherein the expression profile further comprises information about the expression level of CD69, NPM1, SPRY2, TP53, and PTGS1.

9. The method of claim 1, wherein the expression profile comprises information of expression levels of gene transcripts.

10. The method of claim 9, wherein the expression profile is generated by a process involving amplification of gene products.

11. The method of claim 1, wherein the expression profile is generated on an array or microarray.

12. The method of claim 1, wherein the patient is an adult suspected of being Ph+.

13. The method of claim 1, further comprising evaluating the biological sample to determine whether the patient is Ph+.

14. The method of claim 1, further comprising obtaining the biological sample from the patient prior to generating the expression profile.

15. The method of claim 1, wherein the biological sample is enriched or screened for leukemic cells.

16. The method of claim 1, further comprising assessing the level of white blood cells in the patient.

17. The method of claim 1, further comprising determining whether leukemic cells of the patient have abnormal ploidy.

18. The method of claim 1, further comprising determining whether leukemic cells of the patient exhibit an 11q23 rearrangement.

19. The method of claim 1, further comprising reporting the expression profile to a clinician.

20. A method of treating a patient with acute lymphoblastic leukemia (ALL) that is characterized by the presence of Philadelphia chromosome (Ph+) or suspected of being Ph+ comprising:

a) obtaining information about the patient's expression levels of SLC2A3, ITPR1, TCF4, and FLT3 in leukemic cells of the patient;

b) treating the patient for ALL based on whether the expression levels of SLC2A3, ITPR1, TCF4, and FLT3 indicate the patient is an optimal responder or non-responder to ALL chemotherapy or is likely to relapse after ALL chemotherapy.

21. The method of claim 20, wherein the expression levels indicate the patient is likely to be an optimal responder and the patient is treated with standard therapeutic chemotherapy.

22. The method of claim 20, wherein the expression levels indicate the patient is likely to be to be a non-responder or likely to relapse and the patient is not treated with standard therapeutic chemotherapy.

23. The method of claim 22, wherein the patient is treated with a bone marrow or cord blood transplant.

24. The method of claim 20, further comprising obtaining information about the expression levels of one or more of: CD69, NPM1, SPRY2, TP53, or PTGS1.

25. The method of claim 24, wherein information about the expression level of at least CD69 is obtained.

26. The method of claim 24, wherein information about the expression level of at least NPM1 is obtained.

27. The method of claim 24, wherein information about the expression level of at least SPRY2 is obtained.

28. The method of claim 24, wherein information about the expression level of at least TP53 is obtained.

29. The method of claim 24, wherein information about the expression level of at least PTGS1 is obtained.

30. The method of claim 24, wherein information about the expression levels of CD69, NPM1, SPRY2, TP53, and PTGS1 is obtained.

31. The method of claim 24, wherein the information is obtained by taking a patient history or reviewing a report from a laboratory containing the information.

32. The method of claim 20, wherein treatment is determined also based on the level of white blood cells in the patient.

33. The method of claim 20, wherein treatment is determined also based on whether leukemic cells of the patient have abnormal ploidy.

34. The method of claim 20, wherein treatment is determined also based on whether leukemic cells of the patient exhibit an 11q23 rearrangement.

35. The method of claim 20, further comprising obtaining a sample containing leukemic cells from the patient to generate information about expression levels.

36. The method of claim 35, further comprising providing the sample to a laboratory for processing to generate information about expression levels.

37. The method of claim 20, further comprising ordering a test from a laboratory to obtain information about the patient's expression levels.

38. The method of claim 20, further comprising ordering a test from a laboratory that determines whether the patient's ALL is Ph+.

39. A composition comprising a chemotherapeutic agent for use in treating a patient with acute lymphoblastic leukemia (ALL) that is characterized by the presence of Philadelphia chromosome (Ph+) or suspected of being Ph+ , wherein the patient has been prognosed as an optimal responder to chemotherapy based on the patient's expression levels of SLC2A3, ITPR1, TCF4, and FLT3 in leukemic cells of the patient.

40. A composition comprising a chemotherapeutic agent for use in treating a patient with acute lymphoblastic leukemia (ALL) that is characterized by the presence of Philadelphia chromosome (Ph+) or suspected of being Ph+, wherein the patient has evaluated using the method of claim 1.

41. A composition comprising a therapeutic agent that is not a chemotherapeutic for use in treating a patient with acute lymphoblastic leukemia (ALL) that is characterized by the presence of Philadelphia chromosome (Ph+) or suspected of being Ph+ , wherein the patient has been prognosed as not being an optimal responder to chemotherapy based on the patient's expression levels of SLC2A3, ITPR1, TCF4, and FLT3 in leukemic cells of the patient.

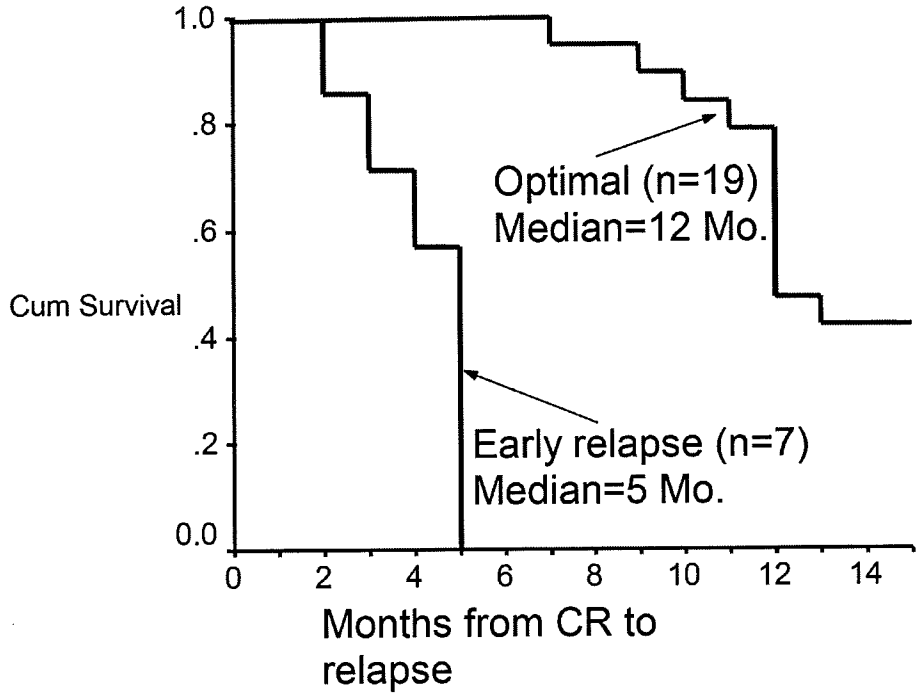

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.6
.4
.2
0.0
Cum Survival
Optimal (n=19)
Median=12 Mo.
Early relapse (n=7)
Median=5 Mo.
0
2
4
6
8
10
12
14
Months from CR to relapse

FIG. 1